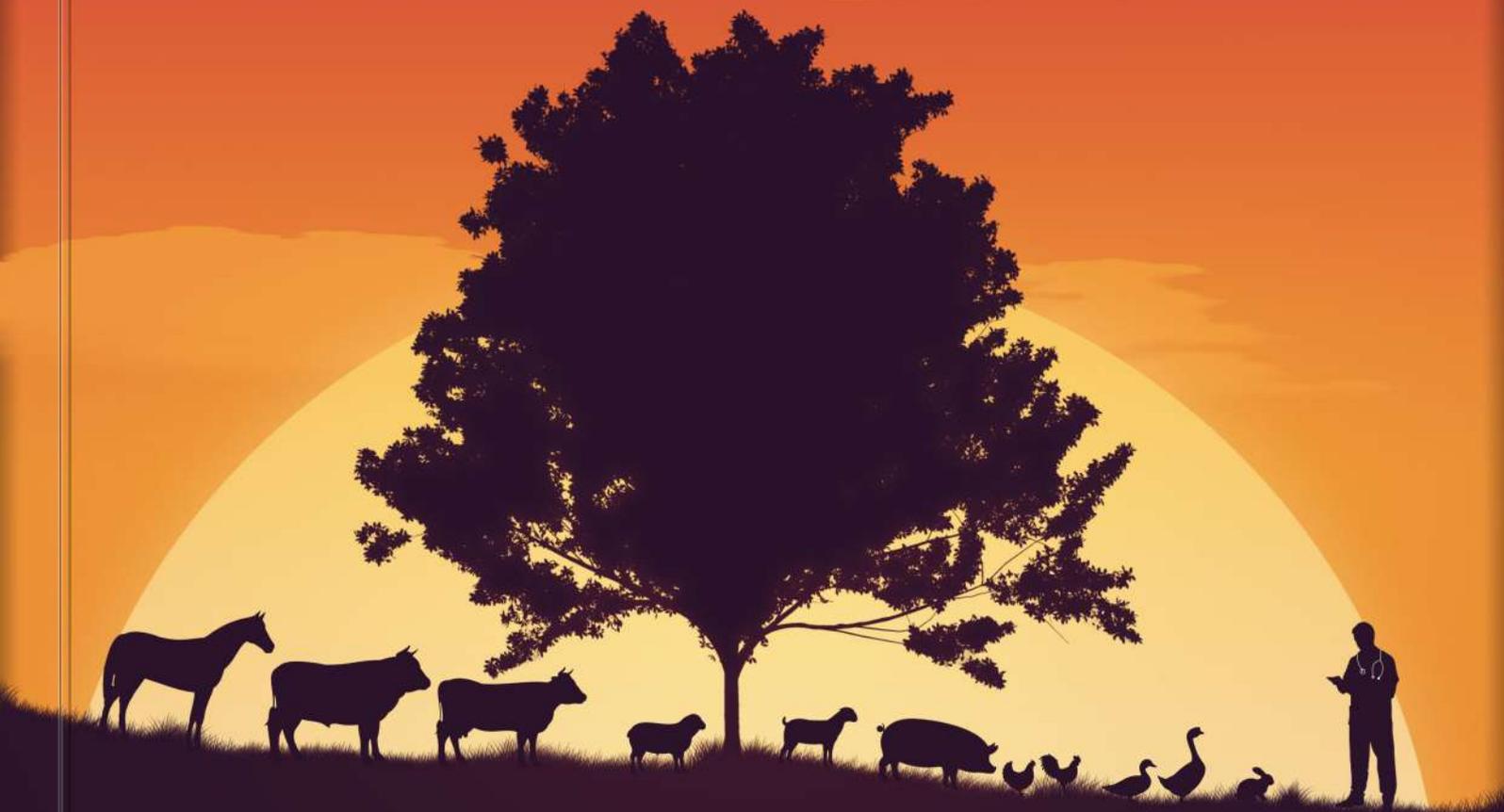




ISSN No. 2594-326x

IVSA MIRROR

Volume #5, June 2021



An Annual Publication of
International Veterinary Students' Association Nepal
(IVSA Nepal)

IVSA MIRROR

Issue #5, June 2021

ISSN No. : 2594-326x

Published By : International Veterinary Students' Association Nepal
(IVSA Nepal)

Email : editor.ivsanepal@gmail.com

Published Date : June, 2021

While the advice and information in this journal are believed to be true accurate at date of publication, neither the authors nor the editor or the publisher can accept legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

EDITOR'S NOTE

It is my great pleasure to bring forward the fifth volume of IVSA Mirror, the annual publication of IVSA Nepal. In spite of various hurdles and unfortunate situation due to COVID pandemic, we have been successful in continuation of the annual journal.

Working as the editor-in-chief of IVSA Mirror has been one of the most memorable period of my life. This responsibility has provided me with an opportunity to develop my skills and confidence as a team person and leader. Even though the editorial board faced many challenges during article collection as well as due to COVID pandemic, we were able to come on top.

I would like to thank the members of my editorial board, especially my assistant editor-in-chief, Miss Premisha Shrestha for her support and innovative ideas for 'IVSA Mirror V'. I would also like to thank the advisors for our publications Dr. Yuvraj Panth Dr. Dipesh Chhetri, Dr. Shankar Raj Barsila and Dr. Binod Pokhrel for their help during editing of the article and their guidance.

I am grateful to IVSA Nepal for providing me with an opportunity to display my skills as an Editor-in-Chief. It was a wonderful journey working as an editor and as a member of IVSA Nepal. I wish our journal could be a source of knowledge to all the readers. I am grateful to all the authors who provided us with their article and had patience and faith in us in this difficult times. IVSA Nepal will provide various platform to bring more knowledgeable and useful activities in future days as well. If you have any suggestion or any comments, please feel free to contact us at the address mentioned below.

Thank you!



Dibek Karki

Editor-in-Chief

IVSA Nepal, 2019/20

dibek118@gmail.com

ivsanepal@gmail.com



Agriculture and Forestry University Office of the Vice Chancellor Chitwan, Nepal



Forewords

It is a great pleasure for me to write a few words about the 5th publication of the scientific journal, IVSA Mirror #5, published by the editorial board and the whole team of International Veterinary Students Association, (IVSA) Nepal. In continuation with the previous publications, IVSA Nepal has been able to bring forward various research, reviews and case studies as well as findings of various authors and researchers in the field of veterinary education and animal husbandry.

Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal has been the first technical university dedicated to the production of highly skilled agricultural scientists, veterinarians as well as forestry graduates. Among the various students' technical organizations, IVSA Nepal has been able to leave a mark among the students as one of the leading technical organization through various programs in the field of veterinary education. The publication of the scientific journals such as IVSA Mirror has brought the knowledge, ideas and innovations of the individuals into the paper for everyone to learn and understand. In fact, it has united various students, teachers, researchers and innovators into a common ground where information is properly shared among the individuals.

IVSA Mirror has been playing a great role in bringing the knowledge of the few to wider audience. The success of such initiative will help to maintain the standard of the university as a true technical university. The journal will be instrumental in bringing together the national and international delegates to recognize the activities conducted by the veterinary students in the university. The journal will certainly promote the recognition of Agriculture and Forestry University in international community.

I would like to congratulate the team of IVSA Nepal for their efforts in bringing yet another informative and useful scientific journal in IVSA Mirror. I am confident that this publication will be highly beneficial to the students, faculty and researchers of the national as well as international community.

I wish for the success of this publication.

Sharada Thapaliya

.....
Prof. Sharada Thapaliya, PhD
Vice Chancellor



Agriculture and Forestry University
Faculty of Animal Science, Veterinary Science & Fisheries

Office of the Dean
Rampur, Chitwan, Nepal

Tel.No.: 056-592131
Fax No.: 056-526014



Message

It is my honor to write a few words to congratulate International Veterinary Students' Association Nepal (IVSA Nepal) on the successful publication of the 5th issue of its annual journal; IVSA Mirror. Being on the responsible position at Agriculture and Forestry University (AFU) for the last few years, I have directly witnessed the improvement of this journal both on quantity and quality, issue after issue. The journal has also started gaining its popularity at the international level. The international articles published in this journal signifies its reach to the global community.

The Faculty of Animal Science, Veterinary Science, and Fisheries (FAVF) is grateful to IVSA Nepal for the publication of this scientific journal, which is the collection of articles from national and international veterinary professionals and students. FAVF is also proud of other various educational activities of the association which are helpful to the students of this faculty. I sincerely praise the editors, the readers, the advisors, and the supporters who contributed to the publication of the journal.

Being in the period of global competition, veterinary students with good academic knowledge should also possess strong writing skills. Good writing skills ease and enhance scientific communication and also the dissemination of research findings. Therefore, IVSA Mirror is a platform for all veterinary students to harness their writing skills. I wish the veterinary students will use this golden platform and hope to see more articles from them in the upcoming issue.

Lastly, I want to appreciate the fact that even during the tough time of the pandemic, IVSA Nepal is successful in publishing such a great journal. I am sure, this journal will be highly beneficial to students, farmers, and foreign delegates. I wish IVSA Nepal an unceasing success.

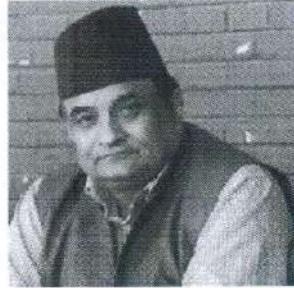
.....
Prof. Matrika Prasad Jamerkatel
Dean
Email: mpjamarkatel@afu.edu.np
Phone no: 9855055670



Tribhuvan University
Institute of Agriculture & Animal Science (IAAS)
Dean's Contact Office
Kirtipur, Kathmandu, Nepal



Liaison Office:
P.O. Box: 984, Kirtipur, Kathmandu, Nepal



Message from Dean

It's my great pleasure to write a few words of appreciation to International Veterinary Students Association Nepal for the publication of the 5th volume of its annual journal; IVSA Mirror. IVSA Nepal is globally recognized and one of the active national MO which has been bonding all the enthusiastic veterinary students from different institutions of Nepal and Global in one single agenda of veterinary sector upliftment.

IVSA Mirror is one of the commendable endeavor of IVSA Nepal which has been helping veterinary students to enhance their knowledge of scientific writings and publications. I believe these series of journals have become a great platform for veterinary students to enrich their writing skills and disseminating veterinary issues too.

Finally, I would like to sincerely praise the editors, the team of IVSA Nepal, the supporters, the valuable readers and authors who have contributed to the publication of this journal. I appreciate the fact that even in these hard times of the pandemic, IVSA Nepal has accomplished this great 5th edition of the journal and I am sure that this journal will be a cornerstone for the aspiring students in their research writing development. Thus, I hope for the continuation of these kinds of programs in the near future and would like to extend best wishes to the team of IVSA Nepal.

I wish for the success of this publication.

Asso. Prof. Sharoj Raj Mishra

Dean, IAAS.



Message

I feel glad to write some words on the publication of IVSA Mirror issue #5, which is a scientific journal annually published by the International Veterinary Students' Association Nepal (IVSA Nepal). It publishes veterinary science and animal husbandry related research articles, review articles, case study, and short communications. I would like to appreciate the IVSA Nepal and its editorial board in publishing this journal which requires hard work and tireless efforts.

I am sure that IVSA Mirror has been a good medium for veterinary students to uplift their writing skills. It has also been a reliable source of veterinary-related information to students, professionals, and farmers. Besides this publication, other activities such as free animal health camps, exchange program, training and workshops, veterinary quiz competitions, international conference and symposium, etc. has been equally admirable.

Being the first private veterinary college of Nepal, Himalayan College of Agricultural Sciences and Technology (HICAST) always supports programs that upgrades the knowledge of veterinary students. HICAST has been supporting the activities of IVSA Nepal, especially the IVSA HICAST from the beginning. I also want to ensure IVSA Nepal that HICAST always encourages and supports such activities that contribute to uplift the skills of veterinary students and veterinary education as a whole.

Finally, I would like to congratulate IVSA Nepal for publishing IVSA Mirror issue #5 and wish for the success of this publication.

Dr. Krishna Bahadur Shrestha
Principal
HICAST, Kirtipur, Kathmandu



पूर्वाञ्चल विश्वविद्यालय तथा प्राविधिक शिक्षा तथा व्यावसायिक तालिम परिषद्बाट सम्बन्धन प्राप्त

नेपाल पोलिटेक्निक इन्स्टिट्यूट लि.

(कलेज अफ इन्जिनियरिङ्ग, एआरएलएचए, केलेजरनरी एण्ड मेडिकल साइन्सेस)

भरतपुर, चितवन



qualityaustria
SYSTEM CERTIFIED
ISO 9001:2015 No.21286/0

प.सं.
च.नं.

मिति २०७७/०६/०१.....

Message from the Acaedemic Director



It is my pleasure to write few words in the "IVSA MIRROR, 5th edition", a scientific annual publication of International Veterinary Students Association (IVSA), Nepal.

I am sure that such publications will help veterinary students to increase their knowledge as reader and also as contributor they can increase their creativity by providing research articles, short notes, case report etc. They get knowledge about how to express their views and ideas on veterinary related matters scientifically.

I know that IVSA-NPI has been working continuously for last 3/4 years organizing different functions and activities such as speech program, work shop, quiz contest, rabies vaccination campaign, tiger day celebration, fare well among students etc which ultimately help students increase their creativities and help to make relationship among veterinarians and societies.

I would like to appreciate the editor, publication team and members of IVSA- Nepal for their hard work in order to bring out such a useful publication.

Thanks.

Dr. Egendra Kr. Shrestha
Academic Director



नेपाल भेटेरिनरी एशोसिएसन NEPAL VETERINARY ASSOCIATION



Message

I would like to congratulate the editorial board and the whole team of IVSA Nepal for their successful publication of 5th edition of IVSA Mirror. IVSA Mirror has been a great platform for various researches in the field of veterinary science among the students as well as seasoned researchers. It has provided the platform to acknowledge the findings in research of the veterinarian and has helped to broaden the horizon of knowledge of the students.

International Veterinary Students' Association Nepal (IVSA Nepal) has done justice to the ethics of the veterinarians and the field of veterinary as a whole. IVSA Nepal from the beginning of its formation has been extraordinary in various veterinary related activities and achievements. In doing so, IVSA Nepal has also been able to make its presence in various international committee. Its activities have benefitted not only veterinary students but also veterinary professionals and semi-professionals.

I would like to extend my best wishes to IVSA Nepal for the success of the publication of the scientific journal, IVSA Mirror. I hope for more of the activities from IVSA Nepal which will bring all the individuals of the veterinary fields together.

Best Wishes

Regards,

Dr. Sital Kaji Shrestha
President



Dear members of IVSA Nepal,

Firstly, I will very quickly introduce myself, my name is Merel Knoops, and I'm the new President of IVSA Global. I'm 22 years old, and I have just finished my fourth year of veterinary medicine at Ghent University, in Belgium.

It is with great pleasure I get to write this foreword for the 5th edition of the IVSA Mirror publication, and I truly hope you will enjoy reading all of the different articles that have been included in this journal.

One of the things I admire most about all IVSA members, is their immense motivation to contribute to the veterinary community, be it through exchanges, events, education or, for example, this journal. It's amazing that IVSA Nepal has worked so hard to get to publish this journal, for all of you to read and learn from. Because that's what IVSA is all about, sharing knowledge, skills and experiences to strengthen the future generation of veterinarians.

I encourage each and every one of you to contribute to the veterinary community in your own way, because together, we will change the world!

A big congratulations to IVSA Nepal for all of their hard work over the years. I hope to meet you all at one of our ISVA events one day.

Lots of love,

Merel Knoops

President 2020-2021





Forewords



It is my great pleasure to write some words on the behalf of IVSA Nepal in the IVSA Mirror volume 5. IVSA Nepal is the national member organization of IVSA which is the largest veterinary students' association in the world. Globally IVSA has about 40 thousands members and 194 member organizations in 73 countries. Since the establishment, along with her daughter organizations (i.e. IVSA HICAST, IVSA NPI, IVSA Paklihawa and IVSA Rampur), IVSA Nepal have been tirelessly attempting for animal and community welfare through releasing various activities including awareness, vaccinations, animal health camps, veterinary quiz competitions, workshops, trainings, Conferences, Symposiums and international exposures via Externship and Exchanges, etc. As a result of persistent hard work and dedication, IVSA Nepal among all the member organizations in 73 countries has been awarded as the "winner of the top MO competition 2020" which is a proud and historic moment never happened before.

IVSA Mirror is a scientific journal which focuses on research articles, review, case study, opinions and short communication related to veterinary science and animal husbandry. It is an annual publication of IVSA Nepal which publishes articles from veterinary professionals, students and other professionals related to Animal Health. The sole objective of the journal is to encourage veterinary students, young graduates, and professionals to write scientific articles and share their experiences, knowledge, and ideas to contribute to the entire veterinary community. Though the journal had to be published in the September, due to the covid-19 pandemic, the publication was delayed. I want to apologize for the late publication and inconvenience it has caused.

At last, my sincere appreciation to the editor and his team for their tremendous and continuous efforts throughout the journey of the publication despite the tough situation. I would like to express my sincere gratitude to our patron Prof. Dr. I.P. Dhakal, our advisors Prof. Dr. Bhuminda Devkota, PG-Coordinator and Assoc.



"Established in 2013 to benefit people and animals of the world"

INTERNATIONAL VETERINARY STUDENTS' ASSOCIATION NEPAL
(IVSA NEPAL)

Ref. No.

Date: - 6th June 2021

Prof. Dr. Rebanta Kumar Bhattarai for their remarkable support and guidance to the publication and moreover throughout the tenure. I am also greatly thankful to the advisors of the journal and the seniors who made major contributions in making this journal more scientific and free of technical indiscretions. I would also like to express my thanks to the executive board members of IVSA Nepal and all the local chapters for their collective efforts in achieving a new height by the organization and for the successful publication of the journal.

.....
Aashirbad Pokharel
President, IVSA Nepal
+977-9843418393
Email: aashirbad.pokharel@gmail.com

Acknowledgement

IVSA Nepal and IVSA Mirror Editorial Board would like to express sincere gratitude to Office of the Dean (FAVF, AFU), Office of the Dean (IAAS), Himalayan College of Agricultural Sciences and Technology (HICAST), Nepal Polytechnic Institute (NPI), Nepal Veterinary Association (NVA) Province number 3 and 5, International Veterinary Students' Association (IVSA), IVSA Rampur, IVSA Paklihawa, IVSA HICAST, IVSA NPI, Prof. Dr. Ishwari Prasad Dhakal, Prof. Dr. Sharada Thapaliya, Prof. Dr. Bhuminanda Devkota, Prof. Dilip Kumar Jha, Assoc. Prof. Shyam Bahadur Raut, Assoc. Prof. Dr. Dipesh Kumar Chhetri, Assoc. Prof. Dr. Rebanta Kumar Bhattarai, Dr. Sital Kaji Shrestha, Asst. Prof. Dr. Ananta Dahal, Asst. Prof. Shanker Raj Barsila, Asst. Prof. Dr. Narayan Neupane, Dr. Shatrughan Shah, Dr. Sanjiv Pandit, Dr. Niraj Shrestha, Dr. Asmita Shrestha, Dr. Nabin Shrestha, Dr. Thaneshwor Bhatta, Dr. Yuvraj Panth, Dr. Bishnu Hari Pokharel, Dr. Ganesh Adhikari, Dr. Manoj Lamichhane Sharma, Mr. Shalikram Kandel, Dr. Anmol Kandel, Dr. Khim Bahadur Ale, Dr. Binod Pokharel, Dr. Amrit Pandey, Dr. Swochhal Prakash Shrestha, Mr. Wang Pakhrin, Ms. Premisha Shrestha, Ms. Suvha Shree Sharma, Mr. Roshan Raj Singh Thakuri and to all the helping hands who directly or indirectly helped us throughout our movement and the publication process.

A big thanks to you all!

IVSA MIRROR

Editorial Board



Dibek Karki
Editor in Chief



Premisa Shrestha
Asst. Editor in Chief



Suvha Shree Sharma
Member



**Roshan Raj Bahadur
Singh Thakuri**
Member



Wang T Pakhrin
Member



Amar Mahat
Member

Advisors



Dr. Dipesh Chettri



Dr. Shankar Raj Barsila



Dr. Yuvraj Panth



Dr. Binod Pokhrel



IVSA NEPAL

Executive Committee 2019/20



Aashirbad Pokharel
President



Pratik Kiju
Exchange Officer



Chakra Oli
General Secretary



Saroj Ojha
Treasurer



Dibek Karki
Editor in Chief



Premisa Shrestha
Asst. Editor in Chief



Tejaswee Pokhrel
Secretary



Sudip Regmi
Member



Prabesh Paudel
Member



Niranjana Yadav
Member



Santosh Panta
Member



IVSA RAMPUR

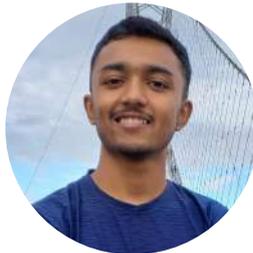
Executive Committee 2019/20



Sudip Regmi
President



Khil Bdr Rana Magar
Exchange Officer



Samín Dahal
Secretary



Pradip Raj Pant
Treasurer



Wang T Pakhrin
Editor



Puspa Rai
SCOH Co-ordinator



Pratibha Poudel
SCoVE Co-ordinator



Kanchan Thapa
SCAW Co-ordinator



Dikshya Khanal
Member



Bishwa Gaire
Member



Kabit Timilsina
Member



Pramod Chaudhary
Member



Som Nath Aryal
Member



IVSA PAKHLIHAWA

Executive Committee 2019/20



Santosh Panta
President



Samikshya Phuyal
Exchange Officer



Aashish Adhikari
Secretary



Pema Sherpa
Treasurer



Suvha Shree Sharma
Editor



Suresh Rokaya
SCOH Co-ordinator



Ashwini Kumar Jha
SCoVE Co-ordinator



Rachana Dangol
SCAW Co-ordinator



Usha Khatri
Member



Alok Dhakal
Member



Anish Balchhaudi
Member



Prakash Rawal
Member



Ram Shankar
Member



IVSA HICAST

Executive Committee 2019/20



Niranjan Yadav
President



Madhav Rawat
Vice President



Sadhana Ghimire
Exchange Officer



Ishwor Sahi
Secretary



Amar Mahat
Editor



Bhuwan Chandra Joshi
Treasurer



Anjan Rijal
Standing Committee
Coordinator



Arya Niraula
Member



Praphulla Panta
Member



Prachanda Tamang
Member



Shitesh Ghimire
Member



IVSA NPI

Executive Committee 2019/20



Prabesh Paudel
President



Anita Poudel
Vice President



Sundar Basnet
Vice President



Prabin Paudel
Exchange Officer



Narayan Poudel
Secretary



**Roshan Raj Bahadur
Singh Thakuri**
Editor



**Shambhu Kumar
Mahato**
Treasurer



Purushottom Kafle
SCOH Coordinator



Sushmita Shah
SCoVE Coordinator



Hasan Bikram Thapa
SCAW Coordinator



Ujjwal Basnet
Member



Rashmi Thapa
Member



Dev Shah
Member



Achyut Acharya
Member



Bipin Regmi
Member



Apekshya Shrestha
Member

IVSA MIRROR

Contents

1	A Case Report on Canine Transmissible Venereal Tumor (CTVT) P. Kiju, I. Tiwari, B. Shrestha	1
2	A Case Report on Leptospirosis in Bull at Pokhara Nepal P. L. Mahato, S. Regmi, I. Devkota, R. P. Neupane, A. Khulal, G. K.C	6
3	A Case Report on Urolithiasis in Dog S. Kunwar, B. Shrestha	10
4	Assisted Reproductive Techniques in Farm animals: Need of the Hour T. Safdar, M. A. Bashir, A. Haseeb	15
5	Calcium Homeostasis and Milk Fever in Dairy Cows R. Yadav, S. Yadav & B. Shrestha	19
6	Canine Parvovirus, a Prevalent Disease of Canine, Its Transmission & Pathophysiology Haseeb A., Bashir, M. A., Rehman, A.U., Abedin, M.Z.U.	24
7	Case Report: Canine Distemper in German Shepherd M. Ahmed, A. Waheed	29
8	Case Report on Management of Acute Gastric Dilation Lacking Volvulus without Surgical Intervention A. Yadav	31
9	A Case Report on: Metastatic Mammary Gland Tumor in 12-year-old Mongrel Dog S. Phuyal, R. Shah, G. P. Yadav	35

IVSA MIRROR Contents

- 10 Case Study of Lumpy Skin Disease in Cattle of Chitwan Nepal** 38
Y. R. Pandeya, C. R. Pathak, G. Pandey, P. Hamal, P. Khanal
- 11 Control, Prevention and Management of Orf in a Goat (*Capra hircus*)** 42
H. M. M. Ali, H. Jawad
- 12 Dephosphorylation of Phytate Complex for Increasing the Phosphorus Availability in Monogastric Animals Diet** 45
S. R. Barsila
- 13 Effect of Feed Processing on Quality: Implications on Ruminants and Non-Ruminants Performance** 48
S. R. Barsila
- 14 Ketosis in Dairy Cattle: a Review** 54
P. Shrestha, R. Pande
- 15 Mastitis in Dairy Cows: Behavioral Changes, Treatment and Control** 58
R. A. Gul, H. Jawad, N. Iqbal, M. A. Nawaz
- 16 Medroxyprogesterone Acetate Induced Prolonged Gestation in German Shepherd: a Case Study** 62
P. Subedi, N. Shrestha
- 17 Metacarpal Fracture in Nilgai: a Classic Case of Human-wildlife Conflict?** 65
S.P. Shrestha, Y. Panth, D. Sapkota
- 18 Milk in Pakistan: from Production to the End Consumer** 70
H. Jawad, A. Tahir, S. Annisa, R. A. Gul

IVSA MIRROR Contents

19 Myiasis and its Public Health Concern in Tropic E. E. Ojowu, R. A. Gul	73
20 Pseudopregnancy in Bitch: a Review N. Shrestha, K. Kaphle	77
21 Pyometra in a Bitch: a Case Report S. Yadav, R. Yadav	82
22 Repeat Breeding in Cattle and Buffalo: Etiology, Diagnosis, and Treatment G. Gautam	86
23 Spiking and Intra-Spiking: a Tool in Breeder Management N. Aryal	92
24 Surgical Correction of Aural Hematoma in Dog B. Pokhrel, S. Shrestha, Y. Panth	96
25 Tibia and Fibula Fracture in Dog: a Case Report S.P. Bhusal	100
26 Learn from the Past: the Zoonotic Perspective of SARS, MERS, and COVID-19 S. Annisa, H. Jawad	103

A CASE REPORT ON CANINE TRANSMISSIBLE VENEREAL TUMOR (CTVT)

P. Kiju*, I. Tiwari, B. Shrestha



Abstract: *Canine Transmissible Venereal Tumor (CTVT) is a unique kind of tumor that is transmitted by viable cell transplantation under natural conditions through coitus, licking, sniffing and fighting with the infected animal. Kale, a 2-year-old male crossbreed spitz dog was presented in Veterinary Teaching Hospital (VTH), Institute of Agriculture and Animal Science (IAAS), Paklihawa Campus with ulcerated and tumorous growth in the caudal part of bulbous glandis. Diagnosis of the case was done and found to be CTVT on the basis of physical and cytological findings of multiple clear cytoplasmic vacuoles in the glass slide. The traditional method of surgery was performed and tumorous growth was excised using blunt equipment with emasculation technique. The patient was treated with two shots of vincristine sulphate after the surgery and in the 3rd week after the surgery. The immune response plays an important role in determining the course of the disease. Combination therapy which includes a combination of different methods of treatment like surgery, chemotherapy, radiotherapy and immunotherapy is the best method in the treatment of CTVT.*

Keywords: chemotherapy, combination therapy, CTVT, vincristine sulphate

Introduction

Canine Transmissible Venereal Tumor (CTVT) is a benign reticuloendothelial tumor of dogs that mainly affects the genitalia and occasionally the extragenital sites. It is also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor. Since coitus is the major way of transmission of CTVT, the tumor generally occurs in young, sexually mature animals (Purohit, 2019). CTVT is the unique kind of tumor transmitted as an allograft by cell transplantation under natural conditions and surviving as autonomous from the original host (Mukaratirwa & Gruys, 2011). Viable CTVT cells are transmitted to host dogs spontaneously through licking, sniffing, fighting/biting, or scratching and can occur anywhere on the body including the skin, oral and nasal cavities (Eze, Anyanwu, & Kene, 2008). The tumor is typically reported in tropical and subtropical countries and found in all breeds of dogs. CTVT is generally a benign tumor but it is also reported as a metastasized form in regional lymph nodes, liver, pancreas, and brain (Eze et al., 2007). The incidence of metastasis is generally low and about 5% or fewer cases are

reported (Saibaba, Dhana Lakshmi, & Phaneendra, 2015). The mutated tumor cell has an abnormal number of chromosomes ranging from 57 to 64, in contrast to the normal tract, the role of TVT in the pathogenesis of Urinary Tract Infection (UTI) is also high. Upon genital examination of male dogs, the tumors are often situated on glans, mucosa and preputial bulb (Nak, Nak, Cangul, & Tuna, 2005) with consequent phimosis (Rashid Dar et al., 2017). Similarly, in females, the tumor is located in the caudal vagina and vaginal vestibule and rarely found in the uterine region (Nak et al., 2005). Moreover, the projection from the vulva leads to the perineal region which generally doesn't interfere with urination in female dogs (Rashid Dar et al., 2017). Hemorrhagic discharge which is often seen after the ulceration lesion in male external genitalia is usually confused with urethritis, cystitis and prostatitis and such lesion is often the cause of anemia in females (Rashid Dar et al., 2017). Cryosurgery, Radiotherapy, surgical therapy and antineoplastic chemotherapy are some of the methods to handle CTVT. (Rashid Dar et al., 2017). The present

IVSA MIRROR V

paper reports CTVT on a mixed breed dog and treatment is done with combination therapy (Surgical therapy with chemotherapy).

Case Description and History

A 2-year-old male dog (Kale) of mixed breed dog (Spitz cross) was brought in Veterinary Teaching Hospital (VTH), IAAS, Paklihawa Campus with the chief complaint of abnormal swelling near bulbus glandis (**Fig. 1**) with oozing of hemorrhagic fluid. Hematuria was one of the main signs of the patient. The patient was black and grey with a weight of 11.5 kg and a rectal temperature of 101.6° Fahrenheit. According to the owner, the dog was a well-supervised home kept dog but it had escaped once in the same month before the appearance of symptoms.

Clinical Signs

The tumor (8-10 cm) was well palpated from the exterior skin surface of the bulbus glandis. Perforation was clearly observed in the tumor site with oozing of sanguineous fluid. The patient was in pain while urinating as it showed curved spinal position as well as hematuria while urinating. Urinary hesitancy and dysuria were also observed. Extra genital lesions were not observed.

Diagnosis

Definite diagnosis of CTVT is based on physical examination and cytological findings typical of CTVT in exfoliated swabs, fine needle aspiration



Fig 1: Ulcerated tumorous growth in the area of bulbus glandis

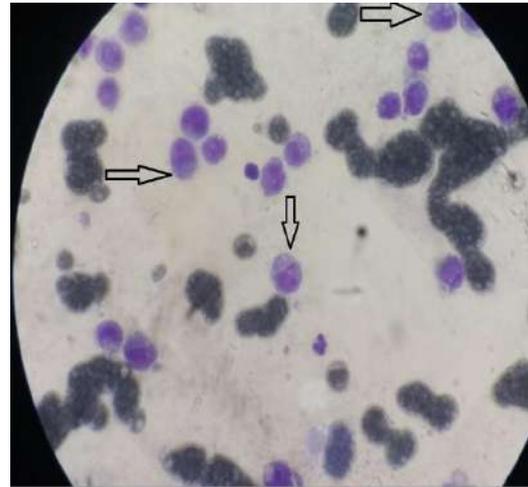


Fig 2: Multiple clear cytoplasmic vacuoles in blood smeared in Giemsa stain

or imprints of the tumor (Purohit, 2019). Imprints of tumor were collected on a glass slide, were air dried and were stained with diluted Giemsa stain (1:20 vol/vol) for 20 min. The slides were then washed off with buffered water (1-2 drops) and left for air drying. After the smear was dried up, it was observed with a 100X microscope. CTVT cells were observed in the treatment microscope and very distinct mitotic figures with chromatin clumping with more than 2 prominent nuclei were observed. Multiple clear cytoplasmic vacuoles were observed with the increased nucleus to cytoplasmic ratio (**Fig. 2**).

Treatment

Surgical treatment:

A. Control and Anesthesia

For Sedation

Xylazine @1mg/kg ml was given intramuscularly and after proper sedation, atropine @0.022 to 0.044 mg/kg was also given to increase heart rate (to oppose the effect of bradycardia due to Xylazine) according to the protocol of VTH, IAAS, Paklihawa Campus.

The dog was positioned in dorsal recumbency, hairs of hypogastric and mesogastric regions were then clipped, properly shaved and the region was made aseptic.

IVSA MIRROR V

For General Anaesthesia

General anesthesia was induced with the combination of Diazepam and Ketamine in the ratio 1:1 @0.25mg/kg Diazepam and @5mg/kg ketamine as induction dose and was maintained as Diazepam and ketamine in the ratio of 1:2 according to the required protocol.

B. Surgery incision and suturing

The traditional approach of surgical intervention was practiced. After all the pre-surgical management, the surgical site was draped and prepped properly (Fig. 4). About 4-5 cm long skin incision was given at the site of the body of the penis taking care not to damage the urethra of penis (Fig. 5). Scalpel surgery was not done in the surgery except during the incision on the skin. Slowly using a blunt object, in this case, fingers were used to undermine the tumorous

nodules around the caudal part of the bulbus glandis of the dog (Fig. 6). Blunt emasculation was done during the removal of tumorous mass using the help of the finger of the surgeon and taking care of the urethra and other vascular healthy tissue. Thus, the cauliflower-like growths attached to external genital mucosa were undermined and removed (Fig.7). Then the muscle and subcutaneous layer was closed in a continuous pattern with absorbable suture. Finally, the skin layer was closed using a non-absorbable suture (polypropylene) in a simple interrupted pattern (Fig. 8). The patient was treated with two doses of vincristine sulphate @0.025mg/kg diluted in 200ml of saline water after the surgery on the first day. The patient was weekly consulted for the follow-up of the case where no growth of the tumorous mass was observed. The tumor

Time	13:33	13:40	13:45	13:51	13:57	14:04	14:10	14:23
Top up(ml)	0.5	0.5	0.5	0.8	0.8	0.8	0.8	0.5

Table 1: Time and Top-up dose of anesthesia used during the surgery.

Time	13:09	13:15	13:23	13:33	13:41	13:47	13:52	13:57	13:25
Heart rate	164	164	132	168	148	176	169	164	150
Respiration rate	9	11	12	16	25	30	27	28	24

Table 2: Different clinical parameters during the surgery.

subsequently decreased during the course of follow up observation. The second dose of vincristine sulphate was given with the same dose as the initial dose in the 3rd week of the surgery.

Case Discussion

CTVT can occur as a single solitary mass or as multiple tumors with cauliflower like growth, lobular, nodular or papillary forms like appearance. CTVT may also develop in extragenital sites such as skin, subcutaneous tissue and in oral and nasal cavities and such localization are difficult to diagnose. Metastases

are rare in CTVT and yet they can occur in puppies and immune compromised dogs (Ks, Narendran, & Ajidhan, 2015). The CTVT cells are contagious as it is transmitted by the transfer of living stable cancer cells. Dogs with CTVT are at high risk of having bacteriuria due to urine retention as the urine will not pass out due to mechanical obstruction of the urethral orifice by the tumor. Occasionally tumor cells may be parasitized by canine *Leishmania* where they both coexist (Eze et al., 2008). The immune response against the disease plays a major role in determining the course of the disease. In most adult dogs, the disease spontaneously regresses after a period of logarithmic growth due to the



Fig 3: Aseptic preparation of surgical site



Fig 6: Removal of Tumor using blunt equipment (here, finger) to undermine tumor nodule



Fig 4: Draping around surgical site



Fig 7: Tumor excised out.



Fig 5: Incision of Penis area to undermine the tumor site



Fig 8: Surgical site after removal of CTVT

development of IgG in the sera of the dog after the infection of CTVT. The prognosis for total cure is good unless metastatic involvement of the central nervous system or eye is involved (Ganguly, Das, & Das, 2013).

In this case, surgical treatment was done as the tumor site was excessively ulcerated and the size of the tumor was blocking the urine passage. Surgical excision is generally done to those cases that are presented with solitary, small, easily accessible and noninvasive tumor nodules. Electrosurgical excision seems to have an edge over the traditional surgical approach (Ganguly et al., 2013). Surgical treatment has a lower rate of efficacy and resulting in a higher rate of recurrence (18-60%) which is influenced by the location and extent of the disease and up to 50-68% relapse occurs solely owing to tumor cell transplantation into the surgical wound (Eze et al., 2008). Excision surgery, chemotherapy, radiotherapy, immunotherapy are some of the treatments to cure CTVT, though combination therapy is considered the best method to cure the tumor. Therefore, a single shot of Vincristine Sulphate was also given to the dog after two weeks of surgery in our case. Chemotherapy has been shown as the most effective and practical therapy where vincristine sulphate is most widely used with 2 to 8 injection slow IV for complete remission (A. K. Das, 1989; U. Das & Das, 2000; Khan et al., 2009). Vincristine resistant CTVTs may be treated with doxorubicin. Antineoplastic treatment can be combined with two or more chemotherapeutic agents (Vincristine and cyclophosphamide combined with methotrexate) (Rashid Dar et al., 2017).

Conclusions

CTVT case is one of the most encountered and prevalent neoplasia of the external genitalia of the dog. The case was presented in the VTH hospital, Paklihaba Campus, IAAS and was diagnosed by distinctive physical and cytological findings. As there was an ulcerative and blood oozing wound with tumorous growth on the surface of the bulbus glandis, the tumorous

growth was surgically emasculated. Then, after a week two shots of vincristine sulphate were given within the interval of two weeks. Combination therapy can be considered the best method to cure the tumor.

References

- Das, A. K. (1989). *Metastasis of canine transmissible venereal sarcoma (CTVS) in a dog*. 10(June), 74–75.
- Das, U., & Das, A. K. (2000). Review of canine transmissible venereal sarcoma. *Veterinary Research Communications*, Vol. 24, pp. 545–556. <https://doi.org/10.1023/A:1006491918910>
- EZE, C., ANAYANWU, H. ., & KENE, R. O. (2007). REVIEW OF CANINE TRANSMISSIBLE VENEREAL TUMOUR(TVT) IN DOGS. *Nigerian Veterinary Journal*, 28(1), 54–70. Retrieved from <http://dx.doi.org/10.4314/nvj.v28i1.3544>
- Eze, C. A., Anyanwu, H. C., & Kene, R. (2008). Review Of Canine Transmissible Venereal Tumour (TVT) In Dogs. *Nigerian Veterinary Journal*, 28(1). <https://doi.org/10.4314/nvj.v28i1.3544>
- Ganguly, B., Das, U., & Das, A. K. (2013). Canine transmissible venereal tumour: a review. *Veterinary and Comparative Oncology*. <https://doi.org/10.1111/vco.12060>
- Khan, L. A., Khante, G. S., Raut, B. M., Bodkhe, A. M., Chavan, M. S., Pagrut, N. S., & Bobde, S. P. (2009). *Incidence of Venereal Granuloma and its Medicinal treatment in stray Dogs of Nagpur City A2LA implements new veterinary laboratory accreditation program to include OIE and ISO / IEC requirements*. 2(1), 13–14.
- Ks, S., Narendran, P. V., & Ajidhan, V. B. (2015). Cronicon VETERINARY SCIENCE Case Report. In *Science* (Vol. 2).
- Mukaratirwa, S., & Gruys, E. (2011). *Canine transmissible venereal tumour: Cytogenetic origin , immunophenotype, and immunobiology. A review*. 2176. <http://doi.org/10.1080/0165216.2003.9695151>
- Nak, D., Nak, Y., Cangul, I. T., & Tuna, B. (2005). A clinico-pathological study on the effect of vincristine on transmissible venereal tumour in dogs. *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine*, 52(7), 366–370. <http://doi.org/10.1111/j.1439-0442.2005.0743.x>
- Purohit, G. N. (2019). Canine Transmissible Venereal Tumour :A Review. *The Internet Journal of Veterinary Medicine*, 6(1).
- Rashid Dar, R., Islam, T., Rouf, A., Wani, J. M., Dogra, P., Amin Sheikh, A., ... Ganaie, M. Y. (2017). Cytological Diagnosis and Treatment of Transmissible Venereal Tumor in Dog-A Case Study. *Int.J.Curr.Microbiol.App.Sci*, 6(10), 1365–1369. <https://doi.org/10.20546/ijemas.2017.610.160>
- Saibaba, M., Dhana Lakshmi, N., & Phaneendra, M. S. S. V. (2015). Successful Chemotherapeutic Management of TVT in Dogs-Report of 24 Cases. In *IJISSET-International Journal of Innovative Science, Engineering & Technology* (Vol. 2). Retrieved from www.ijiset.com

A CASE REPORT ON LEPTOSPIROSIS IN BULL AT POKHARA NEPAL

P. L. Mahato*¹, S. Regmi¹, I. Devkota¹, R. P. Neupane¹,
A. Khulal¹, G. K.C²



Abstract: *Leptospirosis is one of the most commonly occurring zoonotic diseases transmitting from one animal to another through infected urine, soil and water when they come in contact with the mucosal surface of the eye, nostrils, mouth and genital tract. Pyrexia, hemoglobinuria, hemolytic anemia, jaundice, weakness, anorexia are the commonly observed signs. The urine dip-strip test was performed showing increased pH and proteinuria with a reduced specific gravity of the urine. The urine sample was centrifuged at 5000 rpm for 5 minutes to obtain sediment. Thick smear was made from the sediment and was stained with Giemsa stain. Dark pink coloration of elongated coiled thread-like structure was observed under oil immersion microscopy. Successful treatment was performed by intramuscular administration of oxytetracycline at the rate of 20mg per kg body weight for 3 days.*

Keywords: hemolytic anemia, oil immersion microscopy, proteinuria, zoonotic

Introduction

Leptospirosis is an infectious disease of zoonotic importance caused by a spirochete bacterium *Leptospira* which can either be transmitted through infected urine or direct contact of contaminated soil or water on the mucosal surface of eye, nostril, mouth or genital tract. They have periplasmic flagella, and a spiral body which is used to move in a rapid darting motion in liquid media and rotate in its long axis so that they can penetrate the membranes (Allan et al., 2015). Venereal mode of transmission is also recorded in animals (Wakeman, 1945). It is also known as Weil's disease and can lead to multiple organ failure with the potential risk for death. It is transmitted to humans from domesticated cattle, pig and horses whereas dogs can be infected by wild animals like raccoons and porcupines (Lokida et al., 2016). *Leptospira* can survive 6 days in water and 24 days in the soil after being excreted by an infected animal (Allan et al., 2015). Farmers are unaware that *Leptospira* can cause abortion in pregnant females at the second trimester and stillbirth or uterine death of the fetus if infected in the third trimester whereas sterility in infected bulls leading to threat in performance of animal and

production potential (Gompo, Jyoti, Pandit, Sapkota, & Pandey, 2020). The overall incidence of leptospirosis was 10.5% in cattle, buffalo, goat, sheep and pig reported by the **Directorate of Animal Health, Nepal** (Regmi, Pandey, Malla, Khanal, & Pandey, 2017). The main clinical signs in cattle include pyrexia, hemolytic anemia, hemoglobinuria, jaundice and occasionally death. In lactating animals, milk yield was reduced and contains colostrum-like color with high somatic cell count (Ellis, 2015). Diagnosis of disease and its treatment becomes challenging in the absence of well-equipped laboratories. Leptospirosis can be tested by direct examination of blood and/or urine, polymerase chain reaction, ELISA etc. (Picardeau et al., 2014).

Case Report

Urine of a 4-years old Jersey cross bull was brought to Veterinary Laboratory Pokhara from Lamachour, Pokhara-19 reporting that the animal is urinating red-colored urine, weakness, anorexia, restlessness, unable to mount from the last three days. The animal was not brought to the lab, so no vital signs were recorded. The

¹ Agriculture and Forestry University, Rampur, Nepal

² Regional Veterinary Laboratory, Pokhara, Nepal

*Corresponding author: pnamahato1@gmail.com

IVSA MIRROR V

farmer also reported that the same incidence was seen six month ago.

Materials and method of diagnosis

For proper diagnosis, a dip-strip test of urine for the detection of any disorder was performed. Increased level of protein in the urine, increased pH and decreased specific gravity was detected. We took a few ml of urine in a test tube and it was centrifuged at the rate of 5000 rpm for five minutes. The urine became clear with only a few ml of sediment at the bottom of the tube. The clear fluid after centrifugation indicated the presence of blood cells in the urine and the condition was hematuria. This condition can be a result of many diseases, injuries in the urinary system and/or kidney disease.

In an attempt of diagnosis, sediment was observed in a microscope using slide and coverslip. We observed clear round cells in 10X focus of the microscope. On observation, slightly moving spiral organisms were seen.

On observation under a microscope, we found a spirally coiled organism in the urine. The sediment was then stained with Giemsa after fixing with methanol. The smear was cleaned with gentle water current to clean the unused stain. Under an oil immersion microscope the organism was seen on the slide appearing as a thread like dark pink colored elongated, coiled structure arranged in clusters in a different field. The observation of organisms matched previous observations which was the same as *Leptospira*.

Discussion

The most common causes of hematuria can be benign tumor, stones, infection, trauma, renal and iatrogenic cause (Yeoh, Lai, Anderson, & Appadurai, 2013). However, these can easily be ruled out, as we saw an organism in urine sediment. Among all other different approaches in diagnosis, urine was the only sample obtained, the laboratory facilities limited us to the use of cytological techniques, and we ended up doing Giemsa Staining instead of the PCR, ELISA or

other advanced technology. Urine can be used as diagnostic material (Rodríguez, Rodríguez, Fernández, Rodríguez, & Cantillo, 2013) and for Fontana Silver stain technique, blood serum is inappropriate. The standard method for staining of *Leptospira* is Fontana Silver stain from a long time (Rodríguez et al., 2013), though Giemsa staining can also be used for the same purpose (Babudieri, 1961). The silver Fontana stain makes the organism visible as a cylinder, and coiled with our findings in the lab even in Giemsa stain.

The normal value of urine protein (albumen) should be negative or very low (Parrah et al., 2013), but in urine strip dip test it was seen that urine was proteinuric, which is associated with hematuria condition. The specific gravity of urine in the bull was lower than normal value, but the specific measure should be in the form of ratio of specific gravity to the creatinine level because their ratio is to be constant (Gunasekara et al., 2017). However, reduction in specific gravity of urine indicates osmotic diuresis, loss of medullary tonicity, resistance and deficiency of antidiuretic hormone (Gunasekara et al., 2017). Normally the acidic pH doesn't favor the growth of *Leptospira*, but the pH for urine was 9 in this bull which favored the growth. It was reported that grazers have higher pH in comparison to non-grazers, hence occurrence of *Leptospira* is higher in grazers (Ellis, 2015).

Alt, Zuerner, & Bolin, (2001) and Towheed et al.,(2019) reported that drugs like Tilmicosine 10mg/kg body weight S/C single dose, Dihydrostreptomycin-penicillin G 25mg/kg body weight I/M single dose, multiple doses of Ceftiofur sodium 5mg/kg body weight I/M once daily for 5 days or 20mg/kg body weight I/M once daily for 3 days stopped shedding of bacteria from the urine. The animals can also be treated for shedding bacteria with streptomycin 25mg/kg body wt, making (PCR) serologically negative leptospira result after two days of the injection (Gerritsen, Koopmans, & Olyhoek, 1993).

IVSA MIRROR V



Table 1: Physical properties and Urine strip test result with reference value (Parrah et al., 2013)

S.N.	Parameters	Properties	Reference value
1	Color	Slightly red	Pale yellow-dark brown yellow
2	Transparency	Opaque	Clear
3	Specific Gravity	1.01	1.02-1.045 (Herman et al., 2019)
4	pH	9	7-8.4
5	Nitrogen toxicity	Negative	Negative
6	Albumen	30gm/dl	Negative or very low
7	Acetone	Negative	Negative
8	Glucose	Negative	Negative
9	Bilirubin	Negative	Negative
10	Urobilinogen	Negative	Negative-weak positive

Treatment

Oxytetracycline was given at the rate of 20mg per kg body weight intramuscularly for three days in the upper muscular region of hind leg i.e. gluteal region. The prognosis was very good after the treatment with Oxytetracycline in the bull. The farmer was advised to keep the bull in a separate isolation shed, feed vitamins and minerals for better and fast recovery and mating was asked to be paused till three weeks of treatment. To make sure that the bacteria was completely eradicated, re-examination of urine samples was suggested before the mating schedule.

Conclusions

Leptospirosis is a bacterial, zoonotic disease; it is easily transmitted to human and other animals through direct, indirect contact with materials infected with the bacteria. High fever, red urine, anemia, jaundice like symptoms are clinical signs and can be diagnosed by a serological and cytological method. Oxytetracycline, Ceftiofur, Tilmicosin, Dihydrostreptomycin - penicillin, streptomycin are antibiotics effective against the bacteria and stop shedding through urine in one to five days of treatment.

Acknowledgement

I'm highly grateful to the staff working in Regional Veterinary Laboratory, Pokhara for their assistance. I would also like to convey my sincere gratitude to Dr. Bharat Regmi, Dr. Kedar Pandey and Mrs. Bishnu who helped me by providing laboratory facilities during my study.

References

- Allan, K. J., Biggs, H. M., Halliday, J. E. B., Kazwala, R. R., Maro, V. P., Cleaveland, S., & Crump, J. A. (2015). Epidemiology of Leptospirosis in Africa: A Systematic Review of a Neglected Zoonosis and a Paradigm for 'One Health' in Africa. *PLoS Neglected Tropical Diseases*, 9(9). <https://doi.org/10.1371/journal.pntd.0003899>
- Alt, D. P., Zuerner, R. L., & Bolin, C. A. (2001). Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar hardjo. *Journal of the American Veterinary Medical Association*, 219(5), 636–639. <https://doi.org/10.2460/javma.2001.219.636>
- Babudieri, B. (1961). *Laboratory Diagnosis of Leptospirosis**. *Bull. Org. mond. Sante* (Vol. 24).
- Ellis, W. A. (2015). Animal Leptospirosis. *Current Topics in Microbiology and Immunology*, 387, 99–137. https://doi.org/10.1007/978-3-662-45059-8_6
- Gerritsen, M. J., Koopmans, M. J., & Olyhoek, T. (1993). Effect of streptomycin treatment on the shedding of and the serologic responses to *Leptospira interrogans* serovar hardjo subtype hardjobovis in experimentally infected cows. *Veterinary Microbiology*, 38(1–2), 129–138. [https://doi.org/10.1016/0378-1135\(93\)90080-Q](https://doi.org/10.1016/0378-1135(93)90080-Q)
- Gompo, T. R., Jyoti, S., Pandit, S., Sapkota, R. C., & Pandey, A. (2020). Sero-prevalence and risk factors of leptospirosis in commercial cattle herds of Rupandehi district, Nepal. *BioRxiv*, 2020.07.29.226464. <https://doi.org/10.1101/2020.07.29.226464>
- Gunasekara, C. P., Sumaiha, M. H. F., Damayanthi, M. K. S., Weerasekera, M. M., & Fernando, S. S. N. (2017). Utility of a modified silver staining technique for detection of *Leptospira*. *Sri Lankan Journal of Infectious Diseases*, 7(2), 85. <https://doi.org/10.4038/sljid.v7i2.8142>
- Herman, N., Bourgès-Abella, N., Braun, J. P., Ancel, C., Schelcher, F., & Trumel, C. (2019). Urinalysis and determination of the urine protein-to-creatinine ratio reference interval in healthy cows. *Journal of Veterinary Internal Medicine*, 33(2), 999–1008. <https://doi.org/10.1111/jvim.15452>
- Lokida, D., Budiman, A., Pawitro, U. E., Gasem, M. H., Karyana, M., Kosasih, H., & Siddiqui, S. (2016). Case report: Weil's disease with multiple organ failure in a child living in dengue endemic area. *BMC Research Notes*, 9(1), 407. <https://doi.org/10.1186/s13104-016-2210-4>
- Parrah, J. D., Moulvi, B. A., Gazi, M. A., Makhdoomi, D. M., Athar, H., Din, M. U., ... Mir, A. Q. (2013). Importance of urinalysis in veterinary practice - A review. *Veterinary World*, 6(9), 640–646. <https://doi.org/10.14202/vetworld.2013.640-646>
- Picardeau, M., Bertherat, E., Jancloes, M., Skouloudis, A. N., Durski, K., & Hartskeerl, R. A. (2014, January). Rapid tests for diagnosis of leptospirosis: Current tools and emerging technologies. *Diagnostic Microbiology and Infectious Disease*. <https://doi.org/10.1016/j.diagmicrobio.2013.09.012>
- Regmi, L., Pandey, K., Malla, M., Khanal, S., & Pandey, B. D. (2017). Sero-epidemiology study of leptospirosis in febrile patients from Terai region of Nepal. *BMC Infectious Diseases*, 17(1), 628. <https://doi.org/10.1186/s12879-017-2733-x>
- Rodríguez, I., Rodríguez, I., Fernández, C., Rodríguez, J. E., & Cantillo, J. (2013). Detection of leptospires from infected urine and tissue samples in vitro by modified Fontana silver stain. *Jornal Brasileiro de Patologia e Medicina Laboratorial*, 49(1), 39–45. <https://doi.org/10.1590/S1676-24442013000100006>
- Towheed, G., Atwa, S., Eisa, M., Badawy, O., Saad, M., Elbaz, E., & Elbeskawy, M. (2019). Detection of *Leptospira* in cattle and dogs in Dakahlia Governorate. *Benha Veterinary Medical Journal*, 36(2), 1–12. <https://doi.org/10.21608/bvmj.2019.12161.1002>
- Wakeman, D. C. (1945). Leptospirosis (Weil's disease). *Medical Bulletin. United States. Army. Forces in the European Theater. Office of the Theater Chief Surgeon*, (29), 5–7. https://doi.org/10.5005/jp/books/13118_104
- Yeoh, M., Lai, N., Anderson, D., & Appadurai, V. (2013). Macroscopic haematuria A urological approach. *Australian Family Physician*, 42(3), 123–126.

A CASE REPORT ON UROLITHIASIS IN DOG

S. Kunwar*, B. Shrestha



Abstract: *Urolithiasis is a common problem in canid species. Calculi are found majorly in the bladder in case of females and in urethra in case of male dogs. Surgical approach might not provide permanent solution for urolithiasis therefore; prevention should be done with regards diet and other factors. Ruby, a seven years old female dog was presented at Veterinary Teaching Hospital, Paklihawa Campus. It had a history of hematuria and difficulty during urination. After observation of the urinary tract through ultrasonography, urolithiasis was confirmed. Surgical operation was carried out to remove calculi and suturing was done avoiding excessive tissue damage. Antibiotics along with NSAIDS were used during post-operative care. After 2 weeks sutures were removed. The dog remained in normal healthy condition after the surgery during the follow up routine.*

Keywords: bladder, calculi, surgery, urolithiasis

Introduction

Urolithiasis or urinary calculi is the deposition of crystals of various salts in any part of the urinary tract. It is generally observed in renal tubules or urinary bladder.

Uroliths consist of major minerals and organic matrices in minor amounts. Three main contributing factors for urolithiasis are:

1. Matrix- inorganic protein core may facilitate initial urolith formation.
2. Crystallization inhibitors of uroliths may be lacking or dysfunctional.
3. Precipitation crystallization factors as solute and other chemical factors lead to crystallization.

Hepatic diseases also contribute to the deposition of urate crystals. Factors affecting urolith formation may not be the same for different crystals. Struvite crystals are mainly seen due to infection of the urinary tract. Uroliths also occur due to alteration of pH i.e. alkaline pH favors the crystal formation; mineral imbalance, deficiency of vitamin-A and infection lead to desquamation which favors formation of crystal (Bartges & Callens, 2015). Genetic factors as altered urate metabolism also play a role in urolithiasis.

Urolithiasis can be diagnosed through its clinical manifestations such as hematuria, dysuria, abdominal palpation of bladder (Brown, 2019). Diagnosis can also be done through urinalysis. Urinalysis tests can be done for urine pH, urine concentration, presence of cells like WBCs, RBCs and presence of crystals in urine. Serum tests also provide guidelines with the increase in creatinine and urea level in urolithiasis due to obstruction in the flow of urine. Confirmatory diagnosis is made through medical imaging techniques such as radiographic studies and ultrasonography. Prevention of urolithiasis is done through increased water intake, regular exercise, dietary management and early treatment of urinary tract infection. In veterinary practice, open cystotomy is commonly used to remove cystoliths (Pinel, Monnet & Reems, 2013).

History

Ruby, a 7yrs old female of Japanese spitz was brought to Veterinary Teaching Hospital, Tribhuvan University. The owner complained about hematuria and dysuria for nearly over two weeks. The animal was in severe pain and anorectic for the past 4 days. Animal was

IVSA MIRROR V

lethargic and showed abnormal gait. Urinating posture was abnormal along with foul odour of urine. Animal appeared dull and dehydrated. Clinical Findings

Bloody urine (dark red in colour) was seen for 13 days. On palpation of the lower abdomen, the bladder was distended and pain was felt by the animal. Animal showed abdominal discomfort and was unable to urinate. Urinary incontinence was observed. Rectal temperature was 101.0 F. Heart rate was 94 beats per minute and respiration was 24 per minute. The mucous membrane was pink. Blood test reports showed an increase in leukocytes which pointed towards infection.

Clinical Findings

Bloody urine (dark red in colour) was seen for 13 days. On palpation of the lower abdomen, the bladder was distended and pain was felt by the animal. Animal showed abdominal discomfort and was unable to urinate. Urinary incontinence was observed. Rectal temperature was 101.0 F. Heart rate was 94 beats per minute and respiration was 24 per minute. The mucous membrane was pink. Blood test reports showed an increase in leukocytes which pointed towards infection.

Hematological report:

Parameters	Value seen in patient blood	Normal range
WBC	$36.2 \times 10^9/L$	$(6-17) \times 10^9/L$
Lymphocytes	$6.2 \times 10^9/L$	$(0.8-5.1) \times 10^9/L$
Granulocytes	$29 \times 10^9/L$	$(4-12.6) \times 10^9/L$
RBC	$9.29 \times 10^{12}/L$	$(5.5-8.5) \times 10^{12}/L$
HGB	220g/L	(110-190)g/L
HCT	65.2%	(39-56)%

Diagnosis

Diagnosis was made on the basis of clinical signs such as hematuria and dysuria. Confirmatory diagnosis was made through ultrasonography and radiography imaging.

During ultrasonography hyperechoic foci were seen. In USG, bright objects were visible in the bladder. Similarly during radiography, radiodense calculi were seen. Serum biochemistry helped to evaluate the kidney function.

Treatment

With the consent of the owner, surgical intervention was done to remove the uroliths. Surgery is the most effective way to treat uroliths. Dog was kept off feed for 12 hours before surgery.



Fig. 1: Dog with sign of hematuria



Fig 2: Hyperechoic foci seen in bladder

Surgical Preparation

Dog was sedated with xylazine @1mg/kg body weight and the operation site was prepared. Anaesthesia induction was done using diazepam and ketamine in the ratio of 1:1 and dose was maintained in the ratio of 1:2 of diazepam and ketamine. Meloxicam @2mg/kg body weight and ceftriaxone was given according to the protocol of VTH, IAAS, Paklihawa campus. After the sedation of the dog, shaving of the



Fig 3: Incision of Subcutaneous tissue



Fig 7: Suturing of the bladder



Fig 4: Distended Bladder



Fig 5: Incision of Bladder



Fig 8: Suturing of subcutaneous tissue



Fig 6: Uroliths (size of 1.7-3cm)



Fig 9: Suturing of incised skin

ventral abdomen was done. The surgical site was scrubbed with an antiseptic solution. Scrubbing was done in a circular pattern moving outward from the incision site. It prevents the dragging of dirt and hair back in surgical sites and ensures that the site is clean. Animal was placed in

dorsal recumbency and limbs were tied for stabilization.

Surgical procedure

After induction of general anesthesia and proper stabilization of the dog, sterile drapes were

IVSA MIRROR V

placed on the top of the dog to create sterile fields and drapes were clamped in place. Mid ventral celiotomy was done with a single incision of 5-6 cm in skin. Upon confirmation of a distended bladder, cystotomy was performed. After the incision on the bladder, urine was evacuated. While doing cystotomy, thickened layers of the bladder were visible which pointed towards bladder wall inflammation but no signs of necrosis was seen. After the removal of calculi of various sizes (1.7-3 cm), the bladder was flushed initially with normal saline followed by mild concentration of iodine.

After repeated dressing of the bladder, suturing of the bladder was done using a Cushing pattern. After bladder closure, a leak test was performed using sterile saline to ensure no leak in the bladder.

Before closing the incision, bleeding was checked. Continuous pattern of suturing was done in peritoneum, muscle layers and subcutaneous tissue in 3 steps. Finally suturing of outer layer i.e. skin was done using an interrupted pattern with a non absorbable suture to prevent herniation. Then the incised area was made aseptic using a mild solution of iodine. During surgery vital signs such as respiration, heart rate and temperature were frequently taken. Fluid therapy was given during the surgery period.

Post operative care

Animal was given ampicillin to prevent secondary bacterial infection and meloxicam for pain relief. Surgical site was checked regularly for swelling and discharge. After about 2 weeks of healing, the suture was removed. Animal was kept in a proper diet to prevent further formation of calculi. Dogs were not allowed to jump and run during the recovery period. The dog was restricted from licking the incision site.

Discussion

Urinary system removes metabolic wastes in the form of liquid. When there is change in the

content and pH of urine, there is an increase in concentration of certain solute and precipitation of these solutes results in calculi formation. Renal calculi are commonly seen in dogs. Hematuria may be due to the irritation caused by uroliths in bladder mucosa. Most common site of calculi formation is the urinary bladder in females and urethra in male dogs. Elevated level of BUN is found in case of urolithiasis due to prolonged retention of urine and may lead to irreversible change in kidney (Uma, Kumar, Lakkawar & Nair, 2018).

In this case, the dog had the symptoms of hematuria so urolithiasis was suspected. Confirmatory diagnosis was made through ultrasonography and radiography images by the observation of radiodense objects in x-ray and hyperechoic foci in USG. Microscopic examination of crystals helps us to know about the chemical structure of calculi. Urine and bacterial culture sensitivity testing was also helpful in determining the type of urolith present. Calculi appeared enlarged during ultrasonography. So, a surgical approach was taken to relieve the patient. During surgery care should be taken so as to not contaminate surrounding organs and to minimize the blood loss. All the vital signs should be maintained in normal range to avoid complications. Techniques to dissolve calculi for some stones have been developed. Medical dissolution consists of adjusting urine pH, eradicating bacterial infection or diluting urine. Allopurinol is used in dogs with ammonium urate calculi (Bartges & Callens, 2015). If it is not treated in time, the severity of the case will increase which leads to multiple organ failure. Treatment strategies must include removing the underlying causes to prevent chance of recurrence. With advancement in technology, less invasive methods have been evolved for the treatment of urolithiasis in animals. Non surgical approaches such as dissolution, lithotripsy or percutaneous cystolithotomy can be performed. Dissolution of Struvite cystoliths can be done with low risk.

IVSA MIRROR V

Cystotomy will cause suture related re-occurrence of uroliths therefore dissolution has been recommended (Lulich et al., 2016). Specific prevention methods should be followed for different uroliths.

Conclusions

Case of urolithiasis is common in canines. So prevention should be done as regular exercise, adequate hydration, proper diet and frequent check up of urine of crystalluria. Prompt treatment of urinary tract infection also reduces the potential for formation of calculi.

References

- Bartges, J. W., & Callens, A. J. (2015). Urolithiasis. *Veterinary Clinics of North America - Small Animal Practice*, 45(4), 747–768. <https://doi.org/10.1016/j.cvsm.2015.03.001>
- Brown, S. (2019). Urolithiasis in Small Animals - Urinary System - Merck Veterinary Manual. In *Merck Veterinary Manual*. <https://www.merckvetmanual.com/urinary-system/noninfectious-diseases-of-the-urinary-system-in-small-animals/urolithiasis-in-small-animals?query=urolithiasis>
- Lulich, J. P., Berent, A. C., Adams, L. G., Westropp, J. L., Bartges, J. W., & Osborne, C. A. (2016). ACVIM Small Animal Consensus Recommendations on the Treatment and Prevention of Uroliths in Dogs and Cats. *Journal of Veterinary Internal Medicine*, 30(5), 1564–1574. <https://doi.org/10.1111/jvim.14559>
- Pinel, C. B., Monnet, E., & Reems, M. R. (2013). Laparoscopic-assisted cystotomy for urolith removal in dogs and cats -23 cases. *Canadian Veterinary Journal*, 54(1), 36–41.
- Uma, S., Kumar, R., Lakkawar, A. W., & Nair, M. G. (2018). *Cystolith in a dog : A case report*. 6(1), 924–927

ASSISTED REPRODUCTIVE TECHNIQUES IN FARM ANIMALS: NEED OF THE HOUR

T. Safdar^{1*}, M. A. Bashir¹, A. Haseeb²



Abstract: *Assisted Reproductive Techniques are one of the key aspects to improve the efficiency of animal production and maintain the productivity level. Major Assisted Reproductive Techniques (ART) discussed in this brief review are Artificial Insemination (AI), Estrus Synchronization, Superovulation, Semen Sexing, Cryopreservation, and Embryo Transfer (ET). Reproduction is the backbone of any livestock enterprise, and to achieve remarkable results, the use of ART is inevitable. The loss in the economy through various reproductive problems such as infertility, low conception rate, early embryonic death and others can be effectively managed by the use of various assisted reproductive techniques. Although artificial reproductive techniques are becoming more popular among livestock production, these techniques need to be refined to gain more efficiency.*

Keywords: ART, artificial insemination, embryo transfer, estrous synchronization, superovulation, semen sexing, cryopreservation

Introduction

Assisted reproductive techniques are the key to improve the efficiency of animal production in livestock enterprises. Unfortunately, only a few farms are using these techniques to maintain the productivity level. These technologies aim to eradicate common reproductive problems in the dairy industry like infertility, low conception rate, less production, poor genetic makeup of animals, and early embryonic mortality. The livestock industry is suffering from a huge economic loss because of little use of these beneficial techniques. This article aims to cover an overview of major and applicable assisted reproductive techniques (ART) and the need for using these technologies in today's world (Gelayenew & Asebe, 2016; Hafez, 2015; Verma, Kumar, & Chand, 2012).

Artificial Insemination

Artificial insemination is the first and basic assisted reproductive technique which is now being practiced all over the world including developed and under-developed countries. AI is the process of unnatural deposition of semen in the female reproductive tract and was first practiced in bitch by Spallanzin in the year 1784.

Later on, Ivanoff (1922) also used this technique in domestic animals including horses, poultry, cattle, and sheep. Nowadays, almost all corporate and commercial dairy farms are using AI in a routine. Every year almost 100 million cattle, 40 million pigs, 33 million sheep, and 0.5 million goats are artificially inseminated (Boa-Amponsem & Minnozi, 2006). AI is practiced less in small ruminants, especially goats, as compared with large ruminants (cattle and buffalo). AI involves a collection of semen from the male by any method (most commonly artificial vagina and electro-ejaculation methods), examining its vigor and motility, processing it, and then loading into the straws. Either freshly chilled or frozen-thawed semen can be used to inseminate the animal, although freezing and long-term storage requires the use of cryoprotectants. It is also noted that the conception rate is low if the frozen-thawed semen is used. AI is an easy way to improve the genetic makeup of progeny from the side of a male. Also, there is an improved conception rate, less chance of injury, and a low risk of spreading sexually transmitted diseases (STD) from AI (Althouse, n.d.).

¹ Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan

² University of Veterinary and Animal Sciences, Lahore, Pakistan

*Corresponding Author: talhasafdar167@gmail.com

Estrus Synchronization

The sole purpose of estrus synchronization is to induce estrus in a desired group of females at a predestined date and time. While synchronizing the time of induction of heat, we are ultimately scheduling the time of parturition. Detection of heat is a crucial part of the good management of animals and this is even harder when we have to deal with a large herd of hundreds and thousands of animals. Using the estrus synchronization technique, we can relieve this difficulty by bypassing the estrus detection phase. The most commonly used approaches for synchronizing the ovulation time are Prostaglandin Treatment, Controlled internal drug-releasing regimen and GnRH-PGF_{2α}-GnRH protocols. During prostaglandin treatment we use a luteolytic agent (PGF_{2α}) 11 days apart in cyclic females regardless of the stage of estrus in animals and then after the appearance of estrus signs, timely AI is performed. Controlled Internal Drug Releasing (CIDR) device maintains the blood progesterone concentration in the targeted animals and is inserted into the vagina for 7 days. An injection of PGF_{2α} is used on day 6 and estrus is observed on the 8th day. GnRH-PGF_{2α}-GnRH protocol is a good regimen to induce estrus in non-cyclic animals. Usually on day 1, GnRH is injected and then 7 days later, PGF_{2α} injection is administered. Another injection of GnRH is given on the 10th day and AI is performed on day 11. Using estrus synchronization technique eliminates the chance of human error in the detection of estrus. It is also very beneficial for animals, which exhibit low external heat symptoms (silent heat) like sheep and buffalo (Gelayenew & Asebe, 2016; Hafez, 2015).

Superovulation or multiple ovulation

As the name superovulation or multiple ovulation indicates, it is the induction of ovulation of multiple ova from the ovary of an animal using different injectable hormones. Any cyclic animal can be made to ovulate multiple

ova at a single time using synthetic follicle-stimulating hormone (a gonadotropin which is naturally released from the anterior lobe of the pituitary gland). Multiple ovulation techniques combined with embryo transfer can bring revolution in the livestock industry. Although superovulation can be induced in any cyclic animal it is usually applied in animals of superior genetics. As artificial insemination can be used for the improvement of genetic from the male side, multiple ovulation is being used as an improvement of genetics from the female side. Usually, a follicle-stimulating hormone is injected at the end of the luteal phase or before the 1-2 day of completion of estrus synchronization regimen. Superovulation is a crucial technique for embryo biotechnology. Several factors should be considered before inducing superovulation in an animal. Most important are breed, the reproductive health status of the animal and nutrition. Also, repeated super ovulations have a significant effect on the next one (Gelayenew & Asebe, 2016; Hafez, 2015).

Semen Sexing

For producing progeny of desired gender, either male or female, the technique of semen sexing is used. There is a difference in the DNA content of X chromosome bearing spermatozoa and Y-Chromosome bearing spermatozoa. Semen sexing is done by flow cytometric separation of fluorescent labeled X-Chromosome possessing spermatozoa and Y-Chromosome possessing spermatozoa. In recent times, this technology was capable of sorting 15 million spermatozoa into X and Y chromosome bearing chromosomes (Choudhary et al., 2016). The accuracy of predicting the sex of the resulting progeny is 85-95 percent. New generation flow cytometers with the high efficiency of sorting of sperms are also being devised and utilized nowadays. There are some flaws in the sexing of semen like low sperm concentration, reduced motility, viability, decreased life span of spermatozoa and some structural defects. Also, sexed semen needs deep

IVSA MIRROR V

intrauterine insemination. But still, the benefits of this technique outnumber the drawbacks. Major advantages include a high ratio of progeny testing programs, enhanced male production for this purpose, low risk of sex-limited diseases, and the last but not least, conservation of superior and rare genetics of animals (Choudhary et al., 2016).

Cryopreservation

It is a technique in which living cells and tissues are preserved in a state of suspended animation at a very low temperature (-196 °C) for a long period while maintaining the structural integrity of the specimen. At lower temperature, the processes going on in a living cell are dramatically reduced. To alter these processes different cryoprotective agents (CPA) are used like Glycerol, Dimethyl sulfoxide (DSMO) and some polymers. Liquid Nitrogen is used as a freezing agent. Some difficulties like ice crystal formation at a fast cooling rate and osmotic changes due to the high concentration of intracellular and extracellular solutions at a low cooling rate were faced in conventional methods of freezing. These problems can lead to cryo-injury. Vitrification has reduced the risk of cryo-injury very much. Cryopreservation of sperms, oocytes, and embryos has brought a revolution in breeding programs and research systems. Other benefits of cryopreservation are cryosurgery, preservation of organs and tissue for transplantation, and application in food sciences (Choudhary et al., 2016; Verma et al., 2012).

Embryo Transfer

Embryo Transfer is a technique which can improve the genetic worth of a herd at a faster rate than any other techniques discussed above. ET is a process that involves many steps. Mainly, it involves generating and obtaining embryos from females of superior genetics called as the donor and then implanting these embryos, after processing and grading, into the uterus of different females known as recipients.

ET is an expensive technique and is only practiced in animals which can justify its cost. While producing embryos in the donor females, estrus synchronization and superovulation methods are applied to gain better results. By using this technique, genetic combinations of both males and females can be used to enhance genetic gain in the offspring. Surgical, as well as non-surgical embryo transfer techniques are being practiced in elite cattle. Non-surgical embryo transfer is cost-effective, safe, and shows better results. This technique is uncommon as other assisted reproductive techniques. It requires highly skilled technicians and embryologists. The International Embryo Transfer society provides technicians for this purpose. This is the best way devised yet for the rapid expansion of animals of elite genetic makeup (Hafez, 2015; Schatten & Constantinescu, 2007).

Conclusions

Using assisted reproductive techniques in farm animals has escalated over the last few decades. With time, assisted reproductive techniques are drawing attention and becoming popular in the livestock industry due to astonishing results. Yet many technical refinements and public awareness are needed in this field. Artificial Insemination, superovulation, semen sexing, cryopreservation and embryo transfer have proved to be valuable in improving the genetic merit of animals of low genetic worth. Estrus synchronization has eliminated many managerial problems in the reproduction of farm animals. New technologies like stem cell culture technique, Intracytoplasmic sperm injection and nano-technology are also being trialed. To compete with the future crisis, the expansion of major and basic assisted reproductive techniques at all levels in the whole world is the need of the hour (Gelayenew & Asebe, 2016).

IVSA MIRROR V

References

- Althouse, G. (n.d.). Artificial Insemination. *Comparative reproductive biology*, 159–169.
- Boa-Amponsem, K., & Minozzi, G. (2006). The state of development of biotechnologies as they relate to the management of animal genetic resources and their potential application in developing countries. *Background Study Paper*, 33.
- Choudhary, K. K., Kavya, K. M., Jerome, A., & Sharma, R. K. (2016). Advances in reproductive biotechnologies. *Veterinary world*, 9(4), 388.
- Gelayenew, B., & Asebe, G. (2016). Review on Major Assisted Reproductive Technologies.
- Hafez, Y. M. (2015). Assisted reproductive technologies in farm animals. *ICMALPS*
- Schatten, H., & Constantinescu, G. M. (Eds.). (2007). *Comparative reproductive biology* (pp. 117-131). Blackwell Pub.
- Verma, O. P., Kumar, R., Kumar, A., & Chand, S. (2012). Assisted Reproductive Techniques in Farm Animal-From Artificial Insemination to Nanobiotechnology. *Veterinary World*, 5(5).

CALCIUM HOMEOSTASIS AND MILK FEVER IN DAIRY COWS

R. Yadav*, S. Yadav, B. Shrestha



Abstract: Milk fever is an afebrile metabolic disorder that happens at the start of lactation most typically at intervals of 72 hours of calving in adult females. It exposes the animal to different forms of disorders like ketonemia, mastitis (especially coliform mastitis), dystocia, displacement of the abomasum and internal reproductive organ prolapse. The physiological condition of calcium is regulated by thyrocalcitonin, parathyroid hormone, and 1, 25 (OH)₂ D (Vit.D₃). Effects of hypomagnesaemia and hypophosphatemia on periparturient animal health are the factors to understand its pathological condition. Hypomagnesaemia is one of the main risk factors that impair PTH secretion and its ability. Here, calcium physiological condition with its role in milk fever is reviewed. Treatment is often done by the administration of calcium salt resolution largely before the cow is recumbent. Prevention following different management practices to keep normal blood calcium is highly necessary.

Keywords: calcium homeostasis, hypocalcaemia, hypomagnesaemia, hypophosphatemia, milk fever

Introduction

Parturient paralysis (milk fever) is an afebrile metabolic disease occurring at or close to calving typically at intervals 72 hours of calving, particularly in high producing farm cows. Typically, it is sporadic however on individual farms the incidence could seldom reach 25-30% of vulnerable cows (Engdawork, 2019). Its characteristic signs are hypocalcaemia, tetany, generally subnormal temperature, lateral recumbency and circulatory fail and ultimately coma leading to death when left untreated (Chakrabarti, 2014). Normally, milk fever occurs on account of disturbance in the normal calcium homeostasis due to impaired function of parathyroid hormone, imbalance of Dietary Cation Anion Difference (DCAD), hypomagnesaemia and is also associated with other causes. Cows laid low with milk fever has a lot of risk of a secondary problem, e.g: cows recovered from milk fever have associated 8-fold larger incidence of ketonemia and mastitis (especially coliform mastitis) (Curtis et al., 1970). Also, the incidence of dystocia, displacement of the abomasum and female internal reproductive organ prolapse is far larger

due to milk fever (Kahn, 2016). It is an economically important disease that will scale back the productivity lifetime of a lactating cow by 3.4yr (Payne, 1968). Economic losses embody the losses from death (approximately 8% of suffered cows), early culling before mature (approximately 12% of suffering cows), costs of treatment as well as the decline in milk production (Khan et al., 2015).

Etiopathogenesis

The decline of ionized calcium level within the tissue fluids is the basic biochemical defect in parturient paresis. In a normal healthy animal, calcium physiological condition is maintained which is about 2.1-2.5mmol/L (Goff, 2008). Cow within the subclinical hypocalcaemia (total plasma Ca <1.9mmol/L) that happens at the onset of lactation is caused by associated impairment between loss of calcium within the colostrum and inflow of calcium to the extracellular pool from the gut and bone (Blood & Henderson, 1979). A cow producing 10L of milk colostrum will lose 23g of calcium in an exceedingly single milking that is regarding 9

IVSA MIRROR V

times as high as that is found in each cow's plasma calcium pool (Horst et al., 1997). Hypocalcaemia occurs due to a lack of adequate function of the PTH hormone and vitamin D (Horst et al., 1997).

When the calcium level suddenly fall, hyper excitability of the nervous system takes place which reduces the power of the muscle contraction, leading to tetany and paralysis (Kahn, 2016). Plasma Calcium is found to be disturbed in about 5-20% of adult cows and consequently cause severe hypocalcaemia (total plasma calcium, 1.0-1.4 mmol/L) or clinical milk fever (Blood & Henderson, 1979).

Calcium homeostasis and monitoring for hypocalcaemia. In order to stop blood Ca from diminishing at the start of lactation the cow ought to replace extracellular Ca lost in milk and do this by extracting Ca from the bone by increasing the capacity to absorb dietary Ca. The dairy cow mobilizes bone Ca and goes into a state of osteoporosis to help her reach normal calcium level in blood which will result in her losing 9-13% of her bone Ca in the first 30 days of lactation (which is reversible in later lactations) (Ellenberger et al., 1932).

Homeostasis of calcium (Ca) is regulated by calcitonin, parathyroid hormone (PTH), and 1, 25 (OH)₂ Vitamin D₃. Bone Ca uptake and renal tubular reabsorption of Ca is controlled by PTH which is produced whenever blood Ca drops. However, the total amount of Ca that can be recovered by reducing urinal Ca excretion is small as only the minimal amounts of Ca normally loses to urine each day (Goff, 2008). A second hormone, 1, 25 (OH)₂ D₃ is needed to stimulate the intestine to efficiently absorb dietary Ca; this hormone is made by the kidney (convert 25- hydroxyvitamin D to 1, 25 (OH)₂ D₃) from Vitamin D, but only in response to rising in blood PTH (Degaris & Lean, 2009). Hence, when a cow does not extract enough Ca from their bones and diet to restore the Ca lost in milk, it leads to hypocalcaemia and milk fever.

Several nutritional factors such as Ca, Mg, and P are involved in the failure of Ca homeostasis leading to milk fever (Goff, 2008).

Factors impairing Ca homeostasis at the cellular level are as follow:

a. Metabolic alkalosis

Metabolic alkalosis is primarily the result of a diet that provides more cations (K, Na, Ca, and Mg) than anions (Chloride, sulfate, and phosphate) in blood. Metabolic alkalosis alters the PTH receptor conformation and tissue becomes less sensitive to PTH. Lack of PTH response in the target tissue prevents successful use of bone and kidney Ca. More importantly, the kidney fails to convert 25-hydroxyvitamin D into its active form (Goff, 2008).

b. Hypomagnesaemia

Ca metabolism is impaired by hypomagnesaemia in two ways, firstly in response to hypocalcaemia decreasing the PTH secretion (Goff, 2008) and secondly by reducing tissue responsiveness to PTH (Rude, 1998).

Independent to metabolic alkalosis, hypomagnesaemia can also impair PTH's ability to act on the skeleton and the kidney tissue. It activates adenylate cyclase when PTH binds to the receptor of its target tissue, leading to the production of the second messenger, cyclic AMP. The PTH-receptor interactions should also trigger phospholipase C activation in other tissues, resulting in the production of the second messenger diacylglycerol and inositol 1, 4, 5-triphosphate (Goff, 2000, 2008). Both adenylate cyclase and phospholipase C have the binding site of Mg⁺⁺ to be occupied by Mg ion for appropriate activity (Goff, 2000; Rude, 1998). Hypomagnesaemia can cause hypocalcaemia and while Mg therapy-treatment restores serum Ca level alone to normal; Ca and/or vitamin D therapy is unsuccessful (Rude, 1998).

IVSA MIRROR V

c. Hypophosphatemia

The normal plasma inorganic P concentration is 4-8mg/dl (Goff, 2000). With the start of lactation, there is a heavy reduction in ultra-filterable calcium complex. Ultra-filterable calcium complex is the combination of calcium, phosphorus, and bicarbonate ions (HIBBS, 1950). Hypocalcaemia at milk fever leads to the secretion of PTH in heavy amounts which cause loss of P in saliva and urine. Normally, the plasma P decreases at calving and in a cow with milk fever, the range of plasma P is 1-2 mg/dl. Plasma P concentration increases rapidly following treatment of hypocalcaemia due to a reduction in PTH (Goff, 2000). Although, there is an inverse relationship between calcium and phosphorus in the blood, both calcium and phosphorus level goes down together in milk fever (HIBBS, 1950).

d. Reducing diet cation-anion difference to prevent hypocalcaemia

Foods high in Na and K and deficient in Cl and S tend to increase the chance of hypocalcaemia and milk fever (Block, 1984; Ender et al., 1971). The dietary concept of cation-anion difference (DCAD) for the control of milk fever depends on the strong ion model of acid/base balance (Singer & Hastings, 1948). The basic principle of this model is that plasma pH is determined by 4 independent factors; the partial pressure of CO_2 , the solubility of CO_2 in temperature-dependent plasma, the net strong ion charge or strong ion difference (SID+) and the total plasma concentration of non-volatile weak buffers, principally albumin, globulin, and phosphate (ATOT).

The DCAD, a concept used to control milk fever, aims to decrease the (SID+), thereby decreasing plasma PH, resulting in severe ion metabolic acidosis (Degaris & Lean, 2009).

Treatment

Every effort ought to be tried to treat the suffered cow soon after the clinical signs are obvious and

should be done before the cow falls to recumbent in the beginning stage of the disease (Chakrabarti, 2014). The load of the heavyweight cow can cause "crush syndrome" due to ischemic nerves and muscle death from long-term recumbency (Goff, 2008).

In the past days calcium chloride was injected however later it had been found to be terribly irritating to the tissues (Chakrabarti, 2014). Thus, today the fastest and the best way to recover normal plasma Ca is to administer an IV calcium salt resolution (commonly Ca borogluconate) (Horst et al., 1997) from 8.5-11.5 g Ca/500ml (Goff, 2008). They may contain combined sources of Mg, P, and glucose and if supplied IV rapidly, fatal heart disease (arrhythmia) and discontinuation during contraction may occur. It takes 4hr to raise the blood Ca level above normal during IV Ca therapy (Goff, 2008).

There is another alternative treatment too like udder inflation (Chakrabarti, 2014) as it prevents the loss of Ca and P due to the slowdown of further milk secretion and mostly it is suggested to those cows who do not respond to injection.

Prevention

Feeding a calcium-deficient diet

When a diet containing less Ca is fed to the cow than they require, negative Ca balance exists which stimulates the PTH secretion. Afterward, it stimulates bone reabsorption and kidney production of $1, 25 (\text{OH})_2 \text{D}_3$ due to the fact that at calving the bone reabsorption and $1, 25 (\text{OH})_2 \text{D}_3$ are already active in avoiding hypocalcaemia (Green et al., 1981).

Acidic diets

With the addition of CaCO_3 A. I. V. silage can be fed approximately 4 weeks prior to calving and initial 2 weeks after. It leads to intestinal acidity which increases calcium ion and therefore increases calcium absorption (Ender et al., 1971).

IVSA MIRROR V

Ammonium chloride

Inducing acidosis, it helps in prevention with increased Ca absorption from the gut, raised turnover of the available calcium pool and consequently an increase in urinary calcium excretion (Loomis et al., 1966). According to PH value, DCAD should be adjusted by the appropriate use of ammonium chloride with respect to the urine pH (Chakrabarti, 2014).

Short-term calcium administration

Short term calcium administration reduced the incidence of paresis in milk fever-prone cows from 57-3.6% through the administration of calcium chloride in gelatin beginning a few days pre-calving to 2 days post-calving that increases Ca available for uptake (Ringarp et al., 1967).

Vitamin D

Consuming large dose of Vitamin D2 or D3 in short term period relatively 3-7 days prior to calving help in the prevention of hypocalcaemia. Thus, when 30 million IU/day Vitamin D is administered, the chance is reduced but shouldn't continue longer than 7 days because of its toxicity (Ender et al., 1971).

Parathyroid hormone

Hypocalcaemia and milk fever can be prevented with PTH infusions and for effective intramuscular injections, required amount is about 20 times as much PTH as IV infusions (Goff et al., 1989; Goff et al., 1986).

Other management practices are also required for its prevention like Body Condition Score (BCS) management, shortening of the dry period and reducing milking in early lactation (Engdawork, 2019).

Conclusions

Milk fever is caused mainly due to hypocalcaemia, hypomagnesaemia and hypophosphatemia. Calcium salt resolution (commonly Ca borogluconate) is used as an early treatment before a cow reaches the

recumbent stage. Calcium homeostasis is affected by a number of factors and being able to monitor the calcium homeostasis can be one of the important preventive measures.

References

- Block, E. (1984). Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. *Journal of Dairy Science*, 67(12), 2939–2948.
- Blood, D. C., & Henderson, J. A. (1979). *Veterinary Medicine (A Textbook of the disease of cattle, sheep, goats, pigs and horses* (fifth; O. M. Radostits, C. C. Gay, & K. W. Hinchcliff, eds.). Cassell. Ltd.
- Chakrabarti, A. (2014). *Text Book Of Clinical Veterinary Medicine* (fourth). Kalyani.
- Curtis, R. A., Cote, J. F., & Mills, J. A. (1970). *An epizootiological study of parturient paresis (milk fever)*. 81–85.
- Degaris, P. J., & Lean, I. J. (2009). and control principles. *The Veterinary Journal*, 176(1), 58–69. <https://doi.org/10.1016/j.tvjl.2007.12.029>
- Ellenberger, H. B., Newlander, J. A., & Jones, C. H. (1932). Calcium and phosphorus requirement of dairy cows. 2. Weekly balance through lactation and gestation periods. *CABI Agriculture and Bioscience*.
- Ender, F., Dishington, I. W., & Helge-bostad, A. (1971). Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. The solution of the aetiology and the prevention of milk fever by dietary means. *CABI Agriculture and Bioscience*, 28(5), 233–256.
- Engdawork, A. (2019). *Review : Milk Fever and Its Economical Impacts in Commercial Dairy Cattle Production*. (July).
- Goff, J P, Kehrl Jr, M. E., & Horst, R. L. (1989). Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. *Journal of Dairy Science*, 72(5), 1182–1187.
- Goff, J P, Littledike, E. T., & Horst, R. L. (1986). Effect of synthetic bovine parathyroid hormone in dairy cows: Prevention of hypocalcaemic parturient paresis. *Journal of Dairy Science*, 69(9), 2278–2289.
- Goff, Jesse P. (2000). Pathophysiology of Calcium and Phosphorus Disorders. *Veterinary Clinics of North America: Food Animal Practice*, 16(2), 319–337. [https://doi.org/10.1016/S0749-0720\(15\)30108-0](https://doi.org/10.1016/S0749-0720(15)30108-0)
- Goff, Jesse P. (2008). and subclinical hypocalcemia in dairy cows. *The Veterinary Journal*, 176(1), 50–57. <https://doi.org/10.1016/j.tvjl.2007.12.020>
- Green, H. B., Horst, R. L., Beitz, D. C., & Littledike, E. T. (1981). Vitamin D metabolites in plasma of cows fed a prepartum low-calcium diet for prevention of parturient hypocalcemia. *Journal of Dairy Science*, 64(2), 217–226.

IVSA MIRROR V

- HIBBS, J. W. (1950). Milk Fever (Parturient Paresis) in Dairy Cows- A Review. *Journal of Dairy Science*, 33(10), 758–789.
- Horst, R. L., Goff, J. P., Reinhardt, T. A., & Buxton, D. R. (1997). Strategies for Preventing Milk Fever in Dairy Cattle 1 , 2. *Journal of Dairy Science*, 80(7), 1269–1280. [https://doi.org/10.3168/jds.S0022-0302\(97\)76056-9](https://doi.org/10.3168/jds.S0022-0302(97)76056-9)
- Kahn, C. M. (2016). *The Merck Veterinary Manual* (Eleventh). USA: Merck & Co., Inc., Kenilworth, NJ.
- Khan, A., Mushtaq, M. H., Khan, A. W., Chaudhry, M., & Hussain, A. (2015). *Descriptive Epidemiology and Seasonal Variation in Prevalence of Milk Fever in KPK (Pakistan) Department of Epidemiology and Public Health , Faculty of Veterinary Sciences , Veterinary Officer , at Veterinary Hospital Koza Bandai , District Swat , Pakistan. 14(4), 472–477. https://doi.org/10.5829/idosi.gv.2015.14.04.93172*
- Loomis, W. K., Riley, W. F., Westcott, R. W., Neal, P. A., Tyrrell, H. F., Reid, J. T., ... Gordon, C. H. (1966). *Combating Milk Fever I '2. 57(8). https://doi.org/10.3168/jds.S0022-0302(74)84989-1*
- Payne, J. M. (1968). Milk Fever. *Outlook on Agriculture*, 5(6), 266–272.
- Ringarp, N., Rydberg, C., Damberg, O., & Bostrom, B. (1967). Versuch einer vorbugenden Behandlung der puerperal paresis bei kuhen mittels peroraler Zufuhr von Kalziumchloride-Gel. *Zentralblatt Fur Veterinarmedizin Reihe A*, 14(3), 242–251.
- Rude, R. K. (1998). Magnesium deficiency: a cause of heterogenous disease in humans. *Journal of Bone and Mineral Research*, 13(4), 749–758.
- Singer, R. B., & Hastings, A. B. (1948). An improved clinical method for the estimation of disturbances of the acid-base balance of human blood. *Medicine*, 27(2), 223.

CANINE PARVOVIRUS, A PREVALENT DISEASE OF CANINE, ITS TRANSMISSION & PATHO-PHYSIOLOGY



Haseeb, A.^{1*} Bashir, M. A.², Rehman, A.U.², Abedin, M.Z.U.³

Abstract: *Parvovirus is the most prevalent disease of canines that has two pathogenic strains, canine parvovirus 1 (CPV1) and canine parvovirus 2 (CPV2). The virus structural proteins play an important role in antigenicity and host specificity. The infected dog sheds virus for about 4-7 days of post-infection and the major route of transmission is a fecal-oral route. The most common clinical findings of infected dogs are diarrhea, myocarditis, and hemorrhages in the intestine with vomiting. Parvovirus can be diagnosed by serological tests and other molecular tests like PCR, ELISA, Haemagglutination test. Moreover, the main way to control canine parvovirus is through vaccination and proper hygienic measures but sometimes maternal antibodies interfere with active immunization with live attenuated vaccines and results in parvovirus vaccine failure.*

Keywords: canines, hemorrhagic enteritis, myocarditis, vaccination, vomition

Introduction

Canine Parvovirus (CPV) is a non-enveloped linear single-stranded negative-sense DNA virus. Canine Parvovirus was first introduced in 1970. It is structurally composed of two structural proteins and two non-structural proteins. The structural proteins are VP1 and VP2 while Non-Structural Proteins are NS1 and NS2 (Appel, 1978). Canine Parvovirus (CPV) belongs to the family parvoviridae and subfamily parvovirinae. It is structurally identical to Feline Panleukopenia virus (about 98%) but differs only in 6 & 7 number amino acids in structure (Black, Holscher, Powell, & Byerly, 1979). The most prevalent type is canine parvovirus 2 (CPV 2) which has a high morbidity rate of 100% and mortality of 10%. It is a pathogenic virus that mainly causes myocarditis and hemorrhagic enteritis, the symptoms may include bloody diarrhea and vomiting, anorexia, depression, lethargy and fever. Within 24 to 48 hours, most affected dogs start vomiting and hemorrhagic small-bowel diarrhea results from severe dehydration (Burtonboy, Coignoul, Delferriere, & Pastoret, 1979). This disease is highly

prevalent in dogs around the globe and can be transmitted by direct and indirect contact of dogs with the feces of the infected dog. Moreover, CPV 2 are very stable in the environment and their main mode of transmission is fecal-oral route. CPV in the fecal sample can be diagnosed by different methods. On the basis of the availability of resources, main methods of diagnosis include cell culture, hemagglutination (HA), electron microscopy, enzyme-linked immuno-sorbent assay (ELISA) and DNA hybridization. There are also several molecular diagnostic techniques which include PCR, Multiple PCR, Reverse Transcriptase PCR, Real-Time PCR, and DNA Probes (Carmichael, Joubert, & Pollock, 1980).

Transmission

The main route of transmission of this virus is fecal-oral route by direct contact to the infected dog or through the sources contaminated with feces like toys, shoes and infected soil. It also transmits to other dogs when the infected dog is in a group of dogs in close proximity and does

¹University of Veterinary and Animal Sciences, Lahore, Pakistan

²The Islamia University of Bahawalpur, Pakistan

³Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan

*Corresponding author: ahaseeb244@gmail.com

various activities like mating, breeding, sniffing each other's feces and through direct contact in an animal shelter, playgrounds and in parks (Carter, Flores, & Wise, 2006). The infected pets also infect the non living subjects around them like veterinary hospitals, pet shops, boarding kennels and commercial breeding areas; these are all secondary infection sources (Decaro et. al., 2005). The incubation period of Parvovirus in the field is 4-5 days but in experimental observation, it is around 3 days (Cho, Song, Park, & Park, n.d.). Unlike most other viruses, canine parvovirus is stable in all environments and even in harsh environments which makes it more dangerous (Cooper, Carmichael, Appel, & Griesen, 1979).

Clinical Signs

The CP virus mostly affects young pups or immunocompromised dogs which mostly results in the occurrence of the signs of CPV and sudden death within one or two days (Decaro et. al., 2005). During the onset of disease, the main clinical signs of the disease include severe hemorrhagic enteritis which results in severe pain with diarrhea which is often bloody. Diarrhea occurs in dogs of all ages but mostly occurs in pups. At the initial stage there is weakness with mild increase in temperature which then becomes subnormal with the addition of vomiting (Fig-1) (Dogonyaro, 2011).



Fig 1- Dog with CPV infection

Source - Indian J Virol. 2010 Jun; 21(1): 31–44.

In some pups, the sign of myocarditis also appears which may result in pulmonary heart failure and severe heart attack and results in death of the pup under the age of 3 weeks. In CPV, about 70% of the pups die because of

myocarditis but nowadays it is not so common largely due to effective immunization of bitches which provides protection to pups during the early period of life (Dogonyaro, 2011).

Pathophysiology and histopathology

After entry to the oral cavity of the host dog, CPV travels to the lymphoid tissue of the oropharynx. Then they spread to whole blood stream of the body and to the other tissues like bone marrow, lymphatic tissues and epithelial crypts of the intestinal parts; mostly of jejunum and ileum. The virus subdivides there and spreads the disease which results in the hemorrhages in the small intestine and diarrhea (Ettinger & Feldman, 1995). Histopathologically, it is observed that the virus causes the necrosis of crypts of epithelial cells of the target site like intestine and Myocardium (Ettinger & Feldman, 1995). The villi of the small intestine are seen fused and small numbers of fused lymphocytes are seen in the peyer's patches, mesenteric lymph nodes and nodules of spleen are also seen. Due to villi fusion, malabsorption is seen and results in diarrhea with electrolyte imbalance. Necrosis of thymus may also be seen in histopathology of young pups (Ettinger & Feldman, 1995).

Diagnosis

For the diagnosis of CPV, the diagnostic tests should be done when the signs and symptoms of Canine Parvovirus like fever, diarrhea, vomiting, loss of appetite weakness, immunosuppression are seen. The disease is always confirmed by laboratory tests (Jacob, Weiser, Hall, & Kowalsky, 1980).

The diagnostic laboratory test for CPV includes HA (Haemagglutination) (Khan, Line, & Aeillo, 2005), Electron Microscopy (EM), Enzyme Linked Immunosorbent Assay (ELISA), Latex Agglutination Test (LAT), Fluorescent Antibody Test (FAT), CIE test, Virus neutralization test, PCR and RE digestion, real time PCR, loop-mediated isothermal amplification (LAMP) (Minakshi et. al., 2014; Mochizuki, San Gabriel,

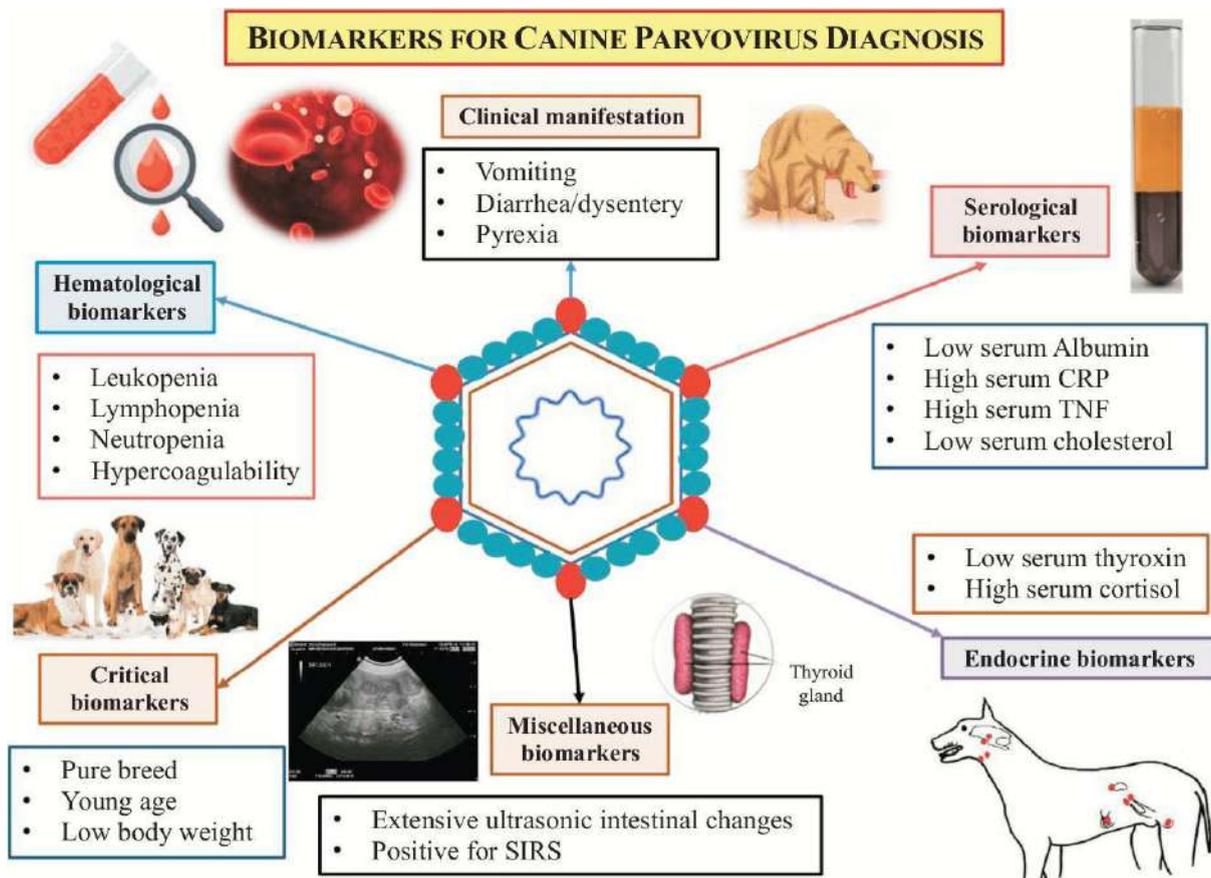


Figure 2- Biomarkers for Canine Parvovirus Diagnosis
Source- Bentham Science, Current Biomarkers, Vol#7,
DOI : 10.2174/2468422807666170810102524

Nakatani, Yoshida, & Harasawa, 1993; Mochizuki et. al., 1996). Various cell cultures can also be isolated for the diagnosis of CPV from the different cell lines like Feline kidney cell line, Madin Darby canine kidney cell line (MDCK), Walter Reed Canine Cell line (WRCC). However, diagnosis through cell culture is time consuming and requires special cell lines and thus, it is not used in daily routine tests. CPV can be isolated from cell culture even after a few days of inoculation (Nandi & Kumar, 2010). The HA test is the most rapid test to be performed for the diagnosis of CPV; it is done by using porcine and feline RBCs (Nandi & Kumar, 2010).

Treatment

Treatment of CPV depends upon the age of the dog and the stage of the animal diagnosed with parvovirus. If the disease is not diagnosed on time, the severity of the disease increases, and

the patient requires more attention and rapid strong treatment (Nandi, Kumar, Anbazhagan, Chidri, & Chauhan, 2007). The treatment includes intravenous drugs and other antimicrobial drugs and supplements (Ogbu et. al., 2017). It has a low mortality rate so it can be treated on time. IV fluids are given to recover from body weakness and electrolyte balance. To overcome diarrhea and vomiting, metoclopramide can be given at the rate of 0.5mg/kg body weight (Ogbu, Chukwudi, Ijomanta, Agwu, & Chinonye, 2016). The hyperimmune serum can be injected and proper vaccination should be done to avoid CPV.

Prevention and Control

As this virus can survive in even harsh environments so it is very difficult to control, the only way to control CPV is vaccination and acquiring hygienic measures. Elimination of the virus can be done by disinfection of the areas

IVSA MIRROR V

like floor with chlorine-based disinfectants (Pereira, Monezi, Mehnert, D'Angelo, & Durigon, 2000). Mechanical decontamination is also helpful, and the area should be dried thoroughly between applications. Potassium peroxymonosulfate can be sprayed on contaminated areas using a pesticide sprayer or other applicator (Scott, 1980).

Conclusions

It is clear that Canine Parvovirus is the killer disease of pups and immuno-compromised dogs and also the most common disease of dogs. The main symptoms of this disease are vomiting and shooting painful diarrhea due to severe hemorrhagic enteritis. Its treatment is just supportive therapy and immunity enhancer supplements with antimicrobial drugs and antibiotics but furthermore it is important to vaccinate all the dogs or injection of hyper-immune serum so that they cannot be infected by this virus. It has a low mortality rate of about less than 10% but it can cause severe high mortality in pups less than age of the 6 months, so vaccination of Canine parvovirus is necessary. The secondary step for control of the disease is to use disinfectants as a precautionary measure in the surrounding areas of the dogs. There should be proper vaccination of pets to protect them from parvovirus infection.

References

- Appel, M. J. G. (1978). Status report: canine viral enteritis. *J Am Vet Med Assoc*, 173, 1516-1518.
- Black, J. W., Holscher, M.A., Powell, H.S., & Byerly C.S., (1979). Parvoviral enteritis and panleukopenia in dogs.
- Burtonboy, G., Coignoul, F., Delferriere, N., & Pastoret, P. P. (1979). Canine hemorrhagic enteritis: detection of viral particles by electron microscopy. *Archives of virology*, 61(1-2), 1-11.
- Carmichael, L. E., Joubert, J. C., & Pollock, R. V. (1980). Hemagglutination by canine parvovirus: serologic studies and diagnostic applications. *American journal of veterinary research*, 41(5), 784-791.
- Carter, G. R., Flores, E. F., & Wise, D. J. (2006). Herpesviridae'. A concise review of veterinary virology.
- Cho, H.S., Song, J.E., Park Y.S., Park N.Y. (n.d.). Diagnosis of the canine parvovirus in faecal

- samples by in situ hybridization. *Int Vet J*. 2004;81:855-859.
- Cooper, B. J., Carmichael, L. E., Appel, M. J., & Greisen, H. E. L. E. N. (1979). Canine viral enteritis. II. Morphologic lesions in naturally occurring parvovirus infection. *The Cornell Veterinarian*, 69(3), 134-144.
- Decaro, N., Campolo, M., Desario, C., Elia, G., Martella, V., Lorusso, E., & Buonavoglia, C. (2005). Maternally-derived antibodies in pups and protection from canine parvovirus infection. *Biologicals*, 33(4), 261-267.
- Decaro, N., Elia, G., Martella, V., Desario, C., Campolo, M., Di Trani, L., ... & Buonavoglia, C. (2005). A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs. *Veterinary microbiology*, 105(1), 19-28.
- Dogonyaro, B. B. (2011). *Molecular characterization of canine parvovirus strains from domestic dogs in South Africa and Nigeria* (Doctoral dissertation, University of Pretoria).
- Ettinger, S. J., & Feldman, E. C. (1995). Textbook of veterinary internal medicine. Section VIII: The Respiratory System.
- Jacobs, R. M., Weiser, M. G., Hall, R. L., & Kowalski, J. J. (1980). Clinicopathologic features of canine parvoviral enteritis. *Journal of the American Animal Hospital Association*, 16(6), 809-814.
- Kahn, C. M., Line, S., & Aiello, S. E. (2005). The Merck veterinary manual. Merck & Co. Inc., Whitehouse Station, NJ.
- Minakshi, P., Ranjan, K., Brar, B., Ambawat, S., Shafiq, M., Alisha, A., ... & Singh, A. (2014). New approaches for diagnosis of viral diseases in animals. *Advances in Animal and Veterinary Sciences*, 2(4S), 55-63.
- Mochizuki, M., Horiuchi, M., Hiragi, H., San Gabriel, M. C., Yasuda, N., & Uno, T. (1996). Isolation of canine parvovirus from a cat manifesting clinical signs of feline panleukopenia. *Journal of Clinical Microbiology*, 34(9), 2101-2105.
- Mochizuki, M., San Gabriel, M. C., Nakatani, H., Yoshida, M., & Harasawa, R. (1993). Comparison of polymerase chain reaction with virus isolation and haemagglutination assays for the detection of canine parvoviruses in faecal specimens. *Research in veterinary science*, 55(1), 60-63.
- Nandi, S., & Kumar, M. (2010). Canine parvovirus: current perspective. *Indian Journal of virology*, 21(1), 31-44.
- Nandi, S., Kumar, M., Anbazhagan, R., Chidri, S., & Chauhan, R. S. (2007). A sensitive method to detect canine parvoviral DNA in the stool samples by polymerase chain reaction. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 28(1and2), 56-57.

IVSA MIRROR V

- Ogbu, K. I., Anene, B., Nweze, N. E., Okoro, J. I., Danladi, M., & Ochai, S. (2017). Canine parvovirus: a review. *International journal of science and applied research (ISSN: 2504-9070)*, 2(2), 74-95.
- Ogbu, K. I., Chukwudi, I. C., Ijomanta, O. J., Agwu, E. O., & Chinonye, C. N. (2016). Prevalence of canine parvovirus in Jos north and south local government areas of plateau state. *Microbiology Research Journal International*, 1-5.
- Pereira, C. A., Monezi, T. A., Mehnert, D. U., D'Angelo, M., & Durigon, E. L. (2000). Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. *Veterinary microbiology*, 75(2), 127-133.
- Scott, F. W. (1980). Virucidal disinfectants and feline viruses. *American journal of veterinary research*, 41(3), 410-414.

CASE REPORT: CANINE DISTEMPER IN GERMAN SHEPHERD

M. Ahmed^{1*}, A. Waheed²



Abstract: *This case report is about the case study of a 2 years old German shepherd breed infected male dog. This dog was taken to the clinic for treatment because the dog was showing some abnormalities. The proper history given by the dog owner was anorexia, depression, nasal discharge, ocular discharge, and hyperkeratinization of the footpad. After the proper history, we clinically examined the dog for proper diagnoses and treatment. On further clinical examination, the patient showed temperature 105°F and heart rate of 120 (bpm). After clinical examination and proper history, we diagnosed that this dog was infected by the viral disease canine distemper. Doctor prescribed good treatment by the expert and the owner told me that the dog is showing good results day by day by the use of medicine that is prescribed by the doctor.*

Keywords: *anorexia, canine distemper, hyperkeratinization, ocular discharge*

Introduction

Canine distemper (CD) is a contagious viral disease of canine species animals in the whole globe that is caused by the canine distemper virus. Canine distemper virus affects all ages of animals but mostly affects young animals. Young unvaccinated puppies and non-immunized dogs are more susceptible to CD disease (Beineke, Puff, Seehusen, Baumgärtner, 2009; Deem, Spelman, Yates, Montali, 2000; Greene, 2006). Canine distemper virus is a relatively large single-strand RNA virus with a lipoprotein envelope (Deem et. al., 2000). This virus is closely related to pes des petits ruminant (PPR), rinderpest virus, and measles (Beineke et. al., 2009). The CD spread through different ways such as air, direct and indirect contact to the infected animals. It initially attacks the dog tonsils and lymph nodes that are the main organ of the lymphatic system. It replicates itself in a week and can survive at low temperatures. Then it attacks the respiratory, urogenital, gastrointestinal, and nervous system (Greene, 2006). In Pakistan, the prevalence of CDV is 11% (Shabbir, Arfanahmad, Aliahmed, Muhammad & Anwar, 2010).

Case Description and Clinical Examination

This report deals with the case of a two years old German shepherd dog that lived in Lahore. The dog was ill for the last one month and it was unvaccinated and deworming was not done. Before it arrived at the clinic, the owner used Dexamethasone and Qbal (Mecobalamine). The canine had a nasal and ocular discharge. The hyperkeratinization footpad was also found as shown in figure 2. The dog had a high temperature of 105°F and a high heart rate of 120 (bpm). An infected dog was weak and also had a loss of appetite, depression, and cough. On the basis of history and clinical examination, it was confirmed as CD disease.

Treatment

Although, there is no proper treatment for the CD infection but for saving the life of the patient, supporting treatment was used. The treatment includes antipyretic Ketoject (Ketoprofen) @ 2mg/Kg, Tri-oxyl LA (amoxicillin) @ 2ml/IM for bactericidal effect

1 Shaheed Benazir Bhutto University of Veterinary and Animal Science, Sakrand, Pakistan

2 Callvet (SMC) Pvt. Ltd., Lahore, Pakistan

*Corresponding author: mahmad118@yahoo.com



Figure 1: Clinical characteristics

and inf. Ringer Lactate @ 15 ml/ kg b.w. (body weight) for diarrhea control. From 2nd day onwards fluid therapy was used and at 3rd day KCL electrolyte was used. At 4th and 5th-day, vitamin injection Methycobal (Mecobalamin) @ 1ml was used to improve health. The same treatment was done on the 6th day and now the dog had almost recovered. The owner was advised to isolate the sick dog from other dogs and clean the surrounding of the dog properly (Rodeheffer et. al., 2010).



Figure 2: Hard Pad Disease

Discussions and Conclusions

The prevalence rate of canine distemper in dogs of Pakistan is 11%. Keratinization of the footpad is considered as the pathognomonic sign of CD. Canine distemper is a viral disease. Different types of vaccines are present to boost up the immune system as it is the best way for the prevention of viral diseases in animals. If viral disease occurs in non-vaccinated animals, then animals can be recovered by promoting the antibodies produced in the body against the

virus. Lymphatic cells and footpad infection can persist and cause hyperkeratinization called footpad disease. At this time the only step that we can take is to prevent animals from different viral diseases. The disease can be prevented by vaccination at a regular interval until the treatment is discovered (Tariq, Shahzad & Tahira, 2013). Similarly, as reported by the researchers the same treatment is used and all the signs and symptoms are similar to other various researches (Greene & Vandeveld, 2012; Jones, Hunt & King; 1997; Hirsh & Zee, 1999; Cho et. al., 2015; de Almeida Curi et. al., 2016).

References

- Deem, S. L., Spelman, L. H., Yates, R. A., & Montali, R. J. (2000). Canine distemper in terrestrial carnivores: a review. *Journal of Zoo and Wildlife medicine*, 31(4), 441-451.
- Greene, C. E. (2006). Infectious diseases of the dog and cat. Canada: Saunders.
- Beineke, A., Puff, C., Seehusen, F., & Baumgärtner, W. (2009). Pathogenesis and immunopathology of systemic and nervous canine distemper. *Veterinary immunology and immunopathology*, 127(1-2), 1-18.
- Rodeheffer, C., Von Messling, V., Milot, S., Lepine, F., Manges, A. R., & Ward, B. J. (2007). Disease manifestations of canine distemper virus infection in ferrets are modulated by vitamin A status. *The Journal of nutrition*, 137(8), 1916-1922.
- Shabbir MZ, Arfanahmad M, Aliahmed, Muhammad K, Anwar I, 2010. Comparative evaluation of clinical samples from naturally infected dogs for early detection of canine distemper virus. *Turk. J. Vet. Anim. Sci.* 34(6): 547– 552
- Tariq, A., Shahzad, A., & Tahira, S. (2013). Clinical Aspects of Canine Distemper in 1.5 Year Old Labrador retriever bitch bit Bitch.
- Greene, CE; Vandeveld, M (2012). "Chapter 3: Canine distemper". In Greene, Craig E. (ed.). *Infectious diseases of the dog and cat* (4th ed.). St. Louis, Mo.: Elsevier/Saunders. pp. 25–42. [ISBN 978-1-4160-6130-4](#).
- Jones, T.C.; Hunt, R.D.; King, N.W. (1997). *Veterinary Pathology*. Blackwell Publishing.
- Hirsh DC, Zee YC (1999). *Veterinary Microbiology*. Blackwell Publishing. [ISBN 978-0-86542-543-9](#).
- Cho, A. R., Roh, Y. S., Lee, H. B., Cho, H. S., Lim, C. W., Kang, S. J., ... & Kim, B. S. (2015). Case report: Canine distemper virus infection in a wild Korean raccoon dog. *Journal of the Preventive Veterinary Medicine*, 39(1), 29-32.
- de Almeida Curi, N. H., Massara, R. L., de Oliveira Paschoal, A. M., Soriano-Araújo, A., Lobato, Z. I. P., Demétrio, G. R., ... & Passamani, M. (2016). Prevalence and risk factors for viral exposure in rural dogs around protected areas of the Atlantic forest. *BMC veterinary research*, 12(1), 21

CASE REPORT ON MANAGEMENT OF ACUTE GASTRIC DILATION LACKING VOLVULUS WITHOUT SURGICAL INTERVENTION



A. Yadav*

Abstract: Dog with history of heavy food intake, showing signs of abdominal distension, ineffective vomiting attempt and salivating revealed abdominal pain on palpation and was confirmed as gastric dilatation by radiography. Initially, the dog was stabilized with fluid therapy at shock rate followed by preparation of the dog for non-invasive management technique. The dog was sedated with xylazine, buprenorphine and ketamine and induction was done by propofol and diazepam followed by maintenance with propofol. Gastric decompression was the life-saving procedure with the help of an orogastric tube through which normal saline was poured and withdrawn with ingesta. Suspension of aluminium hydroxide, magnesium hydroxide and simethicone was introduced through an orogastric tube. The radiograph after the procedure showed improvement and the dog recovered after an hour. Good mental state and normal appetite were noticed on the very next day when a dog was brought in for follow-up. A large breed of dogs immediately on exercise after the feeding should be avoided.

Keywords: abdominal distension, dilation, dog, gastric decompression, orogastric tube, overfeeding

History

A 5-year-old black colour castrated male dog named 'Mitti' was brought to the WVS ITC Clinic. Owners' concern was severe abdominal distention, repeated unproductive retching, ineffective vomiting attempt, salivating, panting, lethargy and excessively enlarged abdomen. Mitti appeared shaky when standing and was not able to stand properly. The dog was left alone at home for approximately 32 hours and then was offered with a large amount of food. The dog was taken for a walk just after feeding. Mitti lacked a happy personality and was very clumsy.

Physical Examination

The clinical parameters of the dog were measured and found to be following:-

Temperature: 102 degrees Fahrenheit

Heart Rate: 120 beats/minute

Respiratory Rate: 22/min

Dehydration %: 5%

Capillary Refill Time: 3 seconds

Mucous Membranes: Pink and Moist

Special examination: Palpation at the right side of abdomen revealed abdominal pain and

observation of the right side of the abdomen revealed distension.

Differential Diagnosis

- Acute pancreatitis lacks abdominal bloat and shows productive vomiting.
- Intestinal intussusceptions are more chronic and less painful.
- Intestinal obstruction presents itself with productive vomiting, lack of salivation and uniform abdominal distension.
- Intestinal volvulus is more chronic and less dramatic.
- Acute gastritis or foreign body is less severe and presents with excessive vomiting.

The dilemma between gastric volvulus dilatation and simple dilatation was ruled out with the help of radiography as a confirmatory diagnosis.

Diagnosis

Radiography: Abdominal radiography was performed after the proper medical stabilization of the dog. The right lateral recumbency is the

IVSA MIRROR V

most relevant position preferred for gastric dilatation with or without volvulus. The radiographic image showed dilatation without any alteration in the position of pylorus and fundus as well the absence of Popeye's arm (double bubble image). The radiographic image helped in confirmation of gastric dilatation without volvulus.

Treatment

Fluid therapy: Initially shock rate was



Fig: Right lateral radiograph before and after gastric decompression

preferred i.e. 180ml (10ml/kg/hr) as a bolus injection for the first 15 minutes followed by 680ml (45ml/kg/hr) remaining in the next 45 minutes. Later, fluid rate was continued with 10ml/kg/hr after sedation.

Anaesthesia: Premedication of xylazine 1 mg/kg IM, buprenorphine 0.02mg/kg IM and ketamine 0.5mg/kg IM was administered based on an estimated weight. The dog was induced with Propofol (1 mg/kg IV) and Diazepam (0.25 mg/kg IV). Maintenance of the anaesthesia was achieved with a propofol bolus (1 mg/kg IV) every 6 to 10 minutes to effect.

Gastric decompression: Before intubation of the orogastric tube, distance from the dog's nose to 13th rib was measured. Gel was applied on the surface as well as at the end of the tube for flexibility during intubation. The animal was placed in dorso-ventral recumbency with the table tilted cranio-caudally upward and intubation was done guided with the help of laryngoscope. After intubation, 500ml of normal saline was poured inside the stomach through a

tube. The table was tilted downward cranio-caudally, the normal saline with ingesta was withdrawn and the procedure continued thrice. Suspension of Aluminium hydroxide, Magnesium hydroxide and Simethicone (Trade name; Gelusil) about 10ml was introduced through the orogastric tube. The radiography after all the procedure clearly showed the improvement and the dog recovered after an hour.

Follow up: On the very next day the dog was brought to the clinic, the dog was in good mentation and normal appetite.

Case Discussions

Introduction

Acute gastric dilatation with or without volvulus is classically a condition of large breed dogs with a deep-chest where the age of the dog is a major concern in which animal's life is in risk (Silverstein, D. & Hopper, K., 2014). Acute gastric dilatation with or without volvulus is a syndrome which is characterized by rapid accumulation of air in the stomach, an increase of gastric pressure, malposition of stomach and shock (Wingfield, W. & Raffe, M., 2002). Increased gastrin concentration, decreased stomach motility, large amounts fed at each feeding, increasing the frequency of feedings (one meal a day), fast eating style and exercise or stress after a meal are the risk factors that can contribute to gastric dilatation with or without volvulus (Monnet, 2012).

Clinical Presentation

Clinical signs of gastric dilatation with or without volvulus present soon after the consumption of food by the dog. Owners notice their dog with the signs like cranial abdominal distention with tympany, retching with the inability to vomit, restlessness, flatulence, excessive salivation and often panting or making grunting signs indicating the animal's pain and discomfort. The duration of the gastric dilatation with or without volvulus relates with the

IVSA MIRROR V

recumbency, at the point of death or even death of an animal. Physical examination findings include a rapid, weak pulse, laboured breathing, pale mucous membranes, prolonged capillary refill time, cranial abdominal distension with tympany (Wingfield, W., & Raffe, M.,2002).

Diagnostic Evaluation

Radiography: Right lateral position is the most revealing position in case of gastric dilatation with or without volvulus (Armbrust, Biller, & Hoskinson, 2000). Abdominal radiography is performed after stabilization of the dog with fluid and medicine. Gastric dilation with or without volvulus is confirmed if a gastric shadow is present due to distension of gas in the stomach. Volvulus is suspected if a tissue density separates the gas-filled gastric shadow into two chambers(Popeye's arm) or the pylorus is dorso-cranial to the fundus in the right lateral view.(Bhatia, Tank, Karle, Vedpathak, & Dhami, 2010; DeYoung, 2017; Monnet, 2012)

Management

Shock management: An intravenous catheter of the largest gauge possible is adjusted in the cephalic veins or jugular vein to deliver shock doses (90 ml/kg) of intravenous fluid. Isotonic fluid is delivered in increments of 25% of the shock dose and the patient's response after each 25% bolus is evaluated. The rate and volume of fluids administered is adjusted according to the assessment of several clinical parameters i.e. heart rate, pulse, mucous membrane colour, capillary refill time and central venous pressure. Colloids at a dose of 4 ml/kg are recommended during hypovolemic shock treatment (Schertel, Allen, Muir, Brouman, & DeHoff, 1997).

Gastric decompression: After initiating aggressive therapy for shock then medical stabilization is maintained followed by decompressing the stomach; decompressing is usually performed with the help of the orogastric tube (Nelson, R. W., & Couto, C. G., 2019). The dog is sedated then gastric decompression is accomplished with orogastric intubation,



Fig: Adjusting orogastric tube and pouring normal saline



Fig: Mitti on the next follow up day

Intubation is desirable to protect the airway and prevent aspiration pneumonia. The smooth orogastric tube should be marked properly from the nares to the caudal edge of the last rib and the lubricated tube should not be passed beyond this point. Dogs with gastric dilatation without volvulus typically do not require surgical intervention (Bhatia et al., 2010; DeYoung, 2017; Fossum, 2015; Monnet, 2012).

Conclusions/ Take Away Points

It is recommended to feed large breed dogs two or three times a day and not to exercise dogs for one hour after feeding.

Quick Diagnosis and Correct Therapeutic Regimen was undertaken on time by the owners and the vets helped to save the patient's life.

References

- Armbrust, L. J., Biller, D. S., & Hoskinson, J. J. (2000). Case Examples Demonstrating the Clinical Utility of Obtaining Both Right and Left Lateral Abdominal Radiographs in Small Animals. *Journal of the American Animal Hospital Association*, 36(6), 531–536 <https://doi.org/10.5326/15473317-36-6-531>
- Bhatia, A. S., Tank, P. H., Karle, A. S., Vedpathak, H. S., & Dhama, M. A. (2010). Gastric dilation and volvulus syndrome in dog. *Veterinary World*, 3(12), 554–557.
- DeYoung, G. F. G. and D. W. (2017). *Gastric Dilatation and Volvulus in the Dog*. 40(1), 1–10. Retrieved from papers3://publication/uuid/BC6CC4D5-1492-40A-A-8C01-6C9A65FBB938
- Fossum, T. (2015). *Gastric Dilatation-Volvulus : What 's New ?* (March).
- Monnet, E. (2012). Gastric dilatationvolvulus: Controlling the crisis. *Veterinary Medicine*, 107(10), 448–455.
- Nelson, R. W., & Couto, C. G. (2019). *Small Animal Internal Medicine-E-Book*. Elsevier.
- Schertel, E. R., Allen, D. A., Muir, W. W., Brouman, J. D., & DeHoff, W. D. (1997). Evaluation of a hypertonic saline-dextran solution for treatment of dogs with shock induced by gastric dilatation-volvulus. *Journal of the American Veterinary Medical Association*, 210(2), 226–230. Retrieved from <http://europepmc.org/abstract/MED/9018357>
- Silverstein, D., & Hopper, K. (2014). *Small Animal Critical Care Medicine-E-Book*. Elsevier Health Sciences.
- Wingfield, W., & Raffe, M. (Eds.). (2002). *The veterinary ICU book*. Teton NewMedia.

A CASE REPORT ON METASTATIC MAMMARY GLAND TUMOR IN 12-YEAR-OLD MONGREL DOG

S. Phuyal*, R. Shah, G. P. Yadav



Abstract: *A 12 years old female dog was presented with a history of the growth of abnormal mass of tissues at the ventral abdominal region, one of large size and another of small size since the last 5 months around the mammary glands. Physical and radiographic examinations of the dog were performed. Heart rate, respiration rate, rectal temperature, and mucous membrane were found 112 beats per minute, 28 breaths per minute, 100.4°F, and pinkish respectively. Radiographic examination revealed the metastatic tumor with nodular growths in the lungs. The case was diagnosed as a metastatic mammary gland tumor. Surgical excision of the tumor was done followed by postoperative medications.*

Keywords: Dog, mammary gland, surgical excision, tumor

Introduction

A tumor may be defined as an abnormal mass of tissue which results when cells divide in an uncontrolled manner than they normally do, even if the original trigger is removed (Samit et al., 2014). Tumors are broadly classified as benign and malignant tumors. Benign tumors grow slowly and locally to great size without invading the nearby tissue and when surgically removed usually do not reoccur. Malignant tumors however grow rapidly and metastases to the other part of the body by invading nearby tissues and have a tendency to recur even after surgical removal. Histologically, canine mammary gland tumors have been categorized as carcinomas (with 6 types and additional subtypes), sarcomas (with 4 types), carcinosarcomas (mixed mammary tumor) or benign adenomas (Susan and Michael, 2016). Although cows, mares, goats, ewes, and sows suffer from mammary gland tumors, the frequency of occurrence is more in dogs i.e. ~50% of all the tumors in intact bitches are mammary tumors (Susan and Michael, 2016). Malignant percentage of mammary tumors is approximately 50% and 85% in dogs and cats respectively, having a higher number of complex and mixed tumors in dogs than cats (Samit et al., 2014). The capacity

of malignant mammary gland tumor to metastasis is greatly influenced by the kind of tumor, histological differentiation, and several clinical prognostic factors (Sorenmo, 2003). The occurrence rate is higher in the posterior-most two mammary glands in comparison to three anterior ones (Sorenmo, 2003). Hormones and growth factors (endogenous and exogenous), food, virus, irradiation, genetic factors are related to their cause (Misdorp, 2002).

History and Clinical Observations

A 12 years old female dog's breed "Mongrel", weighing 16 kg from Tilottama, Butwal, was brought to Veterinary Teaching Hospital, Institute of Agriculture and Animal Science, Paklihawa with the growth of abnormal mass of tissues at the ventral abdominal region, one of large size and another of small size for the last 5 months in the region around the mammary gland (Fig 1). On clinical examination, the large growth was hard in consistency, non-movable while the small one was movable. All the physical parameters were within normal limits. Heart rate, respiration rate, temperature, and mucous membrane were found to be 112 beats per minute, 28 breaths per minute, 100.4°F, and

IVSA MIRROR V

pinkish respectively. Radiographic examination revealed the metastatic tumor showing nodular growths in the lungs (Fig 2). It was diagnosed as a metastatic mammary gland tumor and decided for surgical intervention under general anesthesia.

Treatment

The dog was sedated with xylazine @ 2 mg/kg intramuscularly and prepared aseptically for surgery. Fluid therapy was done and inj. ceftriaxone @ 20 mg/kg and inj. meloxicam @ 0.2 mg/kg was given through the intravenous route. Induction of anesthesia i.e. diazepam (0.5mg/kg) and ketamine (5 mg/kg) in the ratio of 1:1 was given at a dose of 0.8ml each by mixing in the same syringe. Endo-tracheal intubation was done to maintain the patent airway and to ensure the adequate exchange of O₂ and CO₂.

The animal was placed in dorsal recumbency and the surgical site was prepared by removing hair, scrubbing, and draping (Fig 3). An elliptical incision was made through the skin over the growth and affected area including adhesion with abdominal muscles were removed from the inner side (Fig 4 and 5). Bleeding was checked with artery forceps and ligating of blood vessels. Maintenance of anesthesia was done using diazepam and ketamine in the ratio of 1:2 by mixing in the same syringe and top-up was given every 5-10 minutes on the basis of monitoring of anesthesia. After removal of the mass and affected tissues, the site was thoroughly cleaned with normal saline and sutures were applied. Vicryl 2-0 suture was used in muscles and subcutaneous tissues, and skin was sutured with nylon 2-0 suture in ford interlocking pattern and interrupted pattern (Fig 6 and 7). Post-operatively, inj. ceftriaxone @ 20 mg/kg IM BID for 5 days, inj. meloxicam @ 0.2 mg/kg IM OD for 3 days and inj. belamyl @ 1ml IM OD for 5 days was administered. E-collar was placed in the neck until the wound. Dressing of the wound was done with 5 % Povidone-iodine



Fig: SEQ Figure * ARABIC 1: * Abnormal mass of tissue in the abdominal area



Fig SEQ Figure * ARABIC 2: X-ray showing nodular growths in lung and large abnormal growth just behind the xiphoid process



Fig: SEQ Figure * ARABIC 3: Placement of drapes around the tumor



Fig SEQ Figure * ARABIC 4: Skin incision over the tumor



Fig 5: Suturing after removal of tumor



Fig 6: Removing tumor from muscle adhesion



Fig 7: Skin sutured using ford interlocking pattern and interrupted pattern

solution. Sutures were removed after twelve postoperative days.

Discussion

Dogs with mammary gland tumors are typically older, the high incidence was found after 6 years of age with the maximum being 9 to 11 years old (Zatloukal et al., 2005). The risk of developing mammary gland tumor is about 12% in neutered bitches as compared to intact one (Schneider et al., 1969). Those bitches which have been spayed before 1st estrus (approximately 6 months of age), after the completion of 1st estrus and completion of 2nd or more estrus before neutering have risk of about 0.5%, 8% and 26% to suffer from mammary gland tumor respectively (Schneider et al., 1969). Currently, surgery is the only accepted treatment for dogs with mammary gland tumor and there are no established guidelines for treatment beyond surgery.

Conclusion

Mammary gland tumors are more common in female dogs that are either intact or were spayed after 2 years of age. Timely spaying/neutering of dogs prevents the growth of abnormal masses leading to mammary gland tumors. The practice of ovariectomy (OVH) at an earlier age is one of the effective measures to overcome it.

References

- Misdorp, W. (2002). Tumors in the Mammary Gland. In D. J. Meuten (Ed.), *Tumors in Domestic Animals* (4th ed., pp. 575–606). Wiley Online Library. <https://doi.org/10.1002/9780470376928.ch12>
- Nandi, S. K., Halder, S., & Hoque, M. (2014). *A TEXT BOOK ON VETERINARY SURGERY AND RADIOLOGY* (2nd revise). KALYANI.
- Schneider Robert, Dorn C. Richard, T. D. O. N. (1969). Factors Influencing Canine Mammary Cancer Development and Postsurgical Survival 1 . 2. *Journal of the National Cancer Institute*, 43(6), 1249–1261. <https://doi.org/10.1093/jnci/43.6.1249>
- Sorenmo, K. (2003). Canine mammary 58 gland tumors. *Veterinary Clinics: Small Animal Practice*, 33(3), 573–596.
- Susan, E. A., & Michael, A. M. (2016). *THE MERCK VETERINARY MANUAL* (11th editi). Merck & CO, INC. Zatloukal, J., Lorenzova, J., Tichy, F., Necus, A., Kecova, H., & Kohout, P. (2005). Breed and Age as Risk Factors for Canine Mammary Tumours. *ACTA VET. BRNO*, 74, 103–109.

CASE STUDY OF LUMPY SKIN DISEASE IN CATTLE OF CHITWAN NEPAL

Y. R. Pandeya*, C. R. Pathak, G. Pandey, P. Hamal, P. Khanal



Abstract: *Lumpy skin disease (LSD) is a serious skin disease of cattle, buffalo & yak caused by lumpy skin disease virus of genus Capripoxvirus in the family Poxviridae. It causes temporary or permanent loss of milk production, infertility or even sterility in bulls, abortion of pregnant cows, reduced weight gain, permanent damage to hides. Till 2012 it was endemic in most of the African countries but now it has spread to Asian countries too. A 10 year old Holstein Friesian crossbred cow with clinical signs of increased salivation and nodules in most parts of the body was seen at National Cattle Research Program (NCRP) Farm Rampur, Chitwan on 11 August 2020. On the basis of history, clinical findings and ELISA test, it was diagnosed as LSD. Infected cow was treated with a broad spectrum antibiotic, anti-inflammatory and antihistaminic drug for 7 days. Also regular iodine dressing & fly repellent herbal spray over wounds was done till recovery. It took more than a month for the animal. Isolation of animals, comfort bedding, well feeding management, regular monitoring and symptomatic treatment of infected animals were important practices. Prognosis of LSD is good though it takes time for recovery.*

Keywords: cattle, ELISA, lumpy skin disease, nepal

Introduction

Lumpy skin disease (LSD) is a serious skin disease of cattle, buffalo & yak caused by lumpy skin disease virus. Lumpy skin disease virus (LSDV) is a double-stranded DNA virus of genus Capripoxvirus in the family Poxviridae. It is endemic in most African countries. Now, it has spread out of the African continent into the Middle East region. It is one of the most economically significant trans-boundary and OIE listed diseases. It causes temporary or permanent loss of milk production, infertility or even sterility in bulls, abortion of pregnant cows, reduced weight gain, permanent damage to hides, increased cost of treatment and additional feed for diseased animals until their recovery. LSD was first recognized in Zambia in 1929 AD and extended northwards through sub-Saharan West Africa through a series of epizootics in the 1960s. Since 2012 it has spread to Asia and Europe. In 2013 it was confirmed in Turkey. In 2019 its outbreak was reported in neighboring countries India, China & Bangladesh (OIE, 2020). Although Nepal experienced an outbreak

from June 2020 but confirmation by RT PCR was made on 27 July 2020 (Acharya & Subedi, 2020). The incubation period varies from 2 to 5 weeks.

The principal means of transmission is believed to be by arthropod vectors. Though no specific vector has been identified till date, mosquitoes (e.g. *Culex mirificens* and *Aedes natrionus*), biting flies (e.g. *Stomoxys calcitrans* and *Biomys fasciata*) and male ticks (*Rhipicephalus appendiculatus* and *Amblyomma hebraeum*) could play a role in the transmission of the virus (OIE, 2017). Animals can be infected experimentally by inoculation with material from cutaneous nodules or blood. There is a great variation in the morbidity and mortality rates of LSD outbreaks. Generally, morbidity rates vary between 1% and 20%. Mortality rates of 1 to 5% are considered usual (Tuppurainen et al., 2017).

IVSA MIRROR V

Case history and clinical findings

- Species: Cattle
- Breed: Holstein Friesian Cross
- Age: 10 years
- Location: Rampur, Chitwan, Nepal (NCRP Farm)
- Small nodules were present in head, neck, udder, rump, perineum and leg region
- Another cattle was infected with LSD in the same herd 5 days ago
- Temperature: 103.7 ° F
- Increased Salivation
- Increased respiration rate (44 /min)
- Feeding was normal
- Date of first observation of case: 11 August, 2020
- Treatment started from August 15

Diagnosis

By history (Recent outbreak of LSD in same farm and in other parts of same district/ country)

By clinical sign and symptoms (Eruption of nodules in the body and increased salivation)

By ELISA test: Positive (Kit named as ID ScreenR Capripox Double Antigen Multi Species of Lot no. G66 manufactured by ID Vet France) and performed at NCRP laboratory Rampur, Chitwan.

Treatment

1. Inj. Oxytetracycline- SA
Sig: 40ml x OD x IM x 7 days
2. Inj. Meloxicam-P (Meloxicam with Paracetamol)
Sig: 20ml x OD x IM x 7 days
3. Inj. Chlorpheniramine Maleate
Sig: 10 ml x alternate days x IM x 3 days
4. Herbal spray over the body at least 3 times a day.
5. Infected animal was isolated from the rest of the animals and kept in an isolation shed.



Fig 1: Cow with small nodules (neck & head region)-1st day Fig 2: Cow with large nodules (perineum region)-4th day



Fig 3: Nodules ruptured & exposed-8th day
Fig 4: wound in muzzle-9th day
Fig5: Dry and crusty nodule-19th day



Fig6:Scar in the body-60th day

IVSA MIRROR V

Follow up:

2nd day of treatment: Temperature - 103.5° F,

3rd day: 103.0° F,

4th day: 102.4° F,

5th day: 102.0° F,

6th day: 102.4° F,

7th day: 102.8° F,

8th day: 102.7° F

1st day of case - Eruption of few small sized nodules in some parts of the body

4th day - Increased size of nodules (1-4 cm) present over most of the body parts

8th day - Some of the nodules ruptured and became like open wound

19th day - Ruptured and unruptured nodules changed into crust and scar

Discussion

Based on the clinical signs, history and ELISA test result, the current case was diagnosed as LSD which is in accordance with (Al-Salihi, 2014), which indicates clinical manifestations of LSD. The infected animals may show fever commonly rises to 104.0°F-106.7°F (40-41.5°C), lacrimation, increased nasal and pharyngeal secretions, anorexia, dysgalactia, general depression and reluctance to move. Differential diagnosis must be done with Pseudo-lumpy-skin disease, Bovine viral diarrhoea, Bovine malignant catarrhal fever, Demodicosis, Rinderpest, Bovine herpes virus 2 infections, Oncocercariasis, Dermatophilosis and Bovine besnoitiosis (Davies, 1991).

Therefore, laboratory confirmation is needed. Laboratory diagnosis of LSD can be performed by using serological and molecular techniques and by virus isolation in cell cultures (OIE, 2010).

The usual manifestations of LSD are multiple firm circumscribed nodules developed in the skin of the animals primarily on head, neck, perineum, genitalia, udder and limbs. The

regional lymph nodes are easily palpable and enlarged 3-5 times their normal size. Most cases may complicate or extend to other underlying tissues or internal organs and may result in economically significant disorders (Constable et al., 2017). LSD is not associated with high mortalities (1-3%); however, the economic losses accompanying LSD eruption are higher. LSD causes considerable economic losses due to emaciation, damage to hides, infertility, mastitis, loss of milk production and mortality up to 20% (Al-Salihi, 2014).

All cattle breeds appear to be equally susceptible to the disease. In NCRP farms, prevalence of LSD was observed in indigenous Nepalese cattle breeds namely Achhami, Lulu, Terai and exotic breeds; Jersey & Holstein Friesian crossbreeds. Infected indigenous breeds were with mild clinical signs and recovered faster than exotic cattle breeds. Holstein Friesian breeds are more susceptible than indigenous cattle breeds, which is also supported by earlier researchers (Al-Salihi, 2014). Who reported that imported breeds with thin skins, such as *Bos taurus*, Friesland cattle and the Channel Island breeds, were far more susceptible than indigenous breeds with thicker skins.

In this case, Oxytetracycline was provided to check secondary bacterial infection, Meloxicam was provided as anti-inflammatory since nodules were ruptured and developed as open wounds. Antihistaminic drugs were also provided as symptomatic treatment as laboratory tests were delayed. Although antihistaminic drugs were used in this case, some authors have suggested no need of using antihistaminic drugs in case of LSD. But use of Vitamin E and Vitamin C may be useful, as it will boost immunity. Use of fly repellent spray and antiseptic solution checked the myiasis of the wound and helped in wound healing. In this case the animal did not face the anorexic condition. Consequently fever, nodular lesions and other deviations were improved. Skin healing was delayed by forming scars.

There is no specific treatment for LSD. However, supportive treatment should be given to infected animals to relieve clinical signs and to control all secondary complications. Systemic antibiotic and anti-inflammatory drugs are obligatory for skin infections, cellulitis or pneumonia and considerably to avoid further complications and economic losses (Abutarbush et al., 2013).

Immunization of the susceptible animals is the effective methods to control the disease in South Africa, and the effective vaccines are produced from the Neethling strain virus (Ayelet et al., 2014). In Nepal vaccination of LSD has not started yet.

Conclusion

LSD was a new emerging disease for Nepal, first confirmed in July 2020. Although mortality is low it causes significant economic loss due to decreased milk production, infertility, recumbency and long time treatment cost. Strict quarantine, biosecurity and vaccination of animals will be useful for prevention of disease. If an animal is infected, isolation and timely treatment of the animal is very much useful.

Acknowledgement

I would like to acknowledge all the staff of National Cattle Research Program, Rampur for their support in isolation, treatment and care of animals.

References

- Abutarbush, S. M., Ababneh, M. M., Al Zoubi, I. G., Al Sheyab, O. M., Al Zoubi, M. G., Alekish, M. O., & Al Gharabat, R. J. (2013). Lumpy skin disease in Jordan: Disease emergence, clinical signs, complications and preliminary-associated economic losses. *Transboundary and emerging diseases*, 62(5), 549-554.
- Acharya, K. P. & Subedi, D. (2020). First outbreak of lumpy skin disease in Nepal. *Transboundary Emerging Disease*. 2020;00:1-2. <https://doi.org/10.1111/tbed.13815>
- Agonafir, H., Zemene, M., Wondu, B., Getaneh, G., Abebaw, M., Negash, A., & Mamuye, Y. (2016). A review on lumpy skin disease. *Researcher*, 8(11), 73-80.
- Al-Salihi, K. A. (2014). Lumpy skin disease: Review of literature. *Mirror Research in Veterinary Science and Animals*, 3, 6-23.
- Ayelet, G. , Haftu, R., Jemberie, S., Belay, A., Gelaye, E., Sibhat, B., Skjerve, E., & Asmare, K. (2014). Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of lumpy skin disease virus. *Rev. sci. tech. Off. int. Epiz*, 33 (3), 1-23.
- Beard, P. M. (2019). Capripoxviruses, parapoxviruses and other poxviruses of ruminants. *Elsevier Publication*.
- Center for Food Security and Public Health (CFSPH). (2017). Lumpy skin disease. Technical Factsheet. Iowa State University.
- Constable, P.D., Hinchcliff, K. W., Done, S.H., & Gruenberg, W. (2017). *Veterinary Medicine: A Textbook of the diseases of cattle, horses, sheep, pigs, and goats*. Elsevier, UK, p: 1591.
- Davies, F.G. (1991). Lumpy skin disease of cattle: a growing problem in Africa and the Near East. *World Animal Review*, 68, 37-42
- Feyisa, A. F. (2018). A case report on clinical management of lumpy skin disease in bull. *Journal of Veterinary Science and Technology*, 9, 538. doi:10.4172/2157-7579.1000538
- Hamid, M. E. (2016). *Skin diseases of cattle in the tropics: A guide to diagnosis and treatment*. Academic press.
- OIE. (2010). Lumpy skin disease In: *Manual of diagnostic tests and vaccines for terrestrial animals*. Office International des Epizooties, World Organization for Animal Health, Paris, 2010; 1-13.
- OIE. (2010). Lumpy skin disease, Chapter 2.4.14 OIE Terrestrial Manual, Office International des Epizooties, Paris, France, 2010.
- OIE. (2013). *Manual of diagnostic tests and vaccines for terrestrial animals*. World Organisation for Animal Health. Pp 1185-1191.
- OIE. (2017). Lumpy skin disease chapter 2.4.13. OIE manual for diagnostic tests and vaccines for terrestrial animals.
- OIE. (2020). Lumpy Skin Disease. <https://rr-asia.oie.int/en/projects/lumpy-skin-disease-lsd/>
- Tuppurainen, E., Alexandrov, T., & Beltrán-Alcrudo, D. (2017). Lumpy skin disease field manual – A manual for veterinarians. FAO Animal production and health Manual No. 20. Food and Agriculture Organization of the United Nations (FAO).
- http://lrd.spc.int/ext/Disease_Manual_Final/a070_lumpy_skin_disease.html
- <https://www.cabi.org/isc/datasheet/76780#tooverview>
- https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/LUMPY_SKIN_DISEASE_FINAL.pdf
- <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lumpy-skin-disease>

CONTROL, PREVENTION AND MANAGEMENT OF ORF IN A GOAT (*Capra hircus*)



H. M. M. Ali^{1*}, H. Jawad²

Abstract: Scabby mouth is one of the most important diseases that mainly cause economic losses in goats and sheep worldwide. This report is about the control, prevention, and management of Orf in goats aging 1.5 and 3 years in Bahawalpur, Pakistan. Two goats aged 1.5 and 3-years were presented near Bahawalpur, Pakistan with a history of anorexia and various types of cutaneous lesions around the oral cavity. On clinical examination, it was found that both goats had temperatures higher than the normal, dry ulcerated necrotic lesions on muzzle, oral commissures, nostrils and lips. On lungs auscultation, normal sound was revealed. No pneumonic sound and no diarrhea were observed. The clinical picture was pointed towards a scabby mouth. In both cases, Trisomy (trimethoprim and sulfadiazine) at a dose regime of 1ml/30kg for 7 days and Melonac (meloxicam) for 4 days were administered intramuscularly. Topically, Somogel (lignocaine, eucalyptol, alcohol, menthol) was applied over the lesions for 10 days after washing with potassium permanganate solution. Turpentine oil (t.t oil) was also used as a fly repellent. The prognosis was good as after 2 weeks of topical and systemic therapy, both goats recovered.

Keywords: goat, parapox, pyrexia, scabby mouth, ulcerated necrotic lesions

Introduction

Scabby mouth, also known as Orf, contagious pustular dermatitis, sore mouth and contagious ecthyma is a contagious and zoonotic disease (communicable disease between humans and animals) that is caused by virus of genus Parapoxvirus in the subfamily Chordopoxvirinae and family Poxvirida (Kumar et. al., 2015). Parapox is dsDNA (double-stranded DNA virus) about 220-300nm long and 140-170nm wide with a unique property of a unique spiral coat that makes it distinguishable from other poxviruses (Wilson & Miles, 1983). Chances of transmission are more in pasture feeding than the stall feeding because in pasture feeding animal is more likely to be exposed to trauma that may lead to scarified skin which mainly serves as a route of parapox transmission and results in the formation of macule, papule, vesicle, pustule and later that become a scab (Cargnelutti et. al., 2011).

Geological Distribution

Contagious pustular dermatitis has been distributed all over the world that raise goat,

sheep, domesticated and wild ruminants (Adah, Olabode, & Ajayi, 2012).

History

Both goats were anorexic, having dry ulcerated lesions around oral commissure, muzzle and lips from the last 4 days. From previous medication only, diesel oil was applied over the lesions.

Clinical Observation

On clinical examination, it was found that there were ulcerated necrotic lesions around the oral commissures, muzzle and lips. Rectal temperature, pulse rate and respiration were higher than the normal. There was no lesion on the coronet region. Conjunctiva was pale. No pneumonic sound and no diarrhea were observed. So, peculiar lesions and clinical pictures around the oral cavity pointed toward contagious pustular dermatitis (CPD).

Diagnosis

For diagnostic confirmation of the disease, Polymerase chain reaction (PCR) is the preferred diagnostic tool to be performed.

¹University College of Veterinary and Animal Sciences, Lahore, Pakistan

²The Islamia University of Bahawalpur, Bahawalpur, Pakistan

*Corresponding author: mubashir3ali@gmail.com

Differential Diagnosis

Orf should be differentiated from some other abnormalities that are also involved in the development of lesions around the oral cavity. Some of the diseases showing similar clinical signs are:-

- Peste des petits ruminant
- Foot and mouth disease
- Goat pox
- Bluetongue
- Facial eczema

Therapeutic Management

Scabby mouth is a viral disease and thus, there is no specific treatment. Supportive therapy and antibiotics can be given because animals become anorexic and more prone to secondary bacterial infections. In these cases, Trisomy (trimethoprim and sulfadiazine) at a dose regime of 1ml/30kg were administered intramuscularly for 7 days, Melonac (meloxicam) was administered intramuscularly for 4 days. Topically, Somogel (lignocaine, eucalyptol, alcohol, menthol) was applied over the lesions for 10 days after washing with potassium permanganate solution. Turpentine oil (t.t oil) was also used in the site of infection as a fly repellent. After the completion of 2-week therapy, both goats had recovered from the disease.



Fig. 1: Dry ulcerated and scabby lesions around muzzle, nostrils and lips



Fig. 2: Dry, ulcerated necrotic lesions around nostrils and muzzle

Prevention and Control

Orf is not a fatal disease and is self-limiting but from an economical point of view, it is very important because the animal suffering from scabby mouth results in decreased milk production, increased treatment and farm disinfection cost. The following methods are useful for prevention of outbreak of the disease.

- Before introducing a new animal into the flock, it should be placed in quarantine for 10-14 days.
- The suspected animals should be isolated.
- In case of an outbreak, vaccination should be done.
- The shed and equipment should be disinfected after use.

Disinfection can be done by:

- Chemical method
- Heat treatment

For *parapoxvirus*, glutaraldehyde, Virkon, 1% acetic acid and 2% sodium hypochlorite is recommended for its inactivation. Virus can also be inactivated by heating at 59°C for 30 mins.

Prognosis

The prognosis of the disease was good because after 2 weeks of topical and systemic therapy both goats recovered.

Conclusions

Contagious pustular dermatitis is a non-fatal and self-limiting disease but has a great impact on

IVSA MIRROR V

the economy of the farm especially in endemic areas, where the disease has spread to a great extent. In this case, the goats were presented with the signs of dry ulcerated necrotic lesions around the muzzle, nostrils and lips. After two weeks of topical and systemic therapy both goats were fully recovered. Implementation of a contagious ecthyma vaccination in management schemes is still not widely applied by the farmers; nevertheless, that is an important measure that could greatly benefit them if added, as economic losses will be reduced, and production will be improved in the long-term, minimizing greatly the impact of the disease (Spyrou & Veliakos, 2015).

The development of disinfection protocols for animal containment facilities, based on a household disinfectant, such as Lysoform casa, can be a cost-effective means of improving the health and welfare of animals and preventing zoonotic infections (Demiraslan, Dinc, & Doganay, 2017).

Conflict of Interest

The author declares no conflict of interest.

References

- Kumar, R., Trivedi, R. N., Bhatt, P., Khan, S. H., Khurana, S. K., Tiwari, R., ... & Chandra, R. J. A. A. V. S. (2015). Contagious pustular dermatitis (orf disease)–epidemiology, diagnosis, control and public health concerns. *Adv Anim Vet Sci*, 3(4), 560-569.
- Wilson, G.G., & Miles A.A. (1983). Taxonomy and nomenclature. *Topley and Wilson's Principles of Bacteriology, Virology, and Immunity: Virology*, 4, 163.
- Cargnelutti, J. F., Masuda, E. K., Martins, M., Diel, D. G., Rock, D. L., Weiblen, R., & Flores, E. F. (2011). Virological and clinico-pathological features of orf virus infection in experimentally infected rabbits and mice. *Microbial pathogenesis*, 50(1), 56-62.
- Adah, B. M. J., Olabode, H. O. K., & Ajayi, I. E. (2012). A case of contagious pustular dermatitis in Kano brown goats. *Sahel Journal of Veterinary Science*, 11(2), 23-27
- Spyrou, V., & Veliakos, G. (2015). Orf virus infection in sheep or goats. *Veterinary microbiology*, 181(1-2), 178-182.
- Demiraslan, H., Dinc, G., & Doganay, M. (2017). An Overview of ORF virus infection in humans and animals. *Recent patents on Anti-Infective drug discovery*, 12(1), 21-30.

DEPHOSPHORYLATION OF PHYTATE COMPLEX FOR INCREASING THE PHOSPHORUS AVAILABILITY IN MONOGASTRIC ANIMALS DIET



S. R. Barsila*

Abstract: *The phosphorus (P) is the third most important mineral in many plant feed resources, it remains in the form of phytate complex and is not readily available for monogastric animals such as pig and poultry. The organic phosphorus remains in the complex that binds other essential minerals and amino acids in general. The readily available phosphorus remains only 20-40% in feedstuffs. Phytate is a heat stable compound up to 100 °C and thermal processing of feed might be the cause of losses of other essential nutrient elements. As phosphorus remains in the outer peripheral layer of cereals and legumes, the mechanical processing would further the loss of nutrients. The soaking and germination process would reveal a breakdown of the phytate complex, however the availability of phosphorus cannot be guaranteed in pigs and poultry. Fermentation of feeds has been lately proved to be the advantageous method. The addition of phytase enzyme thus is the best way to optimize the P availability in most of the feed categories for monogastric animals. The microbial phytase has been commercially used nowadays in the commercial feed industries.*

Keywords: monogastric animals, phosphorus, phytate complex, phytase enzyme

Introduction

Phytase is an enzyme that helps to break down phytate phosphorus (P), the main form of P in grains to myo-inositol monophosphate allowing better absorption of grain P by poultry and other monogastric animals such as swine and reduces the need for the addition of calcium phosphate supplements. Phytase is produced by bacteria found in the gut of ruminant animals (cattle, sheep) making it possible for them to use the phytic acid found in grains as a source of phosphorus. Non-ruminants (monogastric animals) pigs, rabbit horse birds, etc. do not produce phytase. All Plant sources only contain 60-80% phytate phosphorus while only 20-40% phosphorus in free form (Non-phytate phosphorus). The phytate is a complex of myo-inositol hexaphosphate (free form) that complexes with calcium, magnesium and potassium. In general, it remains as mixed salt of phytic acid (IP₆) in feedstuffs. Monogastric animals and Poultry birds have insufficient ability to degrade this phytate and utilize the phosphorus. Livestock production costs account

for about 60-70 % in the feed (Ravindran, 2013). Phosphorus (P) is the nutrient with the third economic value in monogastric diet formulation after energy and amino acids (Lamid et al, 2018). Up to one-quarter of the feed gets wasted, because the animals lack the enzymes that would allow them to digest it (International poultry production, 2014). The phytase from microbial sources has emerged as the primary commercial feed enzyme worldwide.

There are three basic sources of phytase:

- Plant phytase
- Intestinal phytase
- Microbial phytase
 - Fungus (*Aspergillus niger*, *Aspergillus ficcum*, *Aspergillus oryzae*)
 - Bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Corynebacterium bovis*, *Pseudomonas sp.*)
- Yeast (*Saccharomyces cerevisiae*, *Schwanniomyces castellii*)

Why is Phytate an anti-nutritional factor?

- Binds divalent minerals and affect absorption i.e. P, Ca, Mg, Cu, Zn etc.
- Enhance the excretion of Na.
- Negatively affects energy metabolism.
- Interfere with protein and carbohydrate metabolism.
- Increase indigenous amino acid losses.

Basic methods to increase the P availability

Thermal treatment

Phytate is heat stable up to approximately 100 °C (Bullock et al., 1993). Schlemmer et al. (2001) observed the degradation of only 9% of phytate if soybeans were boiled for 1h. However, as the intrinsic plant phytase is thermolabile, prolonged exposure to high temperatures may inactivate the enzyme (Kumar et al., 2012). In maize, a decrease in the InsP6-P content accompanied by an increase in InsP5- P was observed as a result of extrusion cooking (6.5 MPa, 130–140 °C). In untreated and pelleted (200 kPa, 90 °C) maize 95% of the InsP-P was present in the InsP6 form, whereas the remaining 5% were in the form of InsP5. The value of InsP5-P increased to 10% when maize had been extruded.

Mechanical processing

Mechanical removal of phytate depends on the type of seed as well as on the morphological distribution of phytate in seeds (Hummer et al., 2015). While in a large number of oil seeds and cereals phytic acid is mainly located in the aleurone layer, it seems to be evenly distributed in soybeans (Steiner et al., 2007). Mechanical separation of the phytate containing departments will effectively result in a strong reduction in phytate, but will also lead to a loss of nutrients and valuable bioactive compounds (Schlemmer et al., 2009).

Storage

During long storage, phytate content can decrease, whereby the reduction depends on the storage conditions (especially humidity and

temperature) and the type and age of seeds (Larsson and Sandberg, 1995). However, under dry and cool conditions, no phytate reduction is assumed (Herken et al., 2007).

Soaking and germination

As phytate is water-soluble, a considerable amount of phytate is transferred to the water during soaking and thus may be removed by discarding the soaked water (Gustafsson and Sandberg, 1995). Also, the process enhances the action of naturally occurring phytase in cereals and legumes. It has been shown that phytate hydrolysis during soaking is greatly influenced by temperature and pH. During germination, an increase in phytase activity and thus a decrease in phytate P can be expected in beans.

Fermentation

In recent years, fermentation of dry feeds was effectively used to degrade phytate P and improve mineral and nutrient digestibility in diets for non-ruminants (Kraler et al., 2014). During fermentation, micro-organisms like lactic acid bacteria convert soluble starch and sugars into organic acids, ethanol and carbon dioxide (Prescott et al., 1996). Thus, the acidification of the diet can lead to activation of endogenous plant phytase (Kozłowska et al., 1996). Furthermore, lactic acid bacteria can promote phytate degradation due to their ability to produce phytase (De Angelis et al., 2003)

Conclusions

The scientific evidence has repeatedly shown that phytase increases the availability of phosphorus in diets. The phytase can also increase the availability of other minerals such as calcium. Phytase helps lower the amount of supplemental phosphorus required in the diet. Because of improved dietary phosphorus utilization, less phosphorus is excreted in the manure. Because less phosphorus excreted helps to reduce environmental pollution (eutrophication).

References

- Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U., & Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *Journal of Food Science and Technology*, 48(6), 724-729.
- Bullock, J. I., Duffin, P. A., & Nolan, K. B. (1993). In vitro hydrolysis of phytate at 95 C and the influence of metal ion on the rate. *Journal of the Science of Food and Agriculture*, 63(2), 261-263.
- De Angelis, M., Gallo, G., Corbo, M. R., McSweeney, P. L., Faccia, M., Giovine, M., & Gobbetti, M. (2003). Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. *International journal of food microbiology*, 87(3), 259-270.
- Gustafsson, E. L., & Sandberg, A. S. (1995). Phytate reduction in brown beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, 60(1), 149-152.
- Herken, E. N., İbanoğlu, Ş., Öner, M. D., Bilgiçli, N., & Güzel, S. (2007). Effect of storage on the phytic acid content, total antioxidant capacity and organoleptic properties of macaroni enriched with cowpea flour. *Journal of Food Engineering*, 78(1), 366-372.
- Humer, E., Schwarz, C., & Schedle, K. (2015). Phytate in pig and poultry nutrition. *Journal of Animal Physiology and Animal Nutrition*, 99(4), 605-625.
- Kraler, M., Schedle, K., Domig, K. J., Heine, D., Michlmayr, H., & Kneifel, W. (2014). Effects of fermented and extruded wheat bran on total tract apparent digestibility of nutrients, minerals and energy in growing pigs. *Animal Feed Science and Technology*, 197, 121-129.
- Kumar, V., Sinha, A. K., Makkar, H. P., De Boeck, G., & Becker, K. (2012). Phytate and phytase in fish nutrition. *Journal of animal physiology and animal nutrition*, 96(3), 335-364.
- Larsson, M., & Sandberg, A. S. (1995). Malting of oats in a pilot-plant process. Effects of heat treatment, storage and soaking conditions on phytate reduction. *Journal of Cereal Science*, 21(1), 87-95.
- Prescott, L.M., Harley, J.P., Klein, D.A., 1996. Microbiology. WCB Publishers, p. 935.
- Ravindran, V., Cabahug, S., Ravindran, G., Selle, P. H., & Bryden, W. L. (2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *British Poultry Science*, 41(2), 193-200.
- Rimbach, G., Walter, A., Most, E., & Pallauf, J. (1998). Effect of microbial phytase on zinc bioavailability and cadmium and lead accumulation in growing rats. *Food and Chemical Toxicology*, 36(1), 7-12.
- Schlemmer U, Frolich W, Prieto RM, Grases F (2009) Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Mol Nutr Food Res* 53:S330–S375.
- Schlemmer, U., Jany, K. D., Berk, A., Schulz, E., & Rechkemmer, G. (2001). Degradation of phytate in the gut of pigs—pathway of gastrointestinal inositol phosphate hydrolysis and enzymes involved. *Archives of Animal Nutrition*, 55(4), 255-280.
- Steiner, T., Mosenthin, R., Zimmermann, B., Greiner, R., & Roth, S. (2007). Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Animal Feed Science and Technology*, 133(3-4), 320-334.

EFFECT OF FEED PROCESSING ON QUALITY: IMPLICATIONS ON RUMINANTS AND NON-RUMINANTS PERFORMANCE



S. R. Barsila*

Abstract: *Feed processing is especially done for altering the physical and chemical composition of feed for maximum utilization by animals and enhancing mixing and stability of diet. Feed processing generally enhances nutrient availability and improves digestibility and feed efficiency in animals when done properly. However, it also has some negative impact when processed food is not suitable for animals. Grain processing may influence digestibility rate as well as voluntary intake of forage. Thus processing methods should be efficient as well as feed should be given according to the physiological stage of animals. For the Lambs or calves below 150 kg, whole grains are often given and processing is not required; during dry period processed feed is often a choice because of the high production cost of a concentrate feed and fewer yields. For maintenance of animals with more roughage and less concentrated feed would value economically to the production output too. During the lactating period, medium-coarse ground grain would be better because very fine grinding grains can decrease the fat content in milk and also cause problems such as diarrhoea, laminitis. etc. For other ruminants such as sheep, goat feeding of whole grains is found to be better. Stem flaking with flakes of moderate size seems to be efficient in improving digestibility, energy efficiency and body weightage in cattle. A conventional diet based on corn and soybean meal fed to pigs and poultry is usually provided in a mash form and in most cases, processing other than grinding and mixing is not used. But in the case of poultry, lack of uniform particle size in mash form reduces feed efficiency because of increasing time and energy for chicks to select large particles. Small pellet diet may increase feed intake and weight gain. Optimum particle size for poultry lies between 600 to 900 ppm. Poultry can feed coarse particles as well due to the presence of gizzard but this is not in the case of the pig. Feeding pig diet with roller mill over hammer mill features a considerable advantage in increasing apparent digestibility of DM, N, GE while a decrease in faecal excretion of DM and N. Very fine particles also cause the incidence of stomach ulceration in the pig.*

Keywords: *digestibility, feed, feed processing, nutrients*

Introduction

The feed is a most significant factor in the production of animals. It represents 50% or more production cost in the ruminant though grass consumption rate is high on them and more than 70% production cost on the non-ruminant. Feed processing usually means altering the physical (and sometimes chemical) nature of feed commodities to optimize utilization of nutrients such as amino acid, protein, carbohydrate etc (Köster, 2007). Research studies have also declared that the processed feed significantly lowered rumen ammonia concentration to 50% and increase bacterial nitrogen concentration up to 50 % in comparison to unprocessed feed thus bacteria seems to synthesize more protein on

fermentable ration unchanging the protein quality (Meyer et.al., 1967). Feed processing enhances the digestibility in the broiler to optimize the absorption of major nutrients and also protecting from harmful pathogens maintain the carcass quality better. Feed processing and its cost is associated with receiving, grinding, proportioning, mixing, pelleting, load out and delivery (Behnke & Beyer, 2004). Feed processing simply includes the processing of grains. Grain processing method includes hot and cold processing methods. Popping, micronizing, steamrolling, steam flaking, extruding, pelleting, spraying feed with molasses fall under the hot processing whereas, dehulling,

IVSA MIRROR V

grinding, soaked grains and reconstitution methods are cold processing methods. But 25% of feed seems to be made from a byproduct of these grains rather than concentrated one (Ondarza, 2003).

Hot processing of grains includes the following methods:

1. **Popping:**

Popping is a simultaneous starch gelatinization and expansion process done by exposing to the high temperature of about 300-310 0F for 15-20 sec reducing moisture of 15-20%. In this process superheated vapours produced inside the grains cooks the grains and expands the endosperm suddenly, breaking the outer layer of the grains forming flower-like appearance (Mishra, 2014). Popping and puffing can be done by using dry heat such as sand roasting, roasting using salt, gun puffing, hot oil frying and using a heating medium such as hot air or microwave radiation (Jaybhaye et al., 2014).

2. **Micronizing:**

Infrared micronizing is a short time high-temperature thermal process of cooking of grains accomplished by use of the infrared wavelength of 1.8-3.4 microns which reduce moisture up to 7 % (Deepa & Hebber,2016)

3. **Steam Rolling:**

Grains are steam to 3-5 min before rolling which kills weed seed if present any in grains and may add moisture up to 6%in grains. Grains can be rolled under steel roller without the addition of moisture (dry rolling) but may create the issue of feed loss due to powder (Mathison, 2019).

4. **Steam flaking:**

Steam-rolled grain is usually steamed for 10-15 minutes to increase grain moisture to 12-14%. Then, it is rolled into a thick flake. In some studies, feeding low density (thin) flaked grains has resulted in lower production because of

problems with intake and acidosis (Ondarza, 2003).

5. **Extruding:**

It's the way where the feed is passed through a screw generating large amounts of 20-40 atmospheric pressure and temperature of 138 to 160 degree Celsius for about 30 sec. The feed is expanded and finally dehydrated causing gelatinization of the starch present (Ondarza, 2003).

6. **Roasting:**

It is the complete application of dry heat which may improve feed efficiency and starch degradation by allowing more starch to escape ruminal degradation to be digested in the small intestine. 3 kg increase in milk production when cow fed roasted barley in compared to unroasted (Deghhan-badanaky et.al., 2007).

Cold processing of grains includes the following methods:

1. **Dehulling:**

Removing outer coat of grain for further processing. eg soybean, oat etc. which is usually done by rotating of grains at 200 – 500 rpm for 1 min research study had proved that dehulling increase the feed value of the grain much better and also enhance the digestibility, solubility and palatability (Peltonen,2004).

2. **Grinding:**

Mostly done through a hammer mill, with aid of rotating metal screen. Size may vary from flattened products to finely grind products. Grinding, cracking, rolling, and crimping increase the surface area of the grains and thus microbes can act better on them which enhance the microbial digestion. (Trans et.al., 2011).

3. **Pelleting:**

Pelleting is obtained by using a thick die using a roller with or without stem application. Pelleting may increase starch degradation or through

gelatinization of starch granules (Deghhan-badanaky et.al., 2007).

4. Reconstitution:

It refers to the ensiling soaked grains in the oxygen-limited silo pit for 10-15 days before feeding. Reconstitution shows disorganization of protein matrix surrounding starch granules which improves gelatinization of protein (Hale,1973).

5. Chemical processing

Chemical Processing can be done by adding chemicals on feed such as NaOH, Ammonia/urea, and aldehyde. Application of NaOH increases whole tract digestibility destroying seed coat. Ammonia improves the rate and efficiency of feedlot cattle. Treatment with an aldehyde is expected to protect protein and starch in cereal grain in ruminal degradation (Deghhan-badanaky et.al., 2007).

According to (Ondarza, 2003), processing of feed increases the palatability of feed and also the toxin binder added during processing decreases the amount of toxin and preservative added in the feed to preserve feed, boosting its quality in the future. Grinding, pressure and heat improves the digestibility of nutrients and increases the feed efficiency by optimal utilization of the consumed feed by increasing the surface area where rumen microbes attach and exert their effect for better fermentation. Gelatinization converts the crystalline form of starch into the simpler form which facilitates the digestion of starch into the energy by the easier and simpler breakdown. Also, Sodium hydroxide used during processing disrupts the protein matrix to gelatinize the starch. (Huntington, 1994). Popping and puffing impart acceptable taste and desirable aroma to the product as well as enhance the starch digestibility in the rumen by increasing surface area (Hoke et al., 2005). Micronization of grains during feed processing built some beneficial changes like partial gelatinization of starch, inactivating the enzyme responsible for the quality hindrance of the feed

and denatured anti-nutritional factors from the grains. The partial gelatinization due to micronization improves starch digestibility and palatability (Deepa & Hebbler, 2016). Compared to the other feed processing, rolling decreases the palatability of the feed thus animal may refuse the feed and also it may increase acidosis as well as due to the moisture content its storage time is also comparatively less (Mathison,2019). Pelleting improves the gelatinization of starch granules through starch degradation and is very important in pig and poultry (Theurer, 1986). Very fine feed size causes stomach ulceration in pigs (Liu, 2013).

Effect of different feed processing on the ruminant animal

Grain processing may influence digestibility, rate and site of grain digestion as well as voluntary intake of forage. It has both useful as well as harmful effects when processing is not required, e.g. increase in rumen rate or breakdown of grain or acid production may depress forage intake. Processing is not required for calves below 150 kg of live weight and not for sheep and goats (Campling,1991). Proper processing of grain improves the efficiency of starch digestibility in the rumen in the ruminant animal by disrupting protein matrix during enzymatic process permitting easier enzymatic access to starch granules but there will be the difference between different processing method in digestibility of animal (Hale,1973). Though it also depends upon grain sources and processing method, excessive processing reduces ADG slightly which will reduce DMI intake. Reduce DMI of excessively processed grain formed an excessive rate of acid production in the rumen and cause subclinical acidosis. (Ownes et.al.,1997)

Hale, 1973 explained that utilization of sorghum grain in cattle could be improved by as much as 15% by flaking. Moist heat treatment such as cooking, flaking increase digestibility of nitrogen-free extract by 14% when compared to dry rolled steam grain and when grains are not

IVSA MIRROR V

flaked, it shows denaturation of the protein. However (Ownes et.al.,1997) described that steam flaking was effective in increasing body weight-adjusted ME of wheat but failed to increase ME of barley and oat. Compared to moderate thinness of flakes, very thin flakes tend to reduce ADG and failed to improve efficiency but feed to wt gain ratio was improved by extensive feed processing than dry rolling indicating an improvement in energy efficiency. Dehghan-Banadaky et.al.,2007 also added that finely ground barley grain ferment more rapidly than cracked barley grain which may reduce the productivity of cattle.

Reconstitution shows disorganization of protein matrix surrounding starch granules which improves gelatinization of protein. Flaking reduces acetate to propionate production in the rumen which improves the efficiency of feed utilization in rumen similarly grinding, soaking, crushing, rolling appear to improve palatability in the ruminant animal. Proper processing of grain improves the efficiency of starch digestibility in the rumen in a ruminant animal by disrupting protein matrix during the enzymatic process permitting easier enzymatic access to starch granules (Hale, 1973).

On-body weightage basis, compared to dry rolled, steamrolled large increase in body weight-adjusted ME of corn (15%), milo (21%) and wheat (13%). The energy efficiency of dry rolled was lower than whole shelled corn. ADG was lower in whole than dry rolled grain. Body weight-adjusted ME was higher ($p < 0.05$) for stem rolled than high moisture or whole-grain but body weight-adjusted me was higher in reconstitution than dry rolled. On DMI basis, DMI tends to be lower in wettest rolled grain, ADG to be numerically greater. For maximum efficiency, drier grain should be ground rather than rolled for maximum feed efficiency and utilization (Owens et.al.,1997). Roughages are included in the feed to enhance intake or performance. Ruminal or total tract digestibility is low in a high concentrate diet. With dairy

cattle, long roughages are provided in diet to enhance rumination or time of chewing to enhance the fermentation of acid in the rumen. Corn silage tends to be superior in roughages than dry rolled corn, dry rolled reconstitution milo (Ownes et.al.,1997). Rolled barley tends to form thickened rumen wall with clumped papillae. A medium mixture of barley and oat was slightly superior for milk production than coarsely ground material (Campling,1991). Dry rolling increase digestibility but grain kernel often shatter during processing which may produce may fine particles which cause inconsistent animal performances. Stem flaking use moisture and heat to gelatinize starch but the positive effect of gelatinization more for barley grain than corn or sorghum. Treatment of grain increase ruminal starch digestibility but roasting and aldehyde treatment decrease the rate of crude protein degradation and optimize organic matter degradation in the rumen (Dehghan-Banadaky et.al., 2007).

Effect of feed processing on pig and poultry

Pig and poultry both are monogastric animals and have fundamental differences between gut structure and function. For poultry due to presence of gizzard, there is influence in diet structure on gizzard function and nutrient availability. The gizzard is capable of reducing coarse particle to a certain critical size less than 0.1mm. Optimum particle size for poultry lies between 600 to 900 ppm. However, there is a considerable difference in particle size and feed form. Lack of uniform particle size in mash form reduces feed efficiency because of increasing time and energy for chicks to select large particles. Small pellet diet may increase feed intake and weight gain (Liu,2013).

Poultry feeding diet in pellet form have always been found to improve weight gain and feed efficiency in broilers. Chicken preferred to feed large feed particles which are observed in all ages. Studies also observe that when chicken

IVSA MIRROR V

feed mash diet, feed intake increases in coarser particle than a finer particle, there is also no deleterious effects on feed efficiency (Amerah et al.,2007).

Broiler chicken when fed with a pellet feed of barley with enzyme β -glucanase in food when conditioned with 75°C for 30 sec reduces glucanase activity 66%in compared to controlled mash whereas 15 min conditioning reduces recovery in compared to 30min (Amerah et al.,2011). Incidence of vent pasting reduces comparatively with increased enzyme inclusion rate and was significantly higher when conditioning for 15 min than 30 sec (Inborr and Bedford, 1994).Particle size in pig and poultry both depends upon grain type, feeding system and age of animals. Particle size lower than 400 ppm increases the incidence of gastric ulceration in the pig. Finely ground sorghum reduces starch and protein digestibility coefficient in the distal ileum and changes the balance of digestion in growing and finishing pig. Feeding pig diet with roller mill over hammer mill has a considerable advantage in increasing apparent digestibility of DM, N, GE while a decrease in faecal excretion of DM and N (Liu,2013).

Conclusions

The feed is an important factor for the overall development of the livestock sector. Thus it needed to be processed properly to make it to the form which is highly digestible and palatable to animals and to improve feed efficiency in animals. The feed should be provided according to the digestibility of animals but there is a lack of knowledge about the best feeding method and ideal particle size that provides the best utilization of energy and nutrients. Newer grinding technologies include multistage grinding and stacked rollers which provide more consistent particles should be made popular among farmers. More advancement in the feeding sector, research about it is necessary for the all-round growth of the livestock sector.

References

- Amerah, A. M., Gilbert, C., Simmins, P. H., & Ravindran, V. (2011). Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *World's Poultry Science Journal*, 67(1), 29-46.
- Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007). Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal*, 63(3), 439-455.
- Behnke, K. C., & Beyer, R. S. (2004). Effect of feed processing on broiler performance (Doctoral dissertation, Dissertation, Kansas State University, Manhattan. <http://www.veterinaria.uchile.cl/publicacion/VIIIpatologia/SEMINARIOS/semi2.pdf>).
- Campling, R.C. (1991). Processing cereal grains for cattle—a review. *Livestock Production Science*, 28(3), 223-234.
- Dehghan-Banadaky, M., Corbett, R., & Oba, M. (2007). Effects of barley grain processing on productivity of cattle. *Animal Feed Science and Technology*, 137(1-2),
- Deepa, C., & Hebbar, H.U.(2016). Effect of high-temperature short-Time 'Micronization' of grains on product quality and cooking characteristics. *Food engineering reviews*, 8(2), 201-213.
- Hale, W. H. (1973). Influence of processing on the utilization of grains (starch) by ruminants. *Journal of Animal Science*, 37(4), 1075-1080
- Gayatri Mishraa, D. J. (2014). Popping and Puffing of Cereal Grains: A Review. *Journal of Grain Processing and Storage*, 34-46.
- Huntington, G.B. 1994. Ruminant starch utilization progress has been extensive. *Feedstuffs*. June 6, 1994, p16.
- Hoke K, Houska M, Jirina P, Gabrovska D, Vaculova K and Paulickova I (2007). Optimization of puffing naked barley. *Journal of Food Engineering*, 80, 1016-1022.
- Inborr, J., & Bedford, M. R. (1994). Stability of feed enzymes to steam pelleting during feed processing. *Animal Feed Science and Technology*, 46(3-4), 179-196.
- Jaybhaye RV, Pardeshi IL, Vengaiyah PC and Srivastav PP (2014). Processing and technology for millet based food products: a review. *Journal of Ready to Eat Food*, 1(2), 32-48.
- Köster, D. H. (2007, 6 8). engormix. Retrieved July 25, 2019, from <https://en.engormix.com/feed-machinery/articles/animal-feed-processing-method-t33748.html>:<https://en.engormix.com/feed-machinery/articles/animal-feed-processing-method-t33748.html>.
- Liu, S. Y., Selle, P. H., & Cowieson, A. J. (2013). Strategies to enhance the performance of pigs and poultry on sorghum-based diets. *Animal*

IVSA MIRROR V

Feed Science and Technology, 181(1-4), 1-14.

- Meyer, R. M., Bartley, E. E., Deyoe, C. W., & Colenbrander, V. F. (1967). Feed processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. *Journal of Dairy Science*, 50(8), 1327-1332.
- Mathison, G. (n.d.). Agromedia. Retrieved July 25, 2019, from <http://www.agromedia.ca/AFMG/Section%201%20Nutrition%20&%20Management/1B1%20Processing%20Feed%20Grains.pdf>.
- Ondarza, M. B. (2003, 3 2). De nivel. Retrieved July 25, 2019, from [www.milkproduction.com:http://www.milkproduction.com/Library/Scientific-articles/Nutrition/Grain-processing/](http://www.milkproduction.com/Library/Scientific-articles/Nutrition/Grain-processing/).
- Peltonen-Sainio, P., Kontturi, M., & Rajala, A. (2004). Impact dehulling oat grain to improve quality of on-farm produced feed: 1. Hullability and associated changes in nutritive value and energy content. *Agricultural and Food Science*, 13(1-2), 18-28.
- Tran, T. T., Shelat, K. J., Tang, D., Li, E., Gilbert, R. G., & Hasjim, J. (2011). Milling of rice grains. The degradation on three structural levels of starch in rice flour can be independently controlled during grinding. *Journal of Agricultural and Food Chemistry*, 59(8), 3964-3973.
- Theurer, C. B. (1986). Grain processing effects on starch utilization by ruminants. *Journal of Animal Science*, 63(5), 1649-1662.
- Owens, F. N., Secrist, D. S., Hill, W. J., & Gill, D. R. (1997). The effect of grain source and grain processing on performance of feedlot cattle: A review. *Journal of Animal Science*, 75(3), 18-25

KETOSIS IN DAIRY CATTLE: A REVIEW

P. Shrestha*, R. Pande



Abstract: *Ketosis is an abnormal rise of the ketone bodies which includes acetone, aceto-acetic acid, and β -hydroxy butyric acid in the body. It is the metabolic disorder characterized by ketonemia, ketonuria, and hypoglycemia. The clinical signs in cattle are a rapid loss of body weight, lack of appetite, dry feces, and rapid decrease in milk production and, in some cases, nervous disturbances. The diagnosis is based on animal history, clinical signs, and laboratory findings. Treatment is generally done by glucose replacement therapy or propylene glycol along with glucose. The long-acting glucocorticoid and insulin will keep the glucose level for a long period. It can be prevented by feeding a high quality diet according to need of cattle.*

Keywords: glucose, hypoglycemia, ketone bodies, ketonemia

Introduction

Ketosis is a metabolic disease which occurs when energy demand exceeds the energy intake and results in a negative energy balance in high milk-producing cattle. The result is high concentrations of circulating non-esterified fatty acids (NEFAs) such as acetone, acetoacetate, and β -hydroxybutyrate (BHB) (Morwal & Sharma, 2019). It is a relative or absolute lack of carbohydrate in hepatic cells leading to β -oxidation of fats which leads in the production of ketone bodies and acidosis. It typically occurs in dairy cows in early lactation and is characterized by partial anorexia, depression, ketonemia, ketonuria and hypoglycemia with poor liver glucose content (Chakrabarti, 2014). The condition is worldwide in distribution but is most common in dairy cows which are bred and managed for high production (Kahn, 2016). Most recorded form of ketosis is the wasting and subclinical form of ketosis (McArt et al., 2012). According to Wootton (1992), very little information was available on the nervous form of ketosis.

Aetio-Pathogenesis

Negative energy balance is the main cause of ketosis which occurs shortly after calving in high producing dairy cows. Nervous ketosis appears

to be the same clinical disease as primary ketosis except for the addition of abnormal behavior, such as circling movement, walking blind and abnormal licking (Foster, 1988).

The rumen and liver are two important sites of ketone body formation in the ruminant. In primary ketosis, however, it appears that the liver is the site of the formation of the increased production of ketone bodies. Other tissues form ketones but utilize them at a faster rate than they are formed (Eview, 1955). If sufficient Coenzyme A (CoA) is present then fatty acids can be converted to glucose. In its deficiency, fatty acids are converted into ketones. Carbohydrate is converted into acetic acid, butyric acid, and propionic acid (Eview, 1955). Acetic acid and butyric acid combine to form activated acetate. Activated acetate maybe 1) condensed to fat if glucose metabolism is not impaired. 2) In the normal condition, the other part is utilized for energy via the tricarboxylic acid cycle in the presence of oxaloacetate. 3) Due to the relative absence of oxaloacetate activated acetate is converted to aceto-acetic acid and β -hydroxy butyric acid i.e. ketone bodies (Chakrabarti, 2014).

IVSA MIRROR V

The feed intake does not meet the energy demands of the cattle which causes the insufficient production of propionic acid. Propionic acid is converted to oxaloacetate which turns to glucose and finally, it is converted to lactose (Chakrabarti, 2014). Due to insufficient production of glucose, hypoglycemia i.e. low blood glucose may occur. Low blood sugar results in metabolization of fatty acid and glycerol, oxidized to acetyl CoA. Excess acetyl CoA is converted to ketone bodies which the liver cannot cope with. The excess ketone body is accumulated and excreted in milk and urine.

If non-esterified fatty acid (NEFA) takes oxidation pathways, clinical ketosis is developed. On the other hand, if it undergoes an oxidation esterification pathway fatty liver condition is seen. These conditions depend on glucose availability (Chakrabarti, 2014).

Clinical Signs

Vital signs are within normal range but in last stage of ketosis there is subnormal temperature, loss of appetite, sharp decrease in milk production, feces become dry and covered with mucus, ruminal movement decrease in amplitude and rate, the cow may “Star-gaze”, and characteristic odor of ketone bodies detectable on the breath and in milk (Chakrabarti, 2014). Delirium, circling movement, head pushing, aimless wandering, profuse salivation hyperaesthesia, moderate tremor, and tetany may present (Reddy et al., 2014). Subclinical ketosis where urine and blood contain ketone bodies in excess amounts but there is no obvious sign of ketosis. Milk fever-type, where hypocalcemia accompanies hypoglycemia (Chakrabarti, 2014). This form responds to the treatment with calcium and glucose therapy.

Impact

The studies have reported milk yield losses in ketotic vs. non-ketotic cows of 2.2 to 3.1 lbs of daily milk (4.4 to 6.6%); a milk ketone test was used to diagnose ketosis (Dohoo & Martin, 1984). The negative impact of ketosis in affected

cows is substantial and includes lost milk yield, increased risk for herd removal in early lactation, impaired fertility, and increased risk for displaced abomasum (McArt et al., 2012). The effect of Subclinical ketosis on metritis is difficult to evaluate because both conditions occur at the same time. The total economic loss for a single case of subclinical ketosis is between about \$46 and \$92 (McArt et al., 2012).

Clinical Pathology

In ketosis, acetoacetic, β -hydroxy butyric acid, and free fatty acid level in blood may increase up to 7mg/100ml, 30mg/100ml, and 28mg/100ml respectively. The normal blood glucose level of cattle is reduced (below 40mg/100ml). Normally ketone bodies are not excreted through milk and urine but in ketosis, it may go as high as 40mg/100ml in milk and up to 500-1000mg/100ml in urine (Chakrabarti, 2014). The most conclusive diagnostic test for clinical ketosis is an analysis of urine or milk for ketones (Schultz, 1971). It has been reported that the ketone body (acetone and acetoacetate) concentration in milk is a more reliable sign of clinical ketosis than the ketone body concentration of urine (Schultz, 1971). Serum calcium and magnesium level are below 9-11 mg% and 2-2.5 mg% in ketosis. Hematological changes include eosinophilia, lymphocytosis, and neutropenia (Chakrabarti, 2014).

The diagnosis is based on animal history, clinical signs, and biochemical tests (i.e. Rothera's test), such as detection tests for ketone bodies in urine and milk. Blood test for the detection of blood glucose level and serum test for calcium and phosphorus level is also conducted (Morwal & Sharma, 2019). Subclinical ketosis can be detected by the estimation of β -hydroxybutyric acid in milk. The value greater than 1-2mg/dl will indicate subclinical ketosis which may turn into clinical ketosis (Chakrabarti, 2014). The liver of a cow that died with ketosis was yellow and enlarged and exhibited fatty degeneration (Eview, 1955).

Line of Treatment

Primary ketosis can be differentiated from secondary ketosis by clinical examination, which can be treated by changing the feeding program and administration of glucogenic substances. By the correction of primary disease, secondary ketosis will be managed (Geishauser et al., 2001).

Glucose replacement therapy remains one of the most popular treatments for ketosis (Foster, 1988). Generally, 500-800 c.c. of a 40-50% solution of glucose (Dextrose solution) should be given intravenously at once (Chakrabarti, 2014). Recovery is common after glucose therapy, and up to 40 percent has been reported (Shaw, 1956) but even a 60 percent cure is observed (Foster, 1988). Glucose by intravenous injection of 500 ml of a 50% solution causes a transient hyperglycemia. It releases insulin which interrupts fat mobilization which suppresses both hepatic and mammary ketogenesis for about 5 hours (Kronfeld, 1972).

Propylene glycol is given orally by a stomach tube. As much as 125 to 250gm (4 to 8 ounces) can be given up to two times a day until clinical signs disappear but also reported that doses greater than 250gm can decrease digestion and induce diarrhea (Kronfeld, 1972).

Glucocorticoids reduce ketone body formation by utilization of acetyl-CoA and raising blood glucose level by making greater availability of glucose precursor in the liver (Chakrabarti, 2014). The published research examining the effects of glucocorticoids involves either intramuscular or subcutaneous injections, whereas in clinical practice intravenous administration of glucocorticoids is popular (Foster, 1988). Chronic administration of glucocorticoids can depress the immune system. Single dose of glucocorticoids in the recommended amount is better than several smaller doses for the treatment of ketosis (Gessert, 1955). Glucocorticoid recommendation for treatment of ketosis is 10 mg of

dexamethasone (Foster, 1988). Insulin has been used to facilitate transport of glucose into cells. Insulin is a powerful antiketogenic and suppressor of NEFA mobilization. Generally 200 I.U. protamine zinc is used for large animals. Sometimes 200 I.U. of insulin and 10 mg of dexamethasone (glucocorticoids) can be administered simultaneously. Vitamin B12 and cobalt are sometime administered either as a sole treatment or in conjunction with more standard therapy (Chakrabarti, 2014).

Prevention

Ketosis can be prevented by feeding cows according to nutritional requirements and by genetic selection (Geishauser et al., 2001). Clinical and subclinical ketosis largely owes their origin to negative energy balance during the first eight week of lactation. Prevention of peak lactation ketosis can be achieved through maximizing energy intake and providing sufficient glucose precursor. Oral administration of 2.5mg/d of ionophore compounds such as monensin hydrochloride 3 weeks prepartum helps to increase propionate level and decreases serum NEFA level (Chakrabarti, 2014). Vitamin A, Vitamin B12, niacin, copper, cobalt and phosphorus should be included in the diet. The routine examination of milk and urine for the presence of ketone bodies should be encouraged during the 50-60 days of pregnancy.

Conclusions

The dairy management program that is adequate for the problems of an average producing herd might not be sufficient to handle the problems of a high producing herd. During peak lactation period, high yielding dairy cows enter a stage of negative energy balance to monitor the appropriate glucose level and thus, preventive measures should be followed. Ketotic cattle should be treated properly according to history, clinical signs and laboratory examinations.

References

- Chakrabarti, A. (2014). Text Book Of Clinical Veterinary Medicine (fourth). Kalyani.
- Dohoo, I. R., & Martin, S. W. (1984). Subclinical ketosis: Prevalence and associations with production and disease. *Canadian Journal of Comparative Medicine*, 48(1), 1–5.
- Eview, A. (1955). Ketosis in Dairy Cattle. A Review. *Journal of Dairy Science*, 39(4), 402–434. [https://doi.org/10.3168/jds.S0022-0302\(56\)94765-8](https://doi.org/10.3168/jds.S0022-0302(56)94765-8)
- Foster, L. A. (1988). Clinical Ketosis. *Veterinary Clinics of North America: Food Animal Practice*, 4(2), 253–267. [https://doi.org/10.1016/S0749-0720\(15\)31047-1](https://doi.org/10.1016/S0749-0720(15)31047-1)
- Geishauser, T., Leslie, K., & Kelton, D. (2001). Monitoring for Subclinical Ketosis in Dairy Herds. *23(8)*, 65–71.
- Gessert RA, Shaw JC, Chung AC: Studies on ketosis in dairy cattle: XVII. The value of hydrocortisone therapy. *J Am Vet Med Assoc* 127:215-218, 195.
- Kahn, C. M. (2016). *The Merck Veterinary Manual* (Eleventh). Merck & Co., Inc., Kenilworth, NJ.
- Kronfeld, D.S. (1972). Ketosis in pregnant sheep and lactating cows, A review. *Australian Veterinary Journal*, 48, 680-687.
- McArt, J. A. A., Nydam, D. V., & Oetzel, G. R. (2012). Epidemiology of subclinical ketosis in early lactation dairy cattle. *Journal of Dairy Science*, 95(9), 5056–5066. <https://doi.org/10.3168/jds.2012-5443>
- Morwal, S., & Sharma, S. (2019). Clinico-therapeutic management of ketosis in cow- a field case. *8(5)*, 179–180. <https://doi.org/10.15406/jdvar.2019.08.00264>
- Reddy, B. S., Reddy, B. S. S., Reddy, Y. V. P., & Vennkatasivakumar, R. (2014). Nervous form of ketosis in cows and its treatment. July 2019, 2–4. <https://doi.org/10.14419/ijbr.v2i2.3591>
- Schultz, L.H. (1971). Management and Nutritional Aspects of Ketosis. *Journal of dairy Science*, 54, 962-973.
- Wootton P (1992) Nervous ketosis, *Canadian Veterinary Journal*, Mar, 33(3): 194.



MASTITIS IN DAIRY COWS: BEHAVIORAL CHANGES, TREATMENT AND CONTROL

R. A. Gul^{1*}, H. Jawad¹, N. Iqbal², M. A. Nawaz²

Abstract: Mastitis is one of the most important problems of the dairy sector world-wide. The disease is one of the major causes of economic loss among the dairy production sector. This review article will indicate clinical mastitis and subclinical mastitis, behavioral changes, treatment and total losses caused by mastitis. We will discuss why sub-clinical mastitis is more important in terms of economy. Bacteria causing mastitis, the factors involved for the condition of the mastitis and pathogenesis of mastitis will be discussed in this article. The changes in behavior of cows due to mastitis are also important for identification of the condition. Proper identification of sub-clinical mastitis in herds is very important for timely treatment of the diseased cattle and minimization of economic loss.

Keywords: bacteria, behavioral changes, clinical mastitis, dairy, subclinical mastitis

Introduction

Mastitis is the most common and costly problem of dairy animals. Field surveys of major livestock problems and diseases in Pakistan have ranked mastitis as the number one pathological condition of dairy animals (Khan & Khan, 2006). Research shows that in Nili Ravi Buffalo mastitis can decrease lactation period length by 57 day and milk yield by about 438 kg per lactation (Cady et al., 1983). The total losses caused by clinical mastitis amount to Rs. 240 million per year (Chaudhry and Khan, 1978). Losses occur with discarding of milk, decrease milk yield, culling of animals and replacements. The losses caused by clinical mastitis do not take into account those caused by sub-clinical mastitis (less obvious) and may only be detectable by measuring the milk's somatic cell counts (SCC). Subclinical mastitis is important due to the reason that it is 15 to 40 times more prevalent than the clinical form of mastitis, and it also has longer duration, detection is difficult, affects milk quality and production and constitutes a reservoir of microorganisms.

Pathogenesis

Teat canal is surrounded by a smooth muscle layer which forms a sphincter at the opening of a teat. Entry of bacteria leads to mastitis (Murphy et al., 1989). Keratin layer damage (a layer from inside of teat formed by stratified squamous epithelia) also leads to mastitis as well (Bramley and Dodd, 1984). Bacteria can enter during milking or when there is damage to this layer. After milking, teat remains open for 1-2 hours which is another reason that can cause the entry of bacteria and leads to mastitis (Khan and Khan, 2006). To how much extent inflammatory response would be severe depends on host as well as inflammatory factor.

Table 1: Severity scoring system for mastitis

S. No.	Score	Description
1	Mild	Abnormal milk (e.g. clots, flakes and watery)
2	Moderate	Abnormal milk and signs of udder inflammation (e.g. heat, swelling and pain)
3	Severe	Systemic illness (e.g. fever, dehydration, weakness and in-appetence)

Source: Royster & Wagner, 2015

¹ The Islamia University of Bahawalpur, Punjab, Pakistan

² University of Veterinary and Animal Sciences, Lahore, Pakistan

*Corresponding Author: rriaz3032@gmail.co

Impact of mastitis on behavior

As we all know the first thing which owner notices is behavioral change in the animal. Animals’ pain threshold and physiological and behavioral responses to different pathological conditions and pain can vary depending on how severely it affects animals’ physiological and behavioral functioning (Kemp et al., 2008). When pathogens enter into the body, it increases the body temperature to increase the response of immune system (Johnson, 2002). Then, energy will be diverged from behavioral activities such as feeding, social contact or grooming (Johnson, 2002; Dantzer & Kelly, 2007), and ill animals might have a different set of behavioral priorities, leading to changes in the social and environmental needs of the individuals. So, we can say knowledge of sickness behavior is an important foundation for future design of housing systems for sick dairy cows. It is also reported that mastitis can leads to sickness behavior of animal like animal would be with less rumination, less feed intake, and with minimum resting attitude (Gonzalez et. al., 2008; Siivonen et. al., 2011).

When cows were diagnosed with mastitis they showed classic sign of sick behaviour and these were also noticed by Siivonen et al., 2011; Fogsgaard et al., 2012 & Yeiser et al., 2012 in experimentally induced clinical mastitis..

Mastitis causing bacteria

The most probable causative organisms of mastitis in buffaloes have been reported to be *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli*, *Pseudomonas spp.*, *Corynebacterium spp.*, *Mycoplasma spp.*, *Streptococcus dysgalactiae* and *Mycobacterium tuberculosis* (Khan and Khan, 2006). In Pakistan, etiological agents of mastitis in buffaloes have been reported to be mostly *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Staphylococcus capotus*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus*

pyogenes and *Corynebacterium bovis* (Khan & Khan, 2006).

Treatment

We cannot eradicate mastitis but we can decrease their chance of occurrence. This can be done by good hygienic practice,

Table 2: Effect on milk composition

Constituent	Normal milk	Mastitis milk with high SSC
Fat	3.5	3.2
Lactose	4.9	4.4
Total protein	3.61	3.56
Total casein	2.8	2.3
Whey protein	0.8	1.3 
Serum albumin	0.02	0.07 
Lactoferrin	0.02	0.1 
Immunoglobulin	0.1	0.60 
Sodium	0.057	0.105 
Chloride	0.091	0.147 
Source: Jones (2006)		

teat dipping, dry period management and culling of infected animals. Dry period management has better efficiency for mastitis control than management during lactating period. For the inspection of animals and treatment of infected one, somatic cell count is of much importance. We can control mastitis either by decreasing the exposure of animals from pathogens or increasing the immune response of animals. Dry period management is very important because it can decrease up to seventy percent of streptococcal infection. The animal can be treated with antibiotics like enrofloxacin, NSAIDs like Meloxicam and antihistamine drugs like chlorpheniramine maleate. We can also give supportive therapy (Khan & Khan, 2006).

In a case study, it is also reported that the animal was treated with Inj. Enrocin , Inj. Melonex , Inj. Anistamin, Inj. DNS 5%, Agrimin forte and mineral mixture. The owner was also advised to

IVSA MIRROR V

perform complete frequent milking every 4-5 hours.

On the sixth day, it was completely recovered with complete disappearance of clinical signs; the milk was clear, free from clots or flakes, and white in color (Sharma, Gupta, Sharma & Hussain, 2007).

Treatment of Mastitis during Lactation

Therapeutic response of the cows can be checked by victimization individual somatic cell count, California Mastitis Test (CMT), and bacteriological samples in herds with contagious mastitis. Treating subclinical udder inflammation with antimicrobials is mostly not economical throughout lactation as a result of the price of treatment is high and has poor effectiveness (Pyörälä, 2009).

Controlling Contagious Mastitis

Staphylococcus aureus infections are the main cause of mastitis of farm animals. With antibiotic therapy during lactation, the cure rate is incredibly low and plenty of infected animals become chronic cases and have to be culled. *Strep. agalactiae* respond well to antibiotic therapy and may be eradicated from the farm with sensible mastitis management practices such as teat dipping and dry animal treatment. *Strep. dysgalactiae* can be found in almost all places. It will board the mammary gland, rumen, and feces, and within the barn and may solely be controlled with correct sanitation and management procedures (Khan and Khan, 2006).

Controlling environmental mastitis

It is often achieved by decreasing the amount of microorganism to which the teat end is exposed. The animal's surrounding needs to be clean and dry. The animals need to have minimum to no exposure to manure, mud, or pools of stagnant water. Calving place must be clean. Teat dipping with an antiseptic solution is good practice after milking. Using germicidal attempts to control mastitis during dry periods have been

unsuccessful. In order to control mastitis, we have to follow proper dry period management procedures and when an animal is going to dry off we have to follow devised antibiotic therapy. (Khan & Khan, 2006).

Discussion and Conclusions

Mastitis is an important and prevalent condition in large and small animals. Dairy industry is affected by this condition and economic losses occur due to it. But good farm management and proper dry cow therapy keep cows away from mastitis. Behavior changes help recognizing illness in animals. Behavioral changes can be used as indicator of illness in early lactation cows. Monitoring of somatic cell counts, prompt identification and treatment of mastitis in dairy animals helps in the reduction of mastitis. Dry animal therapy can eliminate 70% of environmental streptococcal infections. *Streptococcus agalactiae* respond well to antibiotic therapy and can be eradicated from dairy herds with good mastitis control practices which include teat dipping and dry animal treatment.

The present results can be used as a basis for further studies on motivational changes, preferences, and behavioral needs of diseased dairy cows, and thereby facilitate improved management of sick animals within the dairy industry.

References

- Bramley, A. J., & Dodd, F. H. (1984). Reviews of the progress of dairy science: mastitis control—progress and prospects. *Journal of Dairy Research*, 51(3), 481-512.
- Cady, R. A., Shah, S. K., Schermerhorn, E. C., & McDowell, R. E. (1983). Factors affecting performance of Nili-Ravi buffaloes in Pakistan. *Journal of Dairy Science*, 66(3), 578-586.
- Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, behavior, and immunity*, 21(2), 153-160.
- Fogsgaard, K. K., Røntved, C. M., Sørensen, P., & Herskin, M. S. (2012). Sickness behavior in dairy cows during *Escherichia coli* mastitis. *Journal of dairy science*, 95(2), 630-638.

IVSA MIRROR V

- González, L. A., Tolkamp, B. J., Coffey, M. P., Ferret, A., & Kyriazakis, I. (2008). Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *Journal of dairy science*, 91(3), 1017-1028.
- Johnson, R. W. (2002). The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary immunology and immunopathology*, 87(3-4), 443-450.
- Jones, G. M., & Bailey, T. L. (2006). Understanding the basics of mastitis. Virginia Cooperative Extension, Publication No. 404-233. *Virginia State University*, 1-7.
- Kemp, M. H., Nolan, A. M., Cripps, P. J., & Fitzpatrick, J. L. (2008). Animal-based measurements of the severity of mastitis in dairy cows. *Veterinary Record*, 163(6), 175-179.
- Khan, M. Z., & Khan, A. (2006). Basic facts of mastitis in dairy animals: a review. *Pakistan veterinary journal*, 26(4), 204.
- Murphy, S. C., Cranker, K., Senyk, G. F., Barbano, D. M., Saeman, A. I., & Galton, D. M. (1989). Influence of bovine mastitis on lipolysis and proteolysis in milk. *Journal of dairy Science*, 72(3), 620-626.
- Pyörälä, S. (2009). Treatment of mastitis during lactation. *Irish Veterinary Journal*, 62(4), 1-5.
- Royster, E., & Wagner, S. (2015). Treatment of mastitis in cattle. *Veterinary Clinics: Food Animal Practice*, 31(1), 17-46.
- Sharma, N., Gupta, S., Sharma, U., & Hussain, K. (2007). Treatment of clinical mastitis in a buffaloes: a case report. *Buffalo Bulletin* 26, 5-6.
- Siivonen, J., Taponen, S., Hovinen, M., Pastell, M., Lensink, B. J., Pyörälä, S., & Hänninen, L. (2011). Impact of acute clinical mastitis on cow behaviour. *Applied Animal Behaviour Science*, 132(3-4), 101-106.
- Yeiser, E. E., Leslie, K. E., McGilliard, M. L., & Petersson-Wolfe, C. S. (2012). The effects of experimentally induced *Escherichia coli* mastitis and flunixin meglumine administration on activity measures, feed intake, and milk parameters. *Journal of dairy science*, 95(9), 4939-4949.
- Chaudhry, N. A. and B. B. Khan, 1978. Estimation of Economic Losses due to Animal Diseases in Punjab. Final Report of Research Project, Univ. Agri., Faisalabad, Pakistan.



MEDROXYPROGESTERONE ACETATE INDUCED PROLONGED GESTATION IN GERMAN SHEPHERD: A CASE STUDY

P. Subedi^{1*}, N. Shrestha²

Abstract: *A thirteen-month old German shepherd was brought to Valley Animal Clinic with history of anorexia and distended abdomen. There was history of medroxyprogesterone acetate (MPA) administration three and half months before and the dog was not involved in any mating since that time. Upon radiological examination, there was presence of fetuses inside the uterus which revealed that mating had already taken place before the administration of MPA. As the gestation length had exceeded beyond its length with no signs of parturition, surgical interference was opted. Upon incising the uterus, it was found that all the fetuses were dead and thus, ovariohysterectomy was performed. Medroxyprogesterone acetate is synthetic progesterone, widely used by humans to control unwanted birth but its extensive use in canines has brought into them various significant complications like pyometra, gametotoxicity and several other disorders. Drugs to abort or prevent pregnancy in bitch and other animals must be based on the safety, efficacy, convenience, compliance in treatment, and cost of the drug. However, ovariohysterectomy is always the best option for those owners who don't want their bitch with unwanted pregnancies.*

Keywords: *depo-provera, ovariohysterectomy, progesterone, prolonged gestation, pyometra*

Introduction

The reproductive endocrinology of bitch is unique among all other animals. They are non-seasonally monoestrous, polytocous having four major phases of estrus cycle viz. proestrus, estrus, diestrus, and anestrus; out of which anestrus is the longest followed by diestrus and estrus being receptive for mating and having two months of the gestation period (Patrick W. Concannon, 2011; Johnston, Kustritz, & Olson, 2001). The estrus cycle of bitch is mainly regulated by progesterone, estrogen, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Johnston et al., 2001). Some indicates that there is no any significant difference between pregnancy and a non-pregnant cycle of bitch, the luteal phases for both last approximately 2 months, with pregnancy having a shorter duration of plasma progesterone elevation and ending with a more abrupt decline of progesterone than the non-pregnant cycle (Chakraborty, 1987). Continuous availability of

progesterone > 2mg/dl should be available throughout the pregnancy which ensures the differentiation of the endometrium and endometrial gland secretion and maintenance of endometrial integrity. Attachment of the placenta also suppresses uterine contractility, particularly by preventing the estrogenic activity of estrogens (Verstegen-Onclin & Verstegen, 2008). As a result of rapid decline in progesterone concentration to <2ng/ml in 12-24 hr interval and increase in prostaglandin, fetal membrane causes pre-partum luteolysis in bitch which initiates normal parturition 64-66 days post-mating (P. W. Concannon, Isaman, Frank, Michel, & Currie, 1988). Failure of luteolysis causes prolonged gestation in bitch and there are various factors responsible for prolonged gestation in bitch, some of them include size of fetuses, age of bitch, cystic corpus luteum, pyometra, fetal death, infectious agents, chromosomal abnormalities, loss of primary

¹Agriculture and Forestry University, Chitwan, Nepal

²Valley Animal Clinic, Koteshwor, Nepal

*Corresponding author: pramila8388@gmail.com

IVSA MIRROR V

uterine inertia and exogenous progesterone implants (Irons, Nöthling, & Volkmann, 1997; Johnston et al., 2001; Noakes, Parkinson, & England, 2019; van der Weijden & Taverne, 1994).

Case Description

History and Clinical Observation

A thirteen-month old German shepherd named Annie was presented to the Valley Animal Clinic, Koteshwor on 9th July, 2020. The bitch was presented with anorexia since four days with slight vaginal mucous discharge and distended abdomen. Owner revealed the history of depo-provera (Medroxyprogesterone Acetate, MAP) injection three and half months before and no activity of mating since. There was no change in vitals of the bitch upon physical examination.

Diagnosis and Treatment

X-ray imaging of bitch revealed fetuses inside the uterus. The case was diagnosed as prolonged gestation on the basis of history of medroxyprogesterone acetate administration and X-ray. The fetuses were assumed to be dead as there was prolonged gestation. Surgical correction (ovariohysterectomy) was done in order to remove the inflamed and distended uterus. Uterus after OVH was immediately incised in order to check for any live fetus was found to be none. The dog was kept in oral antibiotics and NSAIDS for five days to prevent secondary bacterial infection and was fully recovered in a week.

Discussion

The case was presented to Valley Animal Clinic when the owner noticed the distension of the abdomen. The bitch was injected with medroxyprogesterone acetate (depo-provera) to avoid unwanted pregnancy without considering its negative effects on the animal. Medroxyprogesterone Acetate (MPA), synthetic progesterone, has been used to prevent or

suppress estrus through its effect on the Graafian follicles and to suppress false pregnancy through its effect upon the corpora lutea. It suppresses the secretion of FSH, LH and arrests the development of Graafian follicles and corpora lutea within the ovary (Withers & Whitney, 1967). MPA is widely used in human to avoid pregnancy for three months if injected for one time (Mauck et al., 1999). Obliging this fact, it is thought that the depo shots act for a minimum of around two and half months and maintain progesterone level enough to suppress the estrogenic activity and prolong pregnancy in bitch (Bryan, 1973; Jordan, 1994; Withers & Whitney, 1967). The injection is used in canine in some countries during pro-estrus and anestrus to suppress the ovarian function and acts as contraceptive. However, it has been denied in most of the countries due to increased complications like pyometra, acromegaly, diabetes and increased proliferation of mammary tissue causing mammary hyperplasia leading to nodules formation or even mammary cancer (Concannon, 2011). The same was the case with the patient; there was an increased level of progesterone which denied normal parturition and hence complication of prolonged gestation occurred. The owner also complained about post-operative swollen mammary gland which may be due to sudden release of progesterone from blood as discussed above. The same type of case was presented in 2006 where there was prolonged gestation and maceration of the fetus due to inappropriate use of MPA in bitch (González-Domínguez & Maldonado-Estrada, 2006).

Apart from prolonged gestation and mammary gland hyperplasia; progesterone induced pyometra, gametotoxicity, carcinogenicity, reproductive toxicity, atrophy of adrenal gland, pancreas and liver, hyperplasia of gall bladders, uterine cystic hyperplasia and severe uterine lesions are also seen in MPA treated dogs (Eilts, 2002; Fransson & Ragle, 2003; Jordan, 1994; Nelson & Kelly, 1976; Selman, Van Garderen,

Mol, & Van Den Ingh, 1995; Smith, 2006; Von Berky & Townsend, 1993; Withers & Whitney, 1967).

Conclusions

The use of medroxyprogesterone acetate has been increasing in dog owners and dog breeders across the globe irrespective of its detrimental effects to the animal. Uses of medroxyprogesterone injection to prevent unwanted pregnancy are becoming increasingly common due to lack of awareness of its effect on the dogs' health. For the sake of animal health, animal welfare and control of birth, ovariohysterectomy is always the best resort to animals not kept for reproductive performances.

References

- Bryan, H. S. (1973). Parenteral use of medroxyprogesterone acetate as an antifertility agent in the bitch. Retrieved August 8, 2020, from American Journal of Veterinary Research website: <https://pubmed.ncbi.nlm.nih.gov/4703510/>
- Chakraborty, P. K. (1987). Reproductive hormone concentrations during estrus, pregnancy, and pseudopregnancy in the Labrador bitch. *Theriogenology*, 27(6), 827–840. [https://doi.org/10.1016/0093-691X\(87\)90205-6](https://doi.org/10.1016/0093-691X(87)90205-6)
- Concannon, P. W., Isaman, L., Frank, D. A., Michel, F. J., & Currie, W. B. (1988). Elevated concentration of 13,14-dihydro-15-keto-prostaglandin F-2 α in maternal plasma during parturition and parturition in dogs (Canis familiaris). *Journal of Reproduction and Fertility*, 84(1), 71–77. <https://doi.org/10.1530/jrf.0.0840071>
- Concannon, Patrick W. (2011). Reproductive cycles of the domestic bitch. *Animal Reproduction Science*, 124(3–4), 200–210. <https://doi.org/10.1016/j.anireprosci.2010.08.028>
- Eilts, B. E. (2002). Pregnancy termination in the bitch and queen. *Clinical Techniques in Small Animal Practice*, 17(3), 116–123. <https://doi.org/10.1053/svms.2002.34325>
- Fransson, B. A., & Ragle, C. A. (2003). Canine Pyometra: An Update on Pathogenesis and Treatment. *Compendium on Continuing Education for the Practicing Veterinarian*, 25(8), 602–612.
- González-Domínguez, M. S., & Maldonado-Estrada, J. G. (2006). Prolonged pregnancy associated to an inappropriate medroxyprogesterone acetate prescription in a bitch. Is it rational and ethical to use exogenous progestin in the bitch? *Revista Colombiana de Ciencias Pecuarias*, 19(4), 442–450.
- Irons, P. C., Nöthling, J. O., & Volkmann, D. H. (1997). Failure of luteolysis leads to prolonged gestation in a bitch: A case report. [https://doi.org/10.1016/S0093-691X\(97\)00246-X](https://doi.org/10.1016/S0093-691X(97)00246-X)
- Johnston, S. D., Kustritz, M. V. R., & Olson, P. N. S. (2001). *Canine and Feline Theriogenology* (First). <https://doi.org/00-492.29>
- Jordan, A. (1994). Toxicology of depot medroxyprogesterone acetate. *Contraception*, 49(3), 189–201. [https://doi.org/10.1016/0010-7824\(94\)90037-X](https://doi.org/10.1016/0010-7824(94)90037-X)
- Mauck, C. K., Callahan, M. M., Baker, J., Arbogast, K., Veazey, R., Stock, R., ... Gabelnick, H. L. (1999). The effect of one injection of Depo-Provera® on the human vaginal epithelium and cervical ectopy. *Contraception*, 60(1), 15–24. [https://doi.org/10.1016/S0010-7824\(99\)00058-X](https://doi.org/10.1016/S0010-7824(99)00058-X)
- Nelson, L. W., & Kelly, W. A. (1976). Progesterone-Related Gross and Microscopic Changes in Female Beagles. *Veterinary Pathology*, 13(2), 143–156. <https://doi.org/10.1177/030098587601300209>
- Noakes, D. E., Parkinson, T. J., & England, G. C. W. (2019). *Veterinary Reproduction and Obstetrics* (Tenth). <https://doi.org/10.16309/j.cnki.issn.1007-1776.2003.03.004>
- Selman, P. J., Van Garderen, E., Mol, J. A., & Van Den Ingh, T. S. G. A. M. (1995). Comparison of the histological changes in the dog after treatment with the progestins medroxyprogesterone acetate and proligestone. *Veterinary Quarterly*, 17(4), 128–133. <https://doi.org/10.1080/01652176.1995.9694551>
- Smith, F. O. (2006). Canine pyometra. *Theriogenology*, 66(3 SPEC. ISS.), 610–612. <https://doi.org/10.1016/j.theriogenology.2006.04.023>
- van der Weijden, B. C., & Taverne, M. A. (1994). Aspects of obstetric care in the dog. *The Veterinary Quarterly*, 16 Suppl 1(1994). <https://doi.org/10.1080/01652176.1994.9694467>
- Verstegen-Onclin, K., & Verstegen, J. (2008). Endocrinology of pregnancy in the dog: A review. *Theriogenology*, 70(3), 291–299. <https://doi.org/10.1016/j.theriogenology.2008.04.038>
- Von Berky, A. G., & Townsend, W. L. (1993). The relationship between the prevalence of uterine lesions and the use of medroxyprogesterone acetate for canine population control. *Australian Veterinary Journal*, 70(7), 249–250. <https://doi.org/10.1111/j.1751-0813.1993.tb08041.x>
- Withers, A. R., & Whitney, J. G. (1967). The Response of the Bitch to Treatment with Medroxyprogesterone Acetate. *Journal of Small Animal Practice*, 8(5), 265–271. <https://doi.org/10.1111/j.1748-5827.1967.tb04550.x>

METACARPAL FRACTURE IN NILGAI: A CLASSIC CASE OF HUMAN WILDLIFE CONFLICT



S. P. Shrestha^{1*}, Y. Panth², D. Sapkota¹

Abstract: A Nilgai with metacarpal fracture of both forelimbs was presented to Veterinary Teaching Hospital, Paklihawa Campus. External coaptation was carried out with modified Thomas Splint-Cast Combination for repair of the fracture. Among the many methods of fracture repair, external coaptation is one of the aiding and affordable treatment options in the majority of distal fractures. The presented case is a probable example of human-wildlife conflict. With an increase in the number of such cases, there must be a long term tangible solution to protect both humans and wildlife.

Keywords: External coaptation, human-wildlife conflict, metacarpal fracture, Nilgai

Case Description

Species: Nilgai (*Boselaphus tragocamelus*)

- Estimated Weight: 50 kg
- Age: Approximately 3 months
- Sex: Male
- Date: 2076/07/22
- Site: Suryapura, Rupandehi



Figure SEQ Figure * ARABIC 1: Metacarpal fracture of Nilgai

History

The case of Nilgai was presented to Veterinary Teaching Hospital, Paklihawa Campus with metacarpal fracture of both forelimbs. It was brought by the Division Forest Office, Rupandehi. DFO reported to have found the rescued animal in the shed of locals in Suryapura. Locals contacted the local police who came into contact with DFO. Initially, it was taken to Veterinary Hospital and Livestock Service Expert Center (VHLSEC), Rupandehi from where it was referred to Veterinary Teaching Hospital after examination.

In VHLSEC:

- Inj. Meloxicam
Sig: 5ml×IM×OD

Physical Parameters:

- Temperature: 103.50F
- Heart Rate: 52/min

Diagnosis

- Definitive Diagnosis: Fracture of Metacarpal

¹ Tribhuvan University, Kathmandu, Nepal

² Agriculture and Forestry University, Chitwan, Nepal

*Corresponding author: swochhal@gmail.com

IVSA MIRROR V

- Treatment: External Coaptation by Modified Thomas Splint-Cast Combination
- Clinician: Dr. Ganga Prasad Yadav and Dr. Birendra Shrestha (VTH, Paklihawa)
- Ampule placed initially was removed to create the required window for dressing which was temporarily closed using a gauze piece.
- Rope tied to the hoof region was cut.

Fracture Management

Materials Required:

- Aluminum Splint – 6 ft
- Fibreglass cast – 2 pieces
- Cotton – 500 gm
- Zinc Oxide powder – 40 gm
- Jute Rope – 2 pieces
- Shaving blade – 2 pieces
- Bandage (4 inch) – 10 pieces

Procedure:

- Affected portion was cleaned with normal saline and the area around was shaved.
- The limbs were immobilized with the help of gauze and scissor/BP handle tied to the limbs.
- After all the prescribed materials were brought, further treatment was carried out.
- Jute ropes were used to tie the leg at the hoof region.
- Gauze and scissor/BP handle tied to the limb was removed.
- Lignocaine was spread topically around the exposed area.
- Rope was tied at the hoof region which was used to manipulate the orientation of bone by pulling it to maximum extent further.
- Powder was spread around the limb.
- Gauze was tied around the limb.
- An Ampule was placed at the fracture site to create a window for daily dressing.
- Cotton roll was wrapped around the limb.
- Wet gauze was then wrapped around the limb.
- Aluminum splint that was measured to fit from below the knee was fitted at lateral aspect.
- Jute rope was tied to fix aluminum splints.
- Cotton was wrapped around the limbs.
- Fibreglass cast soaked in water was then wrapped around the limb.

Medications:

- Inj. Gentamicin
- Sig. 3ml × IM × BID × 5 days
- Inj. Ceftriaxone 750 mg
- Sig. 3ml × IM × BID × 7 days
- Inj. Meloxicam
- Sig. 4ml × IM × BID × 3 days
- Liq. Calcium (Ossermin)
- Sig. 25ml × PO × OD × 5 weeks

Advices:

- Dressing daily 2 times a day with betadine and application of Gentamicin (1ml) to each limb topically.
- Providing nutritious diet to the animal as much as possible.

Discussion

Metacarpal fractures rank first in order of frequency of long-bone fractures in calves (Belge, Akin, Gülaydin, & Yazici, 2016). Ferguson (1982) and Anderson & St-Jean (2008) reported that the 50% of fracture cases seen in cattle are metacarpus and metatarsus fractures. Köstlin et al. (1990) stated that metacarpal fractures, with an incidence rate of 40.5%, are the most common fractures among the extremity fractures seen among cattle. Because of the large force necessary to cause the fracture and the limited soft tissue supporting structures covering the bone, the fracture frequently is comminuted and open fractures are not uncommon (Tulleners, 1996).

There are many methods that can be used in the repair of a fracture. Open reduction and internal fixation may be an option for a very valuable animal, but the cost of such procedures may render them unaffordable. We must be careful to choose methods that are not harmful and allow patients to return to normal function as soon as possible.



Fig 1: immobilizing the limb



Fig 2: shaving the site of fracture



Fig 3: cleaning the site of fracture



Fig 4: tying rope at the hoof region



Fig 5: applying powder to the limb



Fig 6: tying gauze around the limb



Fig 7: cotton roll wrapped around the limb



Fig 8: ampule placed at the site of fracture



Fig 9: wet gauge wrapped around the limb



Fig 10: making aluminium splint according to limb size



Fig 11: aluminium splint placed at lateral aspect



Fig 12: tying sutri to fix the aluminium splint



Fig 13: fiber glass cast soaked in water wrapped around limb



Fig 14: after application of fiber glass cast



Fig 15: creating window for dressing by removing ampule



Fig 16: closing the window with gauge



Fig 17: repeating the same process in other limb



Fig 18: Nilgai after the treatment

Modified Thomas Splint-Cast Combination

External coaptation can be used as a treatment option in the majority of closed distal fractures. It is best suited for fractures of the phalanx, physeal fractures of the MTC/T III-IV, or simple diaphyseal fractures (transverse or short oblique) (Mulon, 2013). Additional support for the limb, in the form of a modified Thomas splint of transcortical pins, should be used when comminuted or long oblique fractures are diagnosed. The prognosis for long-term pain-free survival is excellent for closed fractures and fair to good for open fractures managed in this manner. In a normal healing time frame for secondary bone, stability of the fracture site is obtained with the fibrocartilaginous callus after three weeks.

The principle aim of external coaptation is to achieve immobilization of the joints adjacent to the fractured bone and to transfer the loading forces directly from the distal portion of the cast to the proximal aspect of it and to the proximal portion of the limb, limiting forces applied to the fracture site.

After application of the protective bandaging, the limb should be maintained in an almost extended position. The fibreglass rolls are applied starting at the fracture site. The casting material should overlap by 50 percent from one layer to the next. The foot is included in the cast in all cases. Fibreglass casting material is weaker in compression compared to tension, so reinforcement of the compression sites of the cast is important to avoid any acute breaking of the material. Calves (less than 150 kg) necessitate six to eight layers of thick cast; adults may require up to 15 layers of thick cast, depending on their weight. The animal should be maintained and confined in a stall during the entire period of coaptation and for an additional four weeks after the cast has been removed.

The combination of external coaptation and a modified Thomas splint allows the bone to be kept in tension and limits the forces applied to

the fracture for the first weeks, allowing time for the cartilaginous callus to bridge the fracture line and provide enough stability so that a full limb cast can be applied subsequently. The modified Thomas splint transfers the forces from the ground to the shoulder or the hip in the anterior or posterior limb, respectively. Thomas splints are made from aluminium rods for calves and from steel rods for juveniles and adults.

Human Wildlife Conflict

Mainly in Terai region of Nepal, wildlife habitat is being replaced by human encroachment (shed and agriculture), while animals are being squeezed into smaller areas of remaining natural habitat, which are surrounded by crops that wild ruminants find more palatable. Among many damages by these animals, crop incursion is the highest problem. This case study also presents a similar situation. Human-wildlife conflict (HWC) is fast becoming a critical threat to the survival of many globally endangered species, in particular to large and rare mammals (Palita & Purohit, 2008). There is a common tendency to perceive human-wildlife conflict as a rural or agricultural problem, but with the expansion and encroachment by human populations into previously forest lands, this conflict has extended to urban areas as well, affecting the rich and poor alike (Messmer, 2000). Hence, there must be tangible long-term solutions so that man and wildlife can mutually coexist together.

References

- Anderson, D. E & St-Jean, G. (1996). External skeletal fixation in ruminants. *Vet Clin North Am Food Anim Pract*, 12, 117–52.
- Anderson, D. E & St-Jean, G. (2008). Management of Fractures in Field Settings. *Vet Clin Food Anim*, 24, 567–582. doi:10.1016/j.cvfa.2008.07.006
- Belge, A., Akin, I., Gülaydin, A., & Yazici, M. F. (2016). The treatment of distal metacarpal fracture with locking compression plate in calves. *Turkish Journal of Veterinary and Animal Sciences*, 40(2), 234–242. <https://doi.org/10.3906/vet-1510-4>
- Benato, L., & Bexton, S. (2011). The management of an injured roe deer (*Capreolus*

- capreolus) with a metacarpal fracture and cortical blindness resulting from a vehicle collision. *Journal of Wildlife Rehabilitation*, 31(1), 15–20.
- Ferguson, J. G. (1982). Management and repair of bovine fractures. *Compend Contin Educ Pract Vet*, 4(1), 128-36.
 - Gillespie, A., Connolly, N., Scarabelli, S., Lipreri, G., & Singer, E. (2018). Internal fixation of a comminuted metacarpal fracture in a bull. *Veterinary Record Case Reports*, 6(2), 1–5. <https://doi.org/10.1136/vetreccr-2018-000630>
 - Kostlin, R. G., Nuss, K., & Elma, E. (1990). Metacarpal and metatarsal fractures in cattle. Treatment and results. *Tierarztl Prax*, 18(2), 131-144
 - Ladefoged, S., Grulke, S., Busoni, V., Serteyn, D., Salciccia, A., & Verwilghen, D. (2017). Modified Thomas splint-cast combination for the management of limb fractures in small equids. *Veterinary Surgery*, 46(3), 381–388. <https://doi.org/10.1111/vsu.12612>
 - Messmer, T. A. (2000). The emergence of human wildlife conflict management: turning challenges into opportunities. *International Biodeterioration & Biodegradation*, 45, 97-102
 - Mulon, P. Y. (2013). Management of long bone fractures in cattle. *In Practice*, 35(5), 265–271. <https://doi.org/10.1136/inp.f2869>
 - Palita, S. K., & Purohit, K. L. (2008). Human-Elephant Conflict: Case Studies from Orissa and Suggested Measures for Mitigation.
 - Tulleners, E. P. (1996). Metacarpal and metatarsal fractures in cattle. *The Veterinary Clinics of North America. Food Animal Practice*, 12(1), 199–209. [https://doi.org/10.1016/S0749-0720\(15\)30443-6](https://doi.org/10.1016/S0749-0720(15)30443-6)

MILK IN PAKISTAN: FROM PRODUCTION TO THE END CONSUMER

H. Jawad^{1*}, A. Tahir², S. Annisa³, R. A. Gul²



Abstract: *The study is basically based on the milk production potential of dairy cattle in Pakistan, milk production systems and how it reaches the end consumer. Despite Pakistan being an agricultural country, there are several bottlenecks like lack of breeding management and advanced production systems, defective infrastructure, quality control mechanisms, drug residues in milk and lack of trained manpower. All these factors need to be looked after for development of the modern dairy sector in Pakistan. Over the last 15-20 years, many new innovations in the dairy industry have put fruitful outcomes like dairy herd enlargements, cross breeding of local high producing animals with the exotic ones, artificial insemination, farmers training and knowledge and comparatively ease to adopt new innovations.*

Keywords: dairy, innovations, milk, Pakistan, production systems, quality assurance

Case Description

Since Pakistan is an agricultural country, the livestock and agriculture sector play a big part in the economy of Pakistan. Agriculture contributes about 18.5% of the country's GDP. Over the past few years, the livestock subsector got a lead on the crop sub sector as a largest contributor to the value added in agriculture. In 2018-19, it contributed to 60.5% in agriculture and 11.5 % in the country's GDP. Gross value addition of livestock has increased from Rs. 1,384 billion (2017-18) to Rs. 1,440 billion (2018-19), showing an increase of 4.0% over the same period last year. The cattle and buffalo population in Pakistan is 47.8 million (2018-19) and 40.0 million (2018-19) respectively. Collective milk production from both animals is 57,871,000 tonnes (2018-19) while the total milk produced from cattle and buffalo which is available for human consumption in Pakistan is 46,297,000 tonnes (2018-19). 20% of milk is wasted; 15% in transportation and 5% in calf feeding (Government of Pakistan, 2019). Among livestock products, milk is the most important single commodity and extensively used product in the country. Milk is considered as a universal

complete diet which has proteins, carbohydrates, lipids, minerals and vitamins in highly digestible form. According to Pakistan's Economic Survey, in 2018, per capita milk consumption was 200 liters per year. Though Pakistan is ranked at fifth position for milk production in the world, still milk production is not enough to meet its self-demand. Buffalo is the principal source of milk production in Pakistan (Burki, Khan, & Bari, 2004). In Pakistan, about 30-35 million of the rural population raise livestock and earn about 30-40% of its income from it. Milk production contributes more to the country's GDP than any other single crop. The main issue about milk production in Pakistan is that milk is being produced on an unorganized non-commercial basis with low milk yield per animal due to poor genetic makeup. Only a little fraction of milk in Pakistan comes through commercial dairy farming (Burki & Khan, 2008).

Milk Production Sources

In Pakistan, milk production falls into five systems. These systems are based on the herd size, location and management level (Tahir, Riaz,

^{1,2}The Islamia University of Bahawalpur, Punjab, Pakistan

³Airlangga University, Surabaya, Indonesia

*Corresponding author: hamzajawad928@gmail.com

IVSA MIRROR V

Bilal, & Nouman, 2019). These five are as follows:

Smallholder subsistence production system:

43% of dairying households operate under this system (Burki, Khan, & Bari, 2004). Milk produced in such a system has limited access to the market, mostly is used by the family and converted into other milk products at home. The average herd consists of three buffalos/cattle with two in lactation and average yield is 3 liters per animal per day. In this system family members act as labor. This system contributes about 0.5% of annual saleable milk in the country (Tahir, Riaz, Bilal, & Nouman, 2019).

Smallholder market oriented production system:

27-28% of dairying households operate under this system (Burki, Khan, & Bari, 2004; Burki & Khan, 2008). About 70% of milk produced is sold out to nearby markets. Herd consists of 5-7 buffalos and cows with 3-4 in lactating. Such producers have milk in excess to family requirements. This system contributes about 70% of annual saleable milk in the country (Tahir, Riaz, Bilal, & Nouman, 2019).

Rural commercial production system:

More than 90% of milk produced in this system is sold out. There are 30 animals out of which 70% are females, and 40% of these females are in lactation most of the year. It is the second largest source of milk collection by dairy companies in Pakistan. This system contributes about 20% in annual saleable milk in the country (Tahir, Riaz, Bilal, & Nouman, 2019).

Peri-urban production system:

Almost all milk is sold directly through the retail shops in the city after decreaming. There are two types of herd sizes; small with 20-200 animals and large with 200 to 2000 animals. These commercial scale units occur in the periphery of urban areas of Pakistan. This system contributes about 9% in annual saleable milk in the country (Silanikove, Leitner, Merin, & Prosser, 2010; Tahir, Riaz, Bilal, & Nouman, 2019).

Large peri-urban commercial dairy farming

These farms produce milk ranging from 0.02 to 0.1 million liters per day with the number of

animals ranging from 2000 to 5000. These farms are based on high inputs-high outputs production systems with good quality feeding and elite animals of Holstein Friesian and crosses of Holstein Friesian and Jersey breeds with milk yield of up to 25 l per day per animal. Farms have their own chilling, pasteurization and packaging systems. This system contributes about 0.5% in annual saleable milk in the country (Bilal, Suleman, & Raziq, 2006; Tahir, Riaz, Bilal, & Nouman, 2019).

Milk collection and distribution

There are two types of milk collection systems in Pakistan; traditional and commercial. About 97.7% of milk produced is collected by traditional system, while the remaining 2.3% is collected commercially by private dairy companies (Bilal, Suleman, & Raziq, 2006; Silanikove, Leitner, Merin, & Prosser, 2010; Tahir, Riaz, Bilal, & Nouman, 2019). In the traditional system, the agent which collects milk and distributes it to either end consumers at home or to the milk retail shops is locally called as Guwala/Dodhi. In a city of Pakistan, Lahore, about 13.2% of milk goes to collection centers or Halwais (the one who makes sweets), 78.2% is collected by Guwala/Dodhi and 8.6% is sold out to nonspecific sources (Jalil, Rehman, Sial, & Hussain, 2009). About 284 million liters of milk is being processed per year according to data collected in 2016 (Tahir, Riaz, Bilal, & Nouman, 2019).

Issues Regarding Milk Production in Pakistan

The main problem about the dairy industry in Pakistan is the low milk yield of buffaloes and cows. This problem highlights one of the most important issues of milk production that is the lack of national breed improvement programs systemically. There is lack of good quality forage, nutrient requirements of animals are not properly fulfilled and poor management practices (Shahid, Shafique, & Shokat, 2012). Milk production potential animals in Pakistan are very low as compared to the developed

IVSA MIRROR V

countries like the U.S.A, Germany and New Zealand. In New Zealand one dairy animal produces milk equal to three dairy animals of Pakistan, in Germany, one dairy animal produces milk equal to six dairy animals in Pakistan, while in U.S.A. one dairy animal produces milk equal to seven dairy animals in Pakistan (Burki & Khan, 2008). Another issue the dairy industry of Pakistan is facing is the lack of dairy management related knowledge, lack of infrastructure and other facilities in deep rural areas (Tahir, Riaz, Bilal, & Nouman, 2019).

Conclusions

Pakistan has a large number of livestock but we cannot meet the milk demand. For this we should imply proper national breeding strategies to improve the genetic potential of the animals. There should be proper extension work to educate the farmers so that they become more inclined to new innovations like artificial insemination, adopting proper hygienic practices and to use new infrastructure. Livestock is dispersed all over the countries. There should be proper development of cattle and buffalo colonies. A proper milk collecting system should be launched to ensure the collection of milk without deteriorating its quality (Bilal, Suleman, & Raziq, 2006; Silanikove, Leitner, Merin, & Prosser, 2010; Tahir, Riaz, Bilal, & Nouman, 2019).

References

- Anderson, D. E & St-Jean, G. (1996). External Burki, A. A., Khan, M. A., & Bari, F. (2004). The state of Pakistan's dairy sector: an assessment. *The Pakistan Development Review*, 149-174.
- Burki, A. A., & Khan, M. A. (2008). Milk supply chain and efficiency of smallholder dairy producers in Pakistan. *Lahore University of Management Sciences*
- Jalil, H., Rehman, H. U., Sial, M. H., & Hussain, S. S. (2009). Analysis of milk production system in peri-urban areas of Lahore (Pakistan): A case study. *Pakistan Economic and Social Review*, 229-242. <http://admin.umt.edu.pk/Media/Site/SPA/FileManager/MrSyedShahidHussain/Analysis%20of%20Milk%20Production.pdf>
- Shahid, H., Shafique, O., & Shokat, A. (2012). Dairy industry of Pakistan. *European Journal of Business and Management*, 4(18), 1-4. https://www.researchgate.net/profile/Owais_Shafique/p

ublication/260595759_Dairy_Industry_of_Pakistan/links/0a85e531b317e678bb000000/Dairy-Industry-of-Pakistan.pdf?origin=publication_detail

- Tahir, M. N., Riaz, R., Bilal, M., & Nouman, H. M. (2019). Current Standing and Future Challenges of Dairying in Pakistan: A Status Update. In *Milk Production, Processing and Marketing*. IntechOpen. <https://www.intechopen.com/books/milk-production-processing-and-marketing/current-standing-and-future-challenges-of-dairying-in-pakistan-a-status-update>
- Silanikove, N., Leitner, G., Merin, U., & Prosser, C. G. (2010). Recent advances in exploiting goat's milk: quality, safety and production aspects. *Small Ruminant Research*, 89(2-3), 110-124.
- Bilal, M. Q., Suleman, M., & Raziq, A. (2006). Buffalo: black gold of Pakistan. *Livestock research for rural development*, 18(9), 140-151.

MYIASIS AND ITS PUBLIC HEALTH CONCERN IN TROPIC

E. E. Ojowu^{1*}, R. A. Gul²



Abstract: Flies are well known biting nuisance of humans and animals. They can invade living tissues causing amputation, disfigurement and rarely death; transmitting infectious disease. Flies can lay eggs on human flesh and their maggots (developing larvae) invade subcutaneous tissues and penetrate external body cavities such as nares, ears and orbits. Myiasis is significant and un-recognized in the world, especially in the tropics. Myiasis is an ectoparasitic infestation of variable necrotic tissues caused by dipterous larvae of human flies. It remains a major economic problem in both animals and human population. It leads to reduced milk production, weight and fertility loss and reduced hide quality with consequent economic losses in animals. The most common clinical presentation is furuncular (subcutaneous) myiasis, wound (superficial cutaneous) myiasis, cavitary (atrial or invasive) myiasis, intestinal myiasis, urinary myiasis, and vaginal. The major curative treatment modality for myiasis is the removal of the intact larva, gentle extraction of the intact larval when it protrudes its abdomen to reach air; occluding the opening using petroleum jelly (Vaseline) to coat. Surgical intervention may be necessary. Myiasis wounds should be cleansed and conservatively debrided during larval removal. Good hygiene, fly controls are ways of preventing myiasis.

Keywords: ectoparasites, flies, hygiene, maggots, myiasis

Introduction

Myiasis refers to an ectoparasitic infestation of viable or necrotic tissues by dipterous larvae of higher flies (Radwan, 2015). Myiasis is a term derived from a Greek word “myia” meaning fly, and “sis” meaning condition (Bhardwaj & Singh, 2010). Myiasis is worldwide in distribution and a common condition in wild and domestic animals (David, & Petri, 2006). In the United State, cases have been reported from rural areas where humans have increased contact with animals both domestic and wild; most cases are associated with travel to tropical and subtropical areas, and in cases with no travel history (David, & Petri, 2006). Infestation is usually associated with wounds. Myiasis cases of human infestation occurs in homeless persons and patients with conditions such as, alcoholism, peripheral vascular disease cancer, and infestation of body areas include; sin, wound, genital, intestine, nasal, aural, oral, ocular (Fydryszewski, 2015).

Flies are in the order Diptera and several genera associated with human myiasis include; *Dermotobia*, *Cochliomyia*, *Chrysoma* and *Cordylobia*. Their site of infestation can vary

based on their species and life cycle as well as their symptoms (Pollack, 2012).

Species associated with human infestation and their common names are; *Dermatobia hominis* (human bot fly), *Cochliomyia hominivorax* (New World screw worm), *Chrysomya bezziana* (Old World screw worm fly) and *Cordylobia anthropophaga* (tumbu fly). They are found in Africa. *Cuterebra*, *Oestrus* and *wohlfahrtia* are genera that primarily infest animals but may occasionally infest humans (Fydryszewski, 2015).

A rare type of myiasis, hematophagous myiasis, is common in infants younger than 9 months, especially in those residing in rural and endemic areas, and the furuncular lesions are usually on the face.

Classification of myiasis:

Based on anatomical or ecological characteristics; the anatomical model described by Bishopp and modified by James and Zumpt is considered the most practical in terms of diagnosis (James, 1947). A common

IVSA MIRROR V

modification of this schema describes myiasis as:

1. Blood sucking or sanguinivorous
2. Cutaneous (furuncular)
3. Wound
4. Cavitory (David & Petri, 2006)

Ecological classification takes into account the level of parasitism of the parasite and the host. It is necessary to take into consideration the ecological classification together with the specific life cycle when designing plague programs for Veterinary medicine, nursing homes or hospitals.

Daiz, (2006), recorded that, most frequently encountered myiasis by humans are:

1. Cutaneous (furuncular and wound)
2. Cavitory (i.e. nasal, oral, genital, ear etc.)

The lesions in furuncular cases are formed at the site of penetration and resemble boils (furuncle) and diagnosis is based on clinical symptoms and patient history. The larvae do periodically emerge from the lesions for respiration (Liebert & Madden, 2004). Cavitory myiasis is associated with infestation of external body orifices and open wounds and cause tissue necrosis with possible sequel such as bacterial infection.

Etiology

- *Dermatobia hominis* (human botfly) causes furuncular myiasis
- *Cordylobia anthropophaga* (tumbu fly) also causes furuncular myiasis.
- *Cochliomyia hominivorax* (America) and *Chrysomya bezziana* (Africa, Australia, Asia) both cause creeping (migratory) myiasis
- *Oestrus ovis* (sheep botfly) causes ophthalmomyiasis.

Epidemiology:

Myiasis is a worldwide infestation with seasonal variation. Its incidence is higher in tropics and subtropics of Africa and the Americas (Fydryszewski, 2015). The flies prefer a warm and humid environment therefore; they are restricted to the summer months in the temperate zones. Any cases reported are usually imported

cases of myiasis from travelers returning from tropical destinations.

Life cycle/ Clinical presentation:

Adults of *Dermatobia hominis* are free-living flies. Radwan, (2015), reported that there are two life cycles, depending on the need for a vector. Adults capture blood-sucking arthropods (such as mosquitoes) and lay eggs on their bodies, using a glue-like substance for adherence (Sloop, Lopez, 2006). Botfly larvae develop within the eggs, but remain on the vector until it takes a blood meal from a mammalian or avian host, newly-emerged botfly larva penetrates the host's tissue (Tamir, Haik, Schwartz, 2003). The larvae feed in a subdural cavity (Bennett et al., 2015) for 5-10 weeks, breathing through a hole in the host's skin (Radwan, 2015). Mature larvae drop to the ground and pupate in the environment. Larvae leave their host during the night and early morning, probably to avoid cycle desiccation. After approximately one month the adults emerge to mate and repeat the cycle (Boggild, Keystone & Kain, 2002).

Other genera of myiasis-causing flies such as *Cochliomyia*, *Cuterebra*, and *Wohlfahrtia* have a more direct life, where the adult flies lay their eggs directly in the host, or in the vicinity of wounds on the host (Puthran, Hegde, Anupama, Andrew, 2012; Radwan, 2015) or in *Cochliomyia* and *Wohlfahrtia* infestations, larvae feed in the host for about a week, and may migrate from the subdermis to other tissues in the body, often causing extreme damage in the process (Radwan, 2015).

Cordylobia anthropophag (tumbu fly) larva penetrate the skin causing pustular lesions that resemble boils or furuncles. The gravid female deposits its eggs on moist sandy soil or on wet clothing (e.g. cloth diapers) hung outside to dry. When the human victim wears the eggs-infested clothing, larva emerges and rapidly burrows into the skin with sharp mandibles for further development. Myiasis lesions are located on the body regions covered with clothing, such as the

IVSA MIRROR V

buttocks and trunks. On the other hand, the female *D. hominis* (botfly) captures blood-feeding insects, usually mosquitoes, and attaches her eggs to the undersurface of the insect which then delivers the botfly eggs to its blood meal victims (Radwan, 2015). Infestations with *D. hominis* are usually characterized by cutaneous swellings on the body exposed area such as the scalp, face, and extremities. This cutaneous swelling may be painful and may produce discharges (Sloop, Lopez, 2006). Sensation of irritation, crawling or episodic lancinating pain may be experienced by the patient.

Cavitary myiasis is often caused by zoonotic screwworm larval deposition in open wounds or external orifices, such as the nares, ears and orbits and may be characterized by deep tissue larval invasion, with secondary infection and extensive tissue necrosis. Patients present epistaxis, nasal obstruction, and facial pain, passage of worms, foul smell, nasal discharge, headache, dysphagia and sensation of foreign body in the nose in nasal myiasis (Radwan, 2015).

Cochliomyia hominivorax infestation, which causes wound myiasis, can be more serious, as this species may travel through living tissue in the body and not stay subdermal like most of the species of flies that cause myiasis. Secondary bacterial and also death may occur during severe infestation of *C. hominivorax*. *C. hominivorax*, the New World screwworm, is a common cause of cavitary myiasis in the Americas; and *Chrysomya bezziana*, the Old World screwworm, is common cause of cavitary myiasis in Africa, Asia, and Indonesia (Tamir, Haik, Schwartz, 2003). Cavitary myiasis must be managed with surgical debridement aggressively and antibiotic therapy for secondary infections (Radwan, 2015).

Oestrus ovis has been known to cause ophthalmomyiasis, which is infection of the eye with fly larvae. Patients usually complain of

severe eye irritation, redness, foreign body sensation, pain, lacrimation, and swelling of the eyelids (Radwan, 2015).

Genitourinary myiasis is uncommon and may present dysuria, hematuria and pyuria, following larval invasion of the urethra (urinary myiasis) or vagina (vaginal myiasis) (Bennett et al., 2015). Intestinal myiasis is also uncommon, usually caused by the accidental ingestion of maggot-contaminated food, and characterized by self-limited nausea, vomiting and diarrhea (Radwan, 2015).

Differential diagnosis:

- Cellulitis
- Abscess
- Foreign body reaction
- Lymphadenopathy
- Tungiasis
- Furunculosis
- Insect bite
- Cutaneous larva migrants
- Cutaneous leishmaniasis

Treatment:

Even though myiasis may be self-limiting, nonfatal in some cases, patients report huge psychological distress. Treatment should be based on few important considerations: (a) larvae are aerobic organisms; (b) maggots separate the necrotic tissue from living tissue, making surgical debridement of wounds easier and (c) treating predisposing factors, (Bhardwaj, Singh, 2010).

Surgical debridement of wound and extraction of larvae is the most commonly used procedure under local anesthetic.

- Noninvasive approaches include placing petroleum jelly, liquid paraffin, heavy oil, or bacon strips and gentle extraction of the intact larva when it protrudes.
- An alternative treatment of for all types of myiasis is oral ivermectin (200ug/kg once a day for 1-2days) or topical

IVSA MIRROR V

ivermectin (1% solution), proven especially helpful with oral and orbital myiasis (Puthran, Anupama, Andrew, 2012).

- Administer tetanus prophylaxis

Complications:

Special care should be taken to avoid laceration of larvae which can produce an undesirable inflammatory responses, bacterial infections or granulomas. Systemic antibiotics are only necessary when a secondary infection is known to be present, (Bhardwaj, Singh, 2010). Antibiotics such as amoxicillin/clavulanic acid are usually indicated in such cases.

Prevention and Control:

- Individuals travelling to rural endemic areas should be covered at all times with long-sleeved shirts, pants, and hats.
- Sleeping on raised beds, in screened rooms, or under a mosquito net is appropriate at night.
- Clothing should be hot-ironed and dried to remove any residual eggs in areas endemic to tumbu flies (Sloop, Lopez, 2006).
- Controlling of domestic and livestock animal larval infestations.
- Minimizing flies preferred breeding grounds by appropriate disposal of offal and carcasses and cementing floors to eliminate maggot flies egg-laying surfaces.
- Wearing long-sleeved shirts and pants which can be pyrethrin or pyrethroid impregnated and spraying exposed skin with diethyltoluamide N-diethyl-metotoluamide [DEET] - containing repellants (Radwan, 2015).
- Use antiseptics to prevent wound myiasis. Wounds should be cleaned and irrigated intermittently and proper dressings should be applied. Patients with wounds

should not be allowed to sleep outside and, if in an indoor or hospital environment, the windows should never be opened unless screened appropriately.

Acknowledgment:

I am very thankful to the College of Veterinary Medicine, Federal University of Agriculture, Makurdi; Faculty of Veterinary and Animal Sciences (FV&AS), Islamia University of Bahawalpur, Pakistan. A special thanks to my colleague, Patrick Emmanuel Yaveni (DVM, final semester) for his assistance and support. The help and guidance of individuals such as, Riaz Ahmad Gul, Victoria Ohechimda Onaah (DVM, 4th year), Hamza Jawad, Hira Anjum (DVM 4th year), and Rawan Osman from Assiut University Assiut, Egypt are also appreciated. I would also like to thank the IVSA team, Pakistan; especially, Muhammad Anas Nawaz and Nasir Iqbal, for their guidance and help.

References

- Bennett, J.E., Dolin, R., Blaser, J.M., (2015). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. (8th ed). Elsevier Saunders, Philadelphia.
- Bhardwaj, K.P., Singh, V., (2010). Oral myiasis: case reports. *Asians journal of oral and Maxillofacial surgery*, 22(2).
- Boggild, A.K., Keystone, J.S., Kain, K.C., (2002). Furuncular myiasis: a simple and rapid method for extraction of intact *Dermatobia hominis* larvae. *Clin Infect Dis*.
- Diaz, J.H., (2006). The epidemiology, diagnosis, management, and prevention of ectoparasitic diseases in travelers. *J Travel Med.*, 13 (2).
- Fydryszewski, N.A., (2013). Myiasis: diagnosis, treatment and medical use of maggot. *Clinical laboratory science*, 26(2).
- Hakeem, M.J., Bhattacharyya, D.N.,(2009). Exotic human myiasis. *Travel Med. Infect. Dis*.
- James, M.T., (1947). The flies that cause myiasis in man. *U.S Department of Agriculture, Washington D.C.*
- Liebert, P.S., Madden, R.C., (2004). Human botfly larva in a child's scalp. *J ped surg*.
- Puthran, N., Hegde V., Anupama, B., Andrew, S., (2012). Ivermectin treatment for massive orbital myiasis in an empty socket with concomitant scalp pediculosis. *Indian J Ophthalmol*.
- Radwan, H.M., (2015). *Myiasis. Tropical medicine and surgery*, 3(4).
- Sloop, G.D., Lopez, F.A., (2006). Clinical case of the month. Furuncular myiasis. *J La State Med. Soc*.
- Tamir, J., Haik, J., Schwartz, E., (2003). Myiasis with Lund's fly (*Cordylobia rodhaini*) in travelers. *J. Travel Medl*.

PSEUDOPREGNANCY IN BITCH: A REVIEW

N. Shrestha*, K. Kaphle



Abstract: *Pseudopregnancy is the physiological condition of non-pregnant pubertal bitch completing the luteal phase of the estrous cycle and characterized by the clinical signs those witnessed throughout the postpartum stage. Although exact etiology is not completely understood; certain hormonal roles, most importantly increased prolactin and decreased serum progesterone level is suggested to be responsible for its development and maintenance. Diagnosis is carried out by clinical signs and aided by abdominal palpation for fetuses, ultrasonography, blood tests, and radiology. Though it is self-limiting, the overt pseudopregnancy should undergo prolactin suppressing therapy and the permanent solution for the condition is ovariohysterectomy. When bitches exhibit repeated signs of pseudopregnancy in every cycle, it predisposes the bitches to pyometra and mammary gland tumor. This article reviews etiopathophysiology, clinical signs, diagnosis, and treatment of pseudopregnancy in bitches.*

Keywords: *bitch, ovariohysterectomy, prolactin, pseudo pregnancy*

Introduction

Pseudopregnancy or pseudocyesis or nervous lactation or false pregnancy is a condition characterized by the clinical signs those witnessed throughout the post-partum stage in bitch (Gobello, Sotaand, & Goya, 2001). Ancel and Bouin (1911) as cited in Weber (1944) first used the term pseudopregnancy for an unusually long luteal phase of estrus cycle in rabbits. The pseudopregnancy in bitch is a physiological reaction experienced by any of the non-pregnant pubertal bitch after completing the luteal phase of an estrus cycle (Kumar, et al., 2018). Pseudopregnancy is obligatory for all non-pregnant domestic and wild canines ensuring their ability to care and nurse the litter (Jochle, 1997). Pseudopregnancy can be classified as a covert meaning normal physiological condition and overt meaning the clinical condition (Root, Parkin, Hutchison, Warnes, & Yam, 2018). Although the exact cause of pseudopregnancy is not completely understood, certain hormonal changes have been suggested to have a role in its development and maintenance (Grunau, Nolte, & Hoppen, 1996).

There is a major role of prolactin hormone in the pathophysiology of pseudopregnancy (Kumar, et al., 2018). Pseudopregnancy is a common finding in domesticated dogs and despite its unknown prevalence, the estimation is as high as 50 to 75% (Johnston, 1980). The article reviews the most significant features of the physiology, clinical findings, diagnosis, and line of treatment and prevention of pseudopregnancy in bitch.

Methodology

The present paper is the review of pseudopregnancy in bitch. The materials to review are accessed from various open access veterinary journal articles, online publications from different websites, Veterinary Obstetrics and Gynaecology by Stephen J Roberts, Veterinary Reproduction and Obstetrics by Arthur, G. H., Reproduction in Farm Animals by Hafez and various unpublished thesis papers.

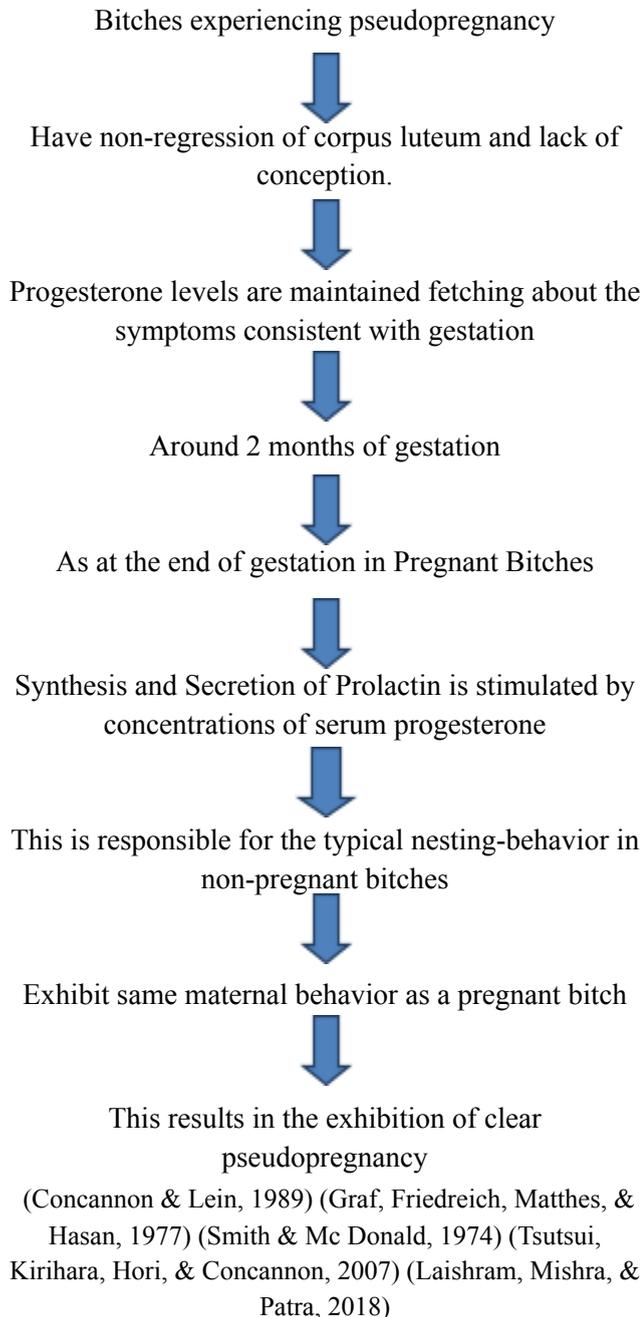
The review tries to compile and concise the concept upon the etiopathogenesis, clinical signs, diagnosis and treatment, and prevention of the

physiological and clinical case of pseudopregnancy in bitches.

Literature review

Etiopathogenesis

The etiopathogenesis of pseudopregnancy is described below in the flowchart:



Clinical Signs

During the mid and late metestrus period, non/pregnant bitch may exhibit signs of pseudopregnancy of variable intensities whereas some show no signs in cohort cases and some show conspicuous signs in overt cases (Gobello,

de la Sota, Castex, Baschar, & Goya, 2001). The pseudopregnancy signs usually start with behavioural signs viz. restlessness, nesting, licking of the abdomen, restlessness and mothering inanimate objects and later show physical signs viz. gain in weight, enlargement of the mammary gland, secretion and let-down of milk, and rarely abdominal contractions like that in parturition (Gobello, Concannon, & Verstegen, Canine Pseudopregnancy: A Review, 2001). The reported symptoms also include anorexia, vomiting, polydipsia, diarrhoea, polyuria, and polyphagia (Johnston, 1980). Overt pseudopregnancy has also been seen after treatment with progestin and 3rd or 4th day after ovariectomy during LP (Johnston, 1980) (Gobello, Sotaand, & Goya, 2001).

Diagnosis:

Diagnosis of pseudopregnancy is based on the history of estrus, observed clinical signs and symptoms, and history of progestin treatment. The major differential diagnosis is made by abdominal palpation where the fetal part is palpable if pregnant, ultrasonography if early diestrual phase or radiology if late diestrual phase and hormone estimation (Laishram, Mishra, & Patra, 2018) (Thangamani, Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018). The relaxin hormone is not detectable in non-pregnant bitch but is detectable in pregnant bitch (5 ng/ml) (Klonisch, Hombach-Klonisch, & Froehlich, 1999) (Laishram, Mishra, & Patra, 2018; Kumar, et al., 2018). Opined that blood test revealed the lower hemoglobin, higher TLC, SGOT, SGPT, and serum alkaline phosphatase in pseudopregnant bitch (Thangamani, Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018).

Treatments

Overt cases of self-limiting physiological pseudopregnancy with mild cases need no treatment and discouraging maternal behaviour and the conventional approach of applying Elizabethan collar for prevention of licking of the mammary gland is highly recommended

IVSA MIRROR V

(Gobello, Sotaand, & Goya, 2001) (Thangamani, Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018). The application of cold or hot packing on mammary glands should be avoided (Thangamani, Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018). Restriction of the water up to 5–7 days during night hours is recommended (Feldman & Nelson, 1996). The prolactin release is stimulated from the anterior pituitary gland due to phenothiazine compounds so these compounds are contraindicated whereas light tranquilization with non-phenothiazine compounds like diazepam is encouraged (Thangamani, Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018; Voith, 1983).

However, it is better to pharmacologically treat the cases of pseudopregnancy in bitches that have repeated episodes as there is a possible relationship between pseudopregnancy and subsequent development of mammary gland tumors that are recently reported (Verstegen, 1999) (Gobello, Concannon, & Verstegen, 2001). Drug therapy for the treatment of mild to severe cases of pseudopregnancy historically includes the use of steroids, including estrogens, androgens, and progestins with the most recent preferred method is the use of dopamine agonists which are effective prolactin suppressing drugs (Gobello, Concannon, & Verstegen, 2001). Sex steroids which are crucial for mammary gland development when used in high doses create a negative effect either by suppressing prolactin release from anterior pituitary or decreasing the sensitivity to prolactin hormone (Allen, 1986) (Gobello, Concannon, & Verstegen, 2001) (Johnston, 1980) (Gobello, Concannon, & Verstegen, 2001).

Estrogens like estradiol benzoate, diethylstilbestrol or estradiol cypionate are used as therapy but their side effects like causing signs of proestrus or estrus, pyometra, and depression of bone marrow resulting in anemia has proved for less recommendation of the use of estrogens as therapy (Gobello, Concannon, &

Verstegen, 2001). Androgens including testosterone and synthetic androgens suppress the lactation and the synthetic androgen mibolerone is reported for reducing the pseudopregnancy duration but clitoral hypertrophy, other forms of virilization, and epiphora are observed side effects (Gobello, Concannon, & Verstegen, 2001) Progestins like megestrol acetate and medroxyprogesterone acetate suppress the overt pseudopregnancy but their use is not recommended as their use results in cystic endometrial hyperplasia-pyometra complex, insulin resistance, mammary gland nodules, mammary gland tumors and acromegaly (Gobello, Concannon, & Verstegen, 2001) (Gobello, Concannon, & Verstegen, 2001).

The inhibition of prolactin secretion by ergot derivatives has been the most recent and advanced form of treatment of pseudopregnancy (Gobello, Sotaand, & Goya, 2001). The common compounds for inhibition of prolactin secretion are bromocriptine and cabergoline and these compounds have a straight action on D2 dopamine receptors of the anterior pituitary, and metergoline, a serotonin antagonist, has a dopaminergic effect when given at high doses (Hamon, et al., 1981) (Janssens, 1986) (Jochle, Arbeiter, & Post, 1989) (Gobello, Sotaand, & Goya, A Review of Canine Pseudocyesis, 2001). Bromocriptine has side effects of vomiting, anorexia and depression while cabergoline has less emesis and metergoline has side effects like anxiety, whining, hyperexcitation and aggressiveness (Gobello, Concannon, & Verstegen, 2001). Homeopathic Thuja D30 can be used safely and effectively in pseudopregnant bitches as an alternative to common pharmacological agents (Ozyurtlu & Alacam, 2005).

The permanent solution for the treatment of pseudopregnancy is the surgical approach by ovariohysterectomy when bitches exhibit repeated signs of pseudopregnancy in every cycle that predisposes the bitches to pyometra and mammary gland tumor (Thangamani,

IVSA MIRROR V

Srinivas, Chandra Prasad, Anusha, & Sadashiva, 2018). Ovariohysterectomy should be done during anestrus as ovariohysterectomy during lactation can extend the length of pseudopregnancy (Allen, 1986) (Gobello, Concannon, & Verstegen, 2001). Ovariohysterectomy in overt pseudopregnant bitches during metestrus (diestrus) may aggravate the pseudopregnancy period from 3 to 7 days post-surgery (Gobello, de la Sota, Castex, Baschar, & Goya, 2001) (Gobello, Concannon, & Verstegen, 2001).

Conclusions

Precise knowledge in physiological and clinical cases of pseudopregnancy aids the pet owners and the clinical practitioners to go through the accurate diagnosis and corrective and preventive therapy of the syndrome. When the importance of the diagnosis and management of pseudopregnancy is not given more serious conditions like pyometra and mammary gland tumor get aggravated in bitches so identifying and differentiating the cohort and overt pseudopregnancy and going through the proper management of the syndrome is essential. The exact etiology, though incompletely understood, the role of progesterone and prolactin is major in the development and maintenance of pseudopregnancy as diestrus/metestrus phase in bitch is longer to show the signs of false pregnancy with phantom whelping. Several treatment strategies are described by different authors with varying results and for the permanent solution of the syndrome and prevention of aggravation of other serious conditions like pyometra and mammary gland tumor, ovariohysterectomy or ovariectomy are recommended as the best approach of treatment.

References

- Allen, W. (1986). Pseudopregnancy in the bitch. The current view on aetiology and treatment. *Small Anim Pract* , 26, 419-424.
- Concannon, P. W. (1986). Canine physiology of reproduction. In T. Burke, *Small Animal Reproduction and Infertility* (pp. 3-27). Philadelphia, USA: Lea and

Febinger.

- Concannon, P., & Lein, D. (1989). Hormonal and clinical correlates of ovarian cycles, ovulation, pseudopregnancy and pregnancy in dogs. In R. Kirk , *Current Veterinary Therapy* (pp. 1269-1282). Philadelphia, USA: WB Saunders.
- Feldman, E., & Nelson, R. (1987). *Canine and Feline Endocrinology and Reproduction*. Philadelphia: WB Saunders Co.
- Feldman, E., & Nelson, R. (1996). *Canine and Feline Endocrinology, Reproduction* (2nd ed.). Philadelphia, USA: WB Saunders.
- Gobello, C., Concannon, P. W., & Verstegen, J. (2001, August 23). *Canine Pseudopregnancy: A Review*. New York: International Veterinary Information Service. Retrieved from www.ivis.org
- Gobello, C., de la Sota, L., Castex, G., Baschar, H., & Goya, R. (2001). Diestrus ovariectomy: A model to study the role of progesterone in the onset of canine pseudopregnancy. *Reprod Fertil* , 57, 55-60.
- Gobello, C., Sotaand, R. d., & Goya, R. (2001). A Review of Canine Pseudocycosis. *Reprod Dom Anim* , 36, 283-288.
- Graf, K., Friedreich, E., Matthes, S., & Hasan, S. (1977). Homologous radioimmunoassay for canine prolactin and its application in various physiological states. *Journal of Endocrinology* , 75, 93-103.
- Grunau, B., Nolte , I., & Hoppen, H. O. (1996). Investigation on the treatment of pseudopregnancy in the bitch with the prolactin inhibitors metergoline and bromocriptine. *Tierarztl Prax.* , 24, 149-155.
- Hamon, M., Mallat, M., Herbet, A., Melson, D., Audinot, M., & Glowinski, J. (1981). H3 Metergoline a new ligand of serotonin receptor in the rat brain. *Neurochem* , 36, 613-626.
- Janssens, L. (1986). Treatment of pseudopregnancy with bromocriptine, an ergot alkaloid. *Vet Rec* , 119, 172-174.
- Jochle , W., Arbeiter, K., & Post, K. (1989). Effects on pseudopregnancy, pregnancy and interoestrous intervals of pharmacological suppression of prolactin secretion in female dogs and cats. *Reprod Fertil* , 39, 199-207.
- Jochle, W. (1997). Prolactin in canine and feline reproduction. *Reproduction in domestic animals* , 32 (4), 183-193.
- Johnston, S. D. (1980). False pregnancy in the bitch. In D. A. Morrow, *Current Veterinary Theriogenology* (1st ed.). pp. 623-624. USA: WB Saunders, Philadelphia.
- Klonisch, T., Hombach-Klonisch, S., & Froehlich, C. (1999). Canine prep relaxin:nucleic acid sequence and localization within the canine placenta. *Biol Reprod* , 60, 551-557.
- Kumar, R., Srivastava, S., Singh, B., Kumar, R., Yadav, S., Kumar, P., et al. (2018). Therapeutic management of overt pseudopregnancy in a bitch: a case report. *Multilogic in Science* , 8, 63-64.
- Laishram, K. S., Mishra, G. K., & Patra, M. K. (2018). Canine pseudopregnancy and its treatment strategies. *Journal of Entomology and Zoology Studies* , 6 (3), 1076-1078.

IVSA MIRROR V

- Ozyurtlu, N., & Alacam, E. (2005). Effectiveness of Homeopathy for the Treatment of Pseudopregnancy in Bitches. *Turk J Vet Anim Sci* , 29, 903-907.
- Root, A. L., Parkin, T. D., Hutchison, P., Warnes, C., & Yam, P. S. (2018). Canine pseudopregnancy: an evaluation of prevalence and current treatment protocols in the U. *BMC Veterinary Research* , 14 (170).
- Smith, M., & Mc Donald, L. (1974). Serum levels of luteinizing hormone and progesterone during the estrous cycle, pseudopregnancy and pregnancy in the dog. *Endocrinology* , 94, 404-412.
- Thangamani, A., Srinivas, M., Chandra Prasad, B., Anusha, K., & Sadashiva, R. K. (2018). Canine Pseudopregnancy: A Review. *Journal of Veterinary Science and Technology* , 7 (1), 7–11.
- Tsutsui, T., Kirihara, N., Hori, T., & Concannon, P. (2007). Plasma progesterone and prolactin concentrations in overtly pseudopregnant bitches: a clinical study. *Theriogenology* , 67, 1032-1038.
- Verstegen, J. (1999). Internet applications des antiprolactiques en la cancerologie chez la chienne. 24th World Small Animal Veterinary Congress. Paris, France.
- Voith, V. (1983). Behavioral disorders. In S. Ettinger, *Textbook of Veterinary Internal Medicine* (pp. 513-522). Philadelphia, USA: WB Saunders.
- Weber, A. F. (1944). Pseudopregnancy in dogs. *Iowa State University Veterinarian*, 7(1), 6.

PYOMETRA IN A BITCH: A CASE REPORT

S. Yadav*, R. Yadav



Abstract: An intact seven year old female street dog with vomiting and regular increase in size of the abdomen without any vaginal discharge was presented to Veterinary Teaching Hospital, Paklihawa. By radiography and ultrasonography it was difficult to find out the exact cause of the abdominal distension. So exploratory laparotomy was performed and it was confirmed as closed pyometra and corrected with ovariohysterectomy after preparing the patient for performing surgery using medical treatment. Pyometra can be a fatal condition in bitch if it is not timely treated and thus, proper diagnosis of the condition and medical or surgical intervention is highly recommended. Ovariohysterectomy is the best treatment of choice for the condition of pyometra in bitch.

Keywords: laparotomy, ovariohysterectomy, pyometra

Introduction

Pyometra, a life-threatening bacterial infection of the uterus, is classified as open or closed cervix pyometra. In closed cervix pyometra, pus and bacterial products accumulate in the uterus, which is thought to induce a more severe illness (Jitpean et al. 2017). It is a hormonally mediated, diestrual disorder that results in abnormal uterine endometrium (Mahesh et al. 2014). The elevated progesterone levels help to create the ideal conditions for infection and stimulate uterine glandular secretions within the uterus, which suppresses uterine contractions and inhibits the effect of fighting blood cells in the uterus (Kumar et al. 2016). The etiology of pyometra is mainly *E.coli*, *Klebsiella*, *Pasteurella*, and *Staphylococcus*; most of the organism is gram-negative bacteria (Fransson and Ragle 2003). In approximately 90% of cases, *Escherichia coli* are the main causative agent and they produce endotoxins that are capable of initiating the cytokine cascade and the release of many inflammatory mediators (Fransson and Ragle 2003). Clinically, the bitch may present with inappetance, depression, polydipsia, lethargy, abdominal distension, and with vaginal discharge in case of open cervix pyometra and without vaginal discharge in closed cervix pyometra.

Methodology

A Seven years old intact female dog was presented in Veterinary Teaching Hospital, Paklihawa with a history of dehydration, depression, distended abdomen, unable to support her weight on a hind limb and Sangini Sui vaccinated to avoid pregnancy. Hematological and biochemical parameters are normal (Table: 1 & 2). Radiographic examination revealed an image of a distended uterus with the appearance of sac-like structure which is suggestive of pyometra (Fig. 1). Based on the history, clinical signs & symptoms, clinical pathology, and radiography report the condition was diagnosed as closed cervix pyometra.

After a diagnosis of closed cervix pyometra, keeping in view of recurrence of pyometra after cessation of treatment, it was planned for ovariohysterectomy (OVH). The animal was sedated with xylazine 2ml intramuscular injection (1-2 mg/kg IM). Ventral abdomen was prepared aseptically and premedicated with atropine sulphate 0.5 ml (0.022-0.044 mg/kg IM) and adrenaline 1ml (1mg/ml in IV). Induction was done with diazepam: ketamine (1:1) given 2ml intravenously and maintenance with

IVSA MIRROR V

diazepam: ketamine (1:2) given 2ml at regular interval of 10 minutes with continues monitoring of heart rate and respiratory rate. The dog was placed on dorsal recumbency after preparing the site of operation. The surgical site was scrubbed with Chlorhexidine and the dog was covered with sterilized drape except at the surgical site. The incision was made just below the umbilicus. Skin and subcutaneous tissue were incised to expose linea alba. The linea alba was pulled up with the help of a rat tooth and stab incision was made into abdominal cavity. The incision of linea Alba was extended both cranially and caudally. As the uterus was large enough to be taken out, the incision was extended up to about 10 cm. The ovary was located and the window was made in the broad ligament and two clamps was placed just below the ovary at a distance of about 0.5 cm and one clamp across the proper ligament of the ovary. Two ligations were made; one below the cranial clamp and one across the cranial clamp and the ovary was taken out. The second ovary was also exteriorized in similar manner. The uterine body was ligated by using transfixation technique and the whole uterus was taken out. Precaution was taken to maintain aseptic condition during surgery. The ligation was made by using catgut of size 1. The abdominal cavity was checked for bleeding and omentalization was done. The abdominal wound was sutured including three layers; abdominal muscles with peritoneum, subcutaneous tissue and skin. The suture material used for abdominal muscles and sub cutaneous tissue was PGA (Polyglycolic Acid). The suture pattern used for abdominal muscles was simple interrupted and subcutaneous tissue was simple continuous. The skin was sutured by using ford interlocking pattern with nylon.

During the entire operative procedure, normal saline @500 ml was given intravenously. Post operatively the animal was given meloxicam 1.5ml (0.2 mg/kg) for 5 days, ceftriaxone 5ml IM bid (15-25 mg/kg BW) for 4 days, tranexamic acid 2ml bid for 3 days intravenously



Fig 1: Surgical site preparation



Fig 2: Midline incision on abdomen



Fig 3: Exteriorization of uterus

IVSA MIRROR V

and suture was removed after 12 days. After 12 days, the dog was found to be completely normal without any complications.

Test	Result	Unit	Reference Value
Urea	19	mg/dl	11-53
BUN	8.87	mg/dl	0.7-30
Creatinine	1.22	mg/dl	0.5-1.8
Sodium (Na)	151.8	mmol/l	139-154
Potassium (k)	4.38	mmol/l	3.6-5.5
Bilirubin, Total	0.89	mg/dl	0.3-1.2
Alkaline Phosphate	85.41	u/l	36-150
SGPT(ALT)	68.21	u/l	10-125
SGOT(AST)	25.31	u/l	23-212
Total Protein	7.4	gm/dl	5.2-8.2
Albumin	3.6	gm/dl	3.5-5.4

Table 1: Biochemistry Report (Reference value taken from MSD manual).

PARAMETER	Result	Unit	Reference Value
WBC	14.1 *10 ⁹	L	6.0-17.0
Lymphocyte	4.1*10 ⁹	L	0.8-5.1
Granulocyte	9.4*10 ⁹	L	4-12.6
Lymphocyte %	28.8%		12-30
Granulocyte %	66.7%		60-83
RBC	7.22*10 ¹²	L	5.50-8.50
MCHC	345	g/dl	300-380
Platelet	107*10 ⁹	L	117-460
PCT	0.120%		
Eosinophil %	4.6%		

Table 2: Hematology Analyzer Report (Reference value taken from MSD manual).

Result and Discussion

The bitch made uneventful recovery without complication. Based on the case history, clinical signs and radiological examination the condition was diagnosed as closed cervix pyometra.

Closed-cervix pyometra is a medical emergency that requires rapid intervention to prevent overwhelming sepsis and the potential of patient



Fig 4: Uterus with pus after exteriorization



Fig 5: Suturing of incision site



Fig 6: Incision site after suturing



Fig 7: Exteriorized uterus of 7 Kg

IVSA MIRROR V

death. Sangini Sui (medroxyprogesterone acetate) is a temporary contraceptive that is used to control unwanted pregnancy in women. Medroxyprogesterone is a synthetic form of the hormone progesterone (protect the embryo and encourage the growth of the placenta). Multiple abnormalities such as bilateral ovarian cyst, cystic endometrial hyperplasia and pyometra, mammary adenoma, fibrosarcoma and cystic papillary adenocarcinoma are identified in animal continually administered with medroxyprogesterone acetate (Keskin et al. 2009). In this presented case, the cause of pyometra is suspected to be long term use of the Sangini injection (MPA). It was recommended that the best prevention for pyometra would be to spay all female dogs that are not meant for breeding purpose.

Conclusion

Canine pyometra is a common reproductive disorder of diestrual bitch. It is hormonally mediated disorder characterized by cystic endometrial hyperplasia with secondary bacterial infection. Use of Sangini injection (medroxyprogesterone acetate) in dog may lead to pyometra. Early diagnosis is important for the treatment of closed cervix pyometra. Diagnosis of closed cervix pyometra can be done by the history of recent estrus, polyuria and polydipsia along with radiography, ultrasound and rectal examination. It was concluded that ovariohysterectomy may be the best treatment of choice in closed pyometra of bitches. Medical treatment includes the injection of prostaglandin along with antibiotics.

References

- Fransson, Boel A., and Claude A. Ragle. 2003. "Canine Pyometra: An Update on Pathogenesis and Treatment." *Compendium on Continuing Education for the Practicing Veterinarian* 25(8): 602–12.
- Jitpean, Supranee, Aime Ambrosen, Ulf Emanuelson, and Ragnvi Hagman. 2017. "Closed Cervix Is Associated with More Severe Illness in Dogs with Pyometra." *BMC Veterinary Research*: 7–13.
- Keskin, Abdulkadir et al. 2009. "Pathological Abnormalities after Long-Term Administration of Medroxyprogesterone Acetate in a Queen." *Journal of Feline Medicine and Surgery* 11(6): 518–21.
- Kumar, U, A. K. Sharma, and L. Kumari. 2016. "Pyometra and Its Complication in Bitch-a Case Report." *Veterinary Practitioner* 17(1): 99–100.
- Mahesh, R., Prasad, V. D., Devarathnam, J., Sumiran, N., Kamalakar, G., & Kumar, R. S. 2014. "Successful Management of a Critical Case of Pyometra in a Bitch: A CaseReport." *Research Journal of Animal, Veterinary and Fishery Sciences* 2(8): 21–23.

REPEAT BREEDING IN CATTLE AND BUFFALO: ETIOLOGY, DIAGNOSIS AND TREATMENT

G. Gautam*



Abstract: Repeat breeding has always become one of the major reproductive problems in dairy cattle and buffaloes. Since the causes of the repeat breeding are multifactorial, it is a challenging job for clinicians to ascertain the exact cause of repeat breeding. Nonetheless, all the possible causes of repeat breeding can be discussed under two broad headings: fertilization failure and early embryonic deaths. Diagnostic approach should be oriented towards evaluating the health of the genital tract, physiological functioning of the ovaries and sequential growth and development of the embryo. The therapeutic regimens in repeat breeding must be aimed at the correction of the most probable causes. This review article discusses the etiology, diagnosis and treatment of repeat breeding in dairy cattle and buffalo.

Keywords: early embryonic death, fertilization failure, repeat breeding, reproductive problem

Introduction

Repeat breeding in cattle and buffalo has been defined as failure to conceive from three or more regularly spaced services in the absence of detectable abnormalities in the reproductive system (Zemjanis 1980; Sah and Nakao, 2006; Azawi et al., 2008a). Clinical examination of such animals may fail to reveal any definite lesion or condition to explain the failure of conception (Roberts, 1971). The repeat breeding syndrome continues to be a major problem in cattle and buffalo breeding, leading to large economic losses to the dairy producers (Bartlett et al., 1986; Lafi et al., 1992; Sah and Nakao, 2006; Yusuf et al., 2010). Repeat breeding syndrome is responsible for long service periods and inter-calving intervals thereby causing low milk and calf production resulting in greater economic losses to the dairy industry (Singh et al., 2008). The incidence of repeat breeding appears to be low in buffaloes compared to cattle (Pattel et al., 2007; Purohit 2008). For various countries, the incidence of the problem has been described in cattle and buffaloes to range from 5 to 35% (Bansal et al., 1978; Narsimha and Kotayya, 1980; Gustafsson and Emanuelson, 2002; Yusuf et al., 2010; Gautam et al., 2017).

Etiology of Repeat breeding

The causes of the repeat breeding are multifactorial (Gustafsson and Emanuelson,

2002; Moss 2002; Yusuf et al., 2010). Etiologies of repeat breeding have been classified in cows in a number of ways, yet failure of fertilization and early embryonic deaths had been one of the oldest classifications of the etiologies (Casida et al., 1961). The cause of repeat breeding may lie with the cow or the bull or a combination of these (Purohit 2008; Perez-Marin et al., 2012) and external factors such as environmental stress and poor breeding management (Perez-Marin et al., 2012). All the causes of repeat breeding can be discussed under two broad headings: fertilization failure and early embryonic deaths.

Fertilization failure

In cattle, the failure of fertilization accounts for a low proportion (10%–20%) of pregnancy losses during the first 21 days post insemination (Diskin and Morris, 2008). Fertilization failure may result from death of the egg before sperm entry, structural and functional abnormality in the egg or sperm, physical barriers in the female genital tract preventing gamete transport to the site of fertilization, ovulatory defects, endometritis, defects in bull (spermatozoa), the technique and timing of insemination when using artificial insemination (AI), ingestion of phytoestrogens etc.

IVSA MIRROR V

1. Abnormal Eggs

Several types of morphologic and functional abnormalities have been observed in unfertilized eggs, e.g., giant egg, oval shaped egg, lentil-shaped egg, and ruptured zona pellucida. Failure to undergo fertilization and normal embryonic development may be due to inherent abnormalities of the egg or to environmental factors.

2. Abnormal Sperms

In most domestic animals, sperm cells can only retain their viability and fertility at a high level for 24 hours or less in the female genital tract (Roberts, 1971). There is a gradual decrease in the fertilizing capacity or aging of spermatozoa in the female genital tract. Sperm aging and injury may cause alterations in the acrosomal cap or leakage of vital intracellular constituents that may prevent defective spermatozoa from fertilizing the egg.

3. Ovulatory defects

Delayed ovulation, anovulation and ovarian cysts are also important (incidence: 25%) causes of fertilization failure (Roberts, 1971) leading to repeat breeding in high producing cattle. Such defects are less frequent in the buffalo (Purohit, 2014) yet can result in fertilization failures. Clinical studies recorded the incidence of 0.5%–1.48% ovarian cysts in buffaloes (Raju et al., 2007) with poor clinical manifestation. Repeat breeders are likely to have delayed ovulation and an extended follicular phase which allows follicle development but postpones the LH surge (Bage, 2003). Hence, there is a longer interval between luteolysis and ovulation and a delayed LH surge resulting in aging of oocytes. Repeat breeder cows are also more likely to have two than three wave estrous cycles further increasing the probability of the presence of aged follicles (Perez et al., 2003).

4. Structural Barriers to Fertilization

Congenital or acquired defects of the female genital tract interfere with transport of the sperm

and/or the ovum to the site of fertilization. Congenital defects are the result of arrested development of the different segments of the Mullerian ducts or of an incomplete fusion of these ducts causally. Oviductal obstructions that probably prevent fertilization can originate from pathologies in the oviduct such as hydrosalpinx, pyosalpinx, salpingitis (Vala et al., 2011) and stenosis or growths in the oviduct (Purohit, 2014). Ovario Bursal adhesions have been mentioned in a few clinical descriptions with incidence varying from 0.04% (Modi et al., 2011) to 6.4% (Azawi et al., 2008b; Vala et al., 2011). Ovulatory disturbances such as delayed ovulation, anovulation and ovarian cysts are less frequent in the buffalo (Purohit 2014) yet can result in fertilization failures.

5. Subclinical endometritis

Some authors argue that subclinical endometritis is a major contributor to the repeat breeding syndrome of bovine sub-fertility (Noakes et al., 2001). However, Pascottini et al., (2017) in their study concluded that as only 25% of repeat breeder cows were affected with subclinical endometritis at AI, it may not be considered a key element associated with the repeat breeding syndrome. The negative effects of endometritis on fertility are mediated directly by bacterial endotoxins or indirectly by inflammatory mediators such as cytokines, nitric oxide and oxidative stress affecting sperm, ovarian, uterine and embryonic function (Gilbert 2011). Endometritis can affect sperm motility and function and results in increased sperm phagocytosis (Gilbert 2011) leading to fertilization failure and repeat breeding.

6. Bull factors

Repeat breeding can originate because of factors related to the bull and semen (Perez-Marin et al., 2012). The age of bull affects the semen volume and the proportion of abnormal spermatozoa; with adult bulls producing the highest volume and lowest abnormal sperms (Khawaskar et al., 2012). The bull breed, semen type (liquid or

IVSA MIRROR V

frozen), quality and source have marked effects on the conception rates (Andrabi, 2009).

7. Errors in technique and timing of insemination

With the increasing use of AI in buffaloes important considerations for optimum fertility include insemination technique, time of insemination and site of semen deposition. The conception rate varies significantly between the inseminators (Thirunavukkarasu and Kathiaravan, 2009). Incorrect inseminations relative to estrus were performed in 30.67% of buffaloes in one study and many repeat breeding buffaloes at insemination had plasma progesterone profiles greater than 1 ng/mL suggesting that buffaloes were inseminated at the wrong time (Sharma et al., 2008).

8. Phytoestrogens

Cows fed estrogenic forage (e.g., subterranean clover and red clover) may suffer impaired ovarian function, often accompanied by reduced conception rates (and increased embryonic loss). In cows, clinical signs resemble those associated with cystic ovaries.

Embryonic mortality

Embryonic mortality denotes the death of fertilized ova and embryos up to the end of implantation. About 25 to 40% of embryos are normally lost in farm species. It is also noted in large litters of swine and during multiple pregnancies in cattle and sheep. Mortality is more common during the early than the late embryonic period (Jainudeen and Hafez, 2013). Embryonic loss that occurs before fetal maternal recognition, and therefore does not involve the elongation of the life of the corpus luteum, is referred to as early embryonic death. Loss after the life of the corpus luteum has been extended is termed late embryonic death (Noakes et al., 2001). In cattle, most embryonic deaths occur during hatching of blastocyst i.e. between days 8 and 16 after insemination without affecting estrous cycle length (Jainudeen and Hafez,

2013). Thus, early embryonic mortality accounts for the major reproductive failure accounting up to 40% mortality of all fertilized eggs (Sreenam and Diskin, 1986) leading to repeat breeding.

Broadly speaking, embryonic loss may be due to either genetic or environmental factors or a combination of the two. Genetic factors causing embryonic loss include single-gene defects, polygenic abnormalities and chromosomal anomalies. A few single-gene mutations are lethal and result in the death of the conceptus and return to estrus (Noakes et al., 2001) leading to repeat breeding. Environmental causes of embryonic mortality can be discussed under following subheadings (Jainudeen and Hafez, 2013).

1. Endocrine Factors

Accelerated or delayed transport of the egg, as a result of estrogen–progesterone imbalance, leads to preimplantation death and return to estrus. Similarly, a diminished response to circulating luteotrophic hormones may contribute to embryonic mortality in subfertile cows (Shelton et al., 1990).

2. Lactation

Embryonic mortality occurs due to stress of lactation in cattle, sheep, and horses and is characterized by return to estrus after breeding.

3. Nutrition of the Dam

Caloric intake and specific nutritional deficiencies affect ovulation rate and fertilization rate, as well as cause embryonic death. Also extremes in the level of feeding are detrimental to embryo survival, so too are extremes in the supply of specific dietary nutrients. In dairy cows, high intakes of rumen degradable protein may lead to embryonic mortality.

4. Overcrowding in Uterus

Because the degree of placental development is primarily influenced by the availability of space and vascular supply within the uterus, increasing

IVSA MIRROR V

the number of implantations decreases the vascular supply to each site and restricts placental development. This results in a high embryonic and fetal mortality rate and probably explains the higher incidence of embryonic mortality in cattle and sheep following twin rather than single ovulation.

5. Thermal Stress

Embryonic mortality increases in a number of species following exposure of the mother to elevated ambient temperatures especially in tropical areas. The effects of thermal stress on the early embryo are not apparent until the later stage of its development.

6. Semen quality

A portion of all embryonic mortality is attributable to the male and the mating system. Genetic factors that are transmitted by the male to the embryo may be inherited, may arise from testicular tissue, or may occur in spermatozoa after they are released from the testis. Infertile matings by highly fertile bulls are primarily due to embryonic mortality, while those of bulls with low fertility are due to fertilization failure and embryonic deaths.

7. Incompatibility

The inherited genotype of the male may include a variety of genetic factors that lead to incompatibility and early embryonic loss. There may be incompatibility between spermatozoa and mother, between spermatozoa and egg, or between zygote and mother.

8. Poor uterine environment

Subclinical endometritis and inadequate uterine milk secretion might not be favorable for embryo survival, leading to repeat breeding. The infectious bovine venereal disease of vibriosis due to *V. fetus*, trichomoniasis due to *Tr. fetus* and probably brucellosis due to *Br. abortus*, IBR-IPV virus and certain pathogenic mycoplasma if introduced into the uterus with semen by artificial insemination can cause

infertility and repeat breeding due to early embryonic deaths probably secondary to an endometritis of variable duration (Roberts, 1971).

9. Age of the Dam

In dairy cows, embryonic mortality decreases with increasing parity up to fifth parity, then increases. The higher incidence of embryonic mortality in old cows may be due to a defective uterine environment.

Diagnostic Approaches

Diagnosing the cause of repeat breeding and pregnancy failure in individual animals is often extremely difficult and evaluations are oriented towards evaluating the health of the genital tract, physiological functioning of the ovaries and sequential growth and development of the embryo (Saraswat and Purohit, 2016). A combination of visual estimates, vaginoscopy, transrectal palpation and transrectal ultrasonography are suggested. More specialized techniques such as endoscopy, uterine biopsies, uterine cytology, uterine microbiology and tubal patency testing have been suggested for detection of pathologies in the genital tract which are not easily traceable (Purohit, 2008). Ovarian and oviductal pathologies such as tumors pyosalpinx, and oviductal obstructions are often not detectable with routine techniques and failure to regain fertility following medical or surgical therapy often limits their use (Purohit, 2014).

Treatment Processes

The therapeutic regimens in a herd with reproductive failures must be aimed at the correction of the most probable causes. Treatment of individual cows/buffaloes at most situations remains difficult as the exact cause is unknown and the most diagnostic modalities described are largely unavailable to the treating clinician. With limited facilities the therapeutic approach usually must be aimed sequentially at

IVSA MIRROR V

(i) combating uterine infection (endometritis), (ii) correcting ovulatory disturbances, (iii) supplementing for luteal insufficiency and (iv) improving management (Purohit, 2008).

Since most of the cases do not reveal any specific conditions, the following guidelines may be adopted.

- Bring the animal into positive energy balance by providing additional ration. Provide mineral-vitamin mixtures to rectify their deficiencies.
- Have proper estrus detection, and do AI at 12-18 hours from the onset of estrus.
- Do AI twice at each estrus preferably at 12 hours interval.
- Check the semen quality - use only high quality semen.
- Temporary replacement/change of the bull may take care of infertility because of the bull.
- Clitoral stimulation for at least three seconds at AI.
- Administration of 100 µg of GnRH or 1000-1500 IU of LH at the time of AI.
- Administration of 500 mg of depot progesterone on the 5th day of AI.
- Skipping of AI, administration of PGF 2 alpha after 9-10 days and fixed time AI twice at 72 and 96 h after PGF 2 alpha injection.
- Although little data are available regarding the efficacy of intrauterine antibiotic use for treating subclinical endometritis, the intrauterine administration of cephalosporin was suggested to be beneficial (Kasimanickam et al., 2005; Denis-Robichaud and Dubuc, 2015).
- Skipping of AI and intrauterine infusion of 2 ml of Lugol's solution diluted in 8 ml of sterile saline or 50 ml of 0.5-1% povidone iodine.
- Flushing of the uterus with normal saline - under moderate pressure as being done in embryo transfer (to remove cellular

debris and also mild block in the uterine tubes).

- Administration of different hormones and antibiotics may preferably be tried at separate estrus.
- Recently, several therapies alternative to the use of antibiotics have been suggested. The intrauterine infusion of immunomodulators such as E. coli lipopolysaccharide (endotoxin), oyster glycogen, infusion of serum, plasma or hyperimmune serum or leukotriene B4 has been reported widely (reviewed by Purohit, 2008), however, their application at routine field practice is yet to come.

References

- Andrabi SM. Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. *Reprod Domest Anim* 2009; 44: 552-569.
- Azawi OI, Ali AJ, Lazim EH. Pathological and anatomical abnormalities affecting buffalo cows reproductive tracts in Mosul. *Iraqi J Vet Sci* 2008b; 22: 59-67.
- Azawi OI, Omran SN, Hadad JJ. A study on repeat breeding of Iraqi buffalo cows. *Buffalo Bulletin* 2008a; 27: 274-283.
- Bage R. Conception rates after AI in Swedish red and white dairy heifers: relationship with progesterone concentrations at AI. *Reproduction in Domestic Animals* 2003; 38:199-203.
- Bansal RS, Gupta SK, Dugwekar YG. Incidence of reproductive disorders in buffaloes. *Journal of Remount and Veterinary Corps* 1978;17:9.
- Bartlett PC, Kirk JH, Mather EC. Repeated insemination in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. *Theriogenology* 1986; 26:309-322.
- Casida LE. Present status of the repeat breeder cow problem. *Journal of Dairy Science* 1961; 44, 12: 2323-2329.
- Denis-Robichaud J, Dubuc J. Randomized clinical trial of intrauterine cephalosporin infusion in dairy cows for the treatment of purulent vaginal discharge and cytological endometritis. *Journal of Dairy Science*. 2015; 98:6856-6864. DOI: 10.3168/jds.2014-9129.
- Diskin MG, Morris DG. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Domest Anim* 2008; 43: 260-267.
- Gautam, G, Gyawali RR, Nath, B and Pant S. Characterization and treatment of infertility in buffaloes of hills and Terai regions of mid Nepal. *Proceedings of the International Buffalo*

- Symposium*, 15-18 Nov 2017, Chitwan, Nepal; pp. 98-102.
- Gilbert RO. The effects of endometritis on the establishment of pregnancy in cattle. *Reprod Fertil Dev* 2011; 24: 252-257.
 - Gustafsson H, Emanuelson U. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. *Acta Veterinaria Scandinavica* 2002; 43:115–25.
 - Jainudeen MR, Hafez ESE. Reproductive failure in females: In: Hafez ESE and Hafez B, editor. *Reproduction in Farm Animals*, 7th ed. Wiley India, New Delhi; 2013. pp. 261–278.
 - Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, et al. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *The Canadian Veterinary Journal* 2005; 46:255-259.
 - Khawaskar MV, Panchal MT, Dhama AJ, Patel JA. Seasonal variation in physic-morphological and functional characteristics of semen of Surti buffalo bulls and their interrelationships. *Indian J Field Vet* 2012; 8: 55-57.
 - Lafi SQ, Kaneene JB, Black JR, Lloyd JW. Epidemiological and economic study of the repeat breeder syndrome in Michigan dairy cattle. II. Economic modeling. *Preventive Veterinary Medicine* 1992; 14:99–114.
 - Modi LC, Patel PA, Patel SP, Patel GG, Joshi AH, Suthar DN. Prevalence of reproductive problems in Mehsana milk shed area of Gujarat. *Int J Anim Vet Med Sci* 2011; 5: 424-428.
 - Moss N, Lean IJ, Reid SWJ, Hodgson DR. Risk factors for repeat-breeder syndrome in New South Wales dairy cows. *Prev Vet Med* 2002; 54:91–103.
 - Narsimha Rao AVN, Kotayya K. Incidence and causes of repeat breeding among cattle and buffaloes under field conditions of Andhra Pradesh. *Indian Journal of Animal Health* 1980; 19:121–4.
 - Noakes DE, Parkinson TJ, England GCW. *Arthur's Veterinary Reproduction and Obstetrics* (8th edition) 2001. W. B. Saunders, UK.
 - Pascottini OB, Hostens M, Opsomer G. Cytological endometritis diagnosed at artificial insemination in repeat breeder dairy cows. *Reproduction in Domestic Animals* 2017; 53(2): 559-561.
 - Patel SB, Hadiya KK, Chavan DB. Incidence of reproductive disorders in cattle and buffaloes in Kaira district of Gujrat. *Indian J Field Vet* 2007; 3: 15-17.
 - Perez I, Rodriguez F, Espana J, Dorado M, Hidalgo JS. Follicular growth patterns in repeat breeder cows. *Veterinary Medicine Czechoslovakia* 2003; 48:1–8.
 - Perez-Marin CC, Moreno LM, Calero GV. Clinical approach to the repeat breeder cow syndrome. In: Perez-Marin CC, editor. *A bird's eye view of vet medicine*. In Tech Open; 2012, pp. 337-362.
 - Purohit GN. Ovarian and oviductal pathologies in the buffalo: occurrence, diagnosis and therapeutic approaches. *Asian Pac J Reprod* 2014; 3: 156-168.
 - Purohit GN. Recent development of in the diagnosis and therapy of repeat breeding cows and buffaloes. *CAB Rev Perspect Agricul Vet Sci Nutr Natur Res* 2008; 3(62): 1-33.
 - Raju KGS, Naidu KV, Rao KS. Incidence of reproductive disorders in buffaloes under field conditions of Andhra Pradesh. *Indian J Anim Reprod* 2007; 28: 46-48.
 - Roberts SJ. The repeat breeder cow. In: Roberts SJ, editor. *Veterinary Obstetrics and Genital Disease*. 2nd Ed. Edwards Brothers, Ann Arbor, MI; 1971. pp. 496–506.
 - Sah SK, Nakao T. Characteristics of repeat breeding buffaloes in Nepal. *Journal of Reproduction and Development* 2006; 52: 335-341.
 - Sharma HC, Dhama AJ, Sharma SK, Sarvariya NP, Kavani FS. Assessment of estrus detection and insemination efficiency of AI workers in buffaloes through plasma progesterone profile under field conditions. *Indian J Anim Sci* 2008; 78: 706-709.
 - Shelton K, Gayerie De Abreu MF, Hunter MG, Parkinson TJ, Lamming GE. Luteal inadequacy during the early luteal phase of subfertile cows. *J Reprod Fertil* 1990; 90:1.
 - Singh J, Dadarwal D, Honparkhe M, Kumar A. Incidences of various etiological factors responsible for repeat breeding syndrome in cattle and buffaloes. *International Journal of Veterinary Medicine* 2008; 6: 220-229.
 - Sreenam JM, Diskin MG. The extent and timing of embryonic mortality in the cow. In: Sreenam JM, Diskin MG, eds. *Embryonic Mortality in Farm Animals*. Boston: Martinus Nijhoff, 1986
 - Thirunavukkarasu M, Kathiaravan G. Factors affecting conception rates in artificially inseminated bovines. *Indian J Anim Sci* 2009; 79: 871-875.
 - Vala KB, Panchal MT, Ghodsara DJ, Hadiya KK, Trangadia BJ, Vagh AA. Studies on histopathological changes in genitalia of culled buffaloes (*Bubalus bubalis*). *Indian J Vet Pathol* 2011; 35: 197-199.
 - Yusuf M., Nakao T, Ranasinghe RBK, Gautam G, Long ST, Yoshida C, Hayashi A. Reproductive performance of repeat breeders in dairy herds. *Theriogenology* 2010; 73: 1220–1229.
 - Zemjanis R. "Repeat Breeding" or conception failure in cattle. In: D.A. Morrow (Editor) *Current Therapy in Theriogenology*, W.B. Saunders, Philadelphia, PA, 1980, 205-213.

SPIKING AND INTRA-SPIKING: A TOOL IN BREEDER MANAGEMENT

N. Aryal*



Abstract: *Spiking simply refers to the periodical addition and/or replacement of the male birds during the production period. It is an excellent technique to improve the mating capability of the male and increase the overall reproductive fertility. The decrease in fertility of the male is compensated by addition of 27-28 weeks old male birds. With decrease in the fertility after 40-45 weeks in case of broiler breeders, the method of spiking and inter-spiking provides a reliable approach to maintain the fertility in the herd. It provides a safe way to compensate for the decline in age related fertility. Spiking and inter-spiking have a major disadvantage in having the breach of the biosecurity of the poultry farm.*

Keywords: *biosecurity, broiler breeder, fertility, inter-spiking, spiking*

Introduction

The main role for broiler breeders is to be an optimal producer of fertile broiler eggs while having large size and weight which can be passed on to their offspring. Due to this selection for higher body weight and the large musculoskeletal frame, broiler breeders have been affected by greater fertility problems (Hocking, 1990; McGary et al., 2003; Bircik et al., 2005). Flock fertility is dependent on the productive status of the birds combined with the bird's interest and capability of mating (Bagley, 1997; Bahr and Bakst, 1987).

Age related decline in fertility

The level of fertility increases gradually with the beginning of the reproductive period in the breeder flock and is maximum i.e. around 95% at 30-40 weeks of age and declines rapidly after 40-45 weeks of age. (Casanovas, 2002)

Both male and female are responsible for age related decline in fertility but the major attribution is on male as fertility can be maintained by artificial insemination. Also one male mate with 8-10 females in a flock so the impact of male in overall fertility is high. (Chung, Smith, & Kattesh, 2012).

The male influence on fertility depends upon two

factors: mating activity and sperm quality. As males age, there is a reduction in the number of spermatozoa in the ejaculate and a reduction in semen. In addition, aging spermatozoa have reduced fertilizing ability in birds. However, studies where fertile eggs could be obtained by artificial insemination with semen from very old roosters suggest that sperm quality may not be as critical a factor as mating behavior (Casanovas, 2002).

In studies at the UGA, it was observed that old males that produce semen still have good sperm quality and acceptable fertilizing potential beyond 50 weeks of age. However, it also seemed true that a significant percentage of the males producing semen at 30 weeks of age did not produce semen at all at 50 or 60 weeks of age. Nevertheless, it was also observed that male mating activity is probably one of the most relevant factors affecting flock fertility.

Two factors can adversely affect mating activity. High yielding males, even in good physical condition, seem to lose mating interest quickly after 40-45 weeks of age. Males in poor physical condition (overweight, crippled) can have both reduced mating efficiency and mating interest. It

IVSA MIRROR V

is therefore critical to have a culling policy for incompetent males.

Management techniques to compensate the decline in mating activity

Assuming optimal environmental condition (including optimum water and feed quality), body weight control and feeding program, male placement and sex ratio, there are two management practices that can be followed to enhance mating activity Spiking and Intraspiking.

Spiking

Spiking is usually defined as the addition of new males to a 40 to 45 week-old broiler breeder flock to compensate for the age-related decline in fertility. As a general rule, males are added to compensate for mortality and to re-establish the original male to female ratio. Alternatively, 5 to 10% of old males are replaced due to their poor physical condition. Generally, the males that are added or used as replacements are 25 to 28 weeks old, have been grown on a pullet farm and moved to a stud farm until needed for spiking.

These young (spiked) males begin breeding while at the same time stimulating older males to resume mating thereby improving overall flock fertility. Spiking will assist in maintaining the optimum male to female ratio and the life-long fertility of the flock.

There are two main types of spiking programs:

- Spike as needed: Fertility is monitored closely and the flock is spiked when fertility values start to decline significantly or reach levels below 90%.
- Spike as a regular practice: All flocks are spiked at approximately 40-45 weeks of age, as a preventive measure, regardless of the fertility level at that moment.

Significant increases in fertility are found two to three weeks post-spiking but a slight decline in fertility is common during the first week

post-spiking. Spiking once in the life of the flock is normally enough. Flocks spiked twice on 8 to 10 week intervals also show good results, but it depends a lot on the quality of the primary males.

How to spike

Option 1 - Add to each house a minimum of 20% new young males that are at least 25 weeks of age and appropriate body weight.

Option 2 - Take out of one house, or compartment, all the good primary males and interspike with the other houses/compartments on the farm. The house with no males will receive the spiking males. This procedure will induce no competition between primary and spiked males and is a very effective way to preserve and utilize the spiking males to their full extent (recommended method).

Option 3 - A third program relates to the use of the heavier males in a young PS flock. The heavier males are pulled out of the flock at 26 weeks of age. They know where to eat and drink and how to mate. Placing these males in a flock with primary males or in a house where primary males have been pulled out normally works very well. This program is in particular popular in farms that have 100% floor operation, and where a higher % of males can be kept to 26 weeks of age (10%-11%) without getting into aggressive male behavior.

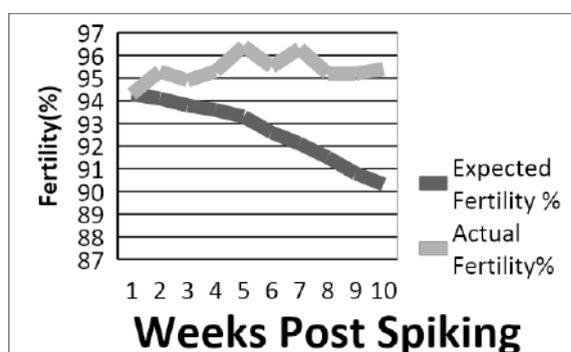


Figure: - Fertility trend after a 25% spiking of a 41week-old Cobb 500 flock with 28 week-old Cobb 500 males (Hypothetical data) Source: Casanovas, P. (2002). Poultry informed professionals.

IVSA MIRROR V

Problems related to Spiking

Spiking causes an increase in aggression and sexual interference (males preventing other males from mating) and results in male mortality.

Biosecurity could be breached because adult males, reared in other farms and housed with several ages on a stud farm, would be incorporated into an established flock.

Spiking programs generally require stud farms that not only represent an extra cost but also are not normally the best conditions to house future breeder males. On stud farms males do not have contact with females; their sexual behavior and mating skills are likely compromised or at least require a learning period in the breeder house before they mate efficiently.

If roosters are housed under the stud farm conditions after 25 weeks of age, aggression and high mortality will result, reducing the number of males available for spiking purposes.

Intra-spiking

As a solution to the spiking problems (mainly the biosecurity risks) and the observation that spiking with young males produced a stimulatory effect on old male's mating activity, the idea of intra-spiking was developed.

“Intra” (“within” in Latin) means that there is no importation of males to the farm. Males from the same age are exchanged between houses of the same farm or between pens of the same house at 40-45 weeks of age, in order to achieve a disruption of the established hierarchy and stimulate mating activity on the original males similar to that created by the young males in regular spiking. Intra-spiking should have three advantages over regular spiking:

- Almost zero cost of the practice (no extra males need to be housed).
- No biosecurity risks.

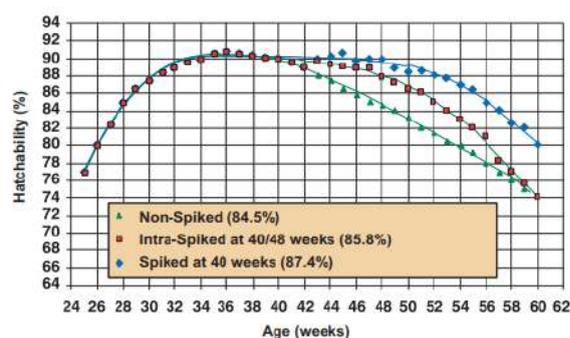
- Quicker and more efficient use of the added males since they have mating experience and a similar conformation as the established males. That should allow them to successfully compete and mate from the beginning.

One possible disadvantage is that in intra-spiking there are no young males to take over after the 6 weeks of stimulation and, therefore, its effects cannot be expected to last as long as a regular spiking.

Mating activity increases significantly after intra-spiking. The effects may last between 6 and 8 weeks. One advantage is that with intra-spiking the males exchanged are already trained in mating and usually have similar weight and maturity as the existing males, improving their chances to compete successfully.

Intra-spiking increases male aggression for two weeks after mixing. There are usually no problems with male or female mortality.

Hatchability does not go up dramatically after intra-spiking. However, the persistency of hatchability is improved and with a double intra-spiking procedure one can expect an increase between 1 and 1.5% in the overall hatchability of the flock.



Source: Breeder Management Guide (2016), Cobb Vantress

References

- Bilcik B, Estevez I and Russek-Cohen E (2005). Reproductive success of broiler breeders in natural mating systems: the effect of male-male competition, sperm quality, and morphological characteristics. *Poultry Science*, 84, 1453-1462.
- Casanovas, P. (2002). Poultry informed professionals.

IVSA MIRROR V

- Chung, K. M., Smith, M. O., & Kattesh, H. G. (2012). The influence of double interspiking on production and behavior in broiler breeder flocks in elevated temperature conditions. *Journal of Applied Poultry Research*, 21(1), 63–69.
- Hocking P (1990). The relationships between dietary crude protein, body weight, and fertility in naturally mated broiler breeder males. *British Poultry Science*, 31, 743- 757
- Ottinger MA, Duchala CS and Masson M (1983). Age related reproductive decline in the male Japanese quail. *Horm. Behav*, 17, 197-207.
- McGary S, Estevez I and Russek-Cohen E (2003). Reproductive and aggressive behavior in male broiler breeders with varying fertility levels. *Applied Animal Behaviour Science*, 82, 29-44

SURGICAL CORRECTION OF AURAL HEMATOMA IN DOG

B. Pokhrel^{1*}, S. Shrestha², Y. Panth¹



Abstract: A 3 years old dog named Leo was presented to Himalayan Animal Rescue Trust (HART) with an aural hematoma on right ear. It was treated with surgical procedures so as to overcome the chance of recurrence that is very common in other techniques like non-surgical methods. Induction was done using ketamine and diazepam (1:1), a longitudinal incision was given along the length of the pinna and after curetting the cavity, parallel horizontal mattress sutures were used throughout the pinna so as to obliterate dead space efficiently. The animal was provided with oral antibiotics and NSAIDs to check bacterial infection and pain post operatively. E-Collar was used to prevent scratching and further aggravation of the wound.

Keywords: ear, HART, hematoma, surgical

Introduction

Aural Hematoma reflects collection of blood in the inner or the outer aspect of the ear flap between skin and cartilage giving soft and fluctuating swelling on concave part of the pinna and generally characterized by purplish round swelling (Kuwahara, 1986a; Nandi, Halder & Hoque, 2009). Aural hematomas can be caused by direct damage (eg, bite wounds, vehicular trauma), allergic reactions, Otodectes cynotis infection, hyperadrenocorticism, hypothyroidism, immunogenic reactions but are more common from head shaking and ear scratching associated with otitis externa or atopy (Joyce & Day, 1997; Pereira, 2006; Gyorffy & Szijarto, 2014; Seibert & Tobias, 2013; Wobeser, 2002). Aural hematoma can occur in any animal, but are more common in animals with long and pendulous ears like Dog and Pig (Drolet, Hélie, & Allaire, 2016). In a research conducted by Mikawa et. al. (2005), around 66% of the dogs that had aural hematoma were dogs with pendulous ears. The pressure of accumulated blood in an aural hematoma is often painful, and it prevents the tissue from remaining in apposition. Hematomas can occur very quickly, within a few minutes of a vessel rupturing. Hematomas are initially soft but become firm and thickened over time as a result of fibrosis.

Fibrosis can ultimately result in the pinna assuming a curled-up conformation often called ‘cauliflower ear’ (Brown, 2010), so these conditions should be repaired very quickly by either surgical or non-surgical method. The therapeutic plan must include proper identification and treatment of underlying cause of irritation, facilitation of drainage, tissue apposition maintenance and recurrence prevention (Hall, Weir & Ladlow, 2016).

Case Description

History and Clinical Observation

A 3 years old dog, named Leo, was brought to Himalayan Animal Rescue Trust (HART) with a unilateral (right side) ear swelling on it for a week. The animal had a history of scratching its ear for a week and the owner drained the swelling area but that recurred after a week again.

Upon physical examination there was no change in vitals of the animal. Upon palpation in the swollen area, we found some fluctuating mass inside the skin of the right ear.

Diagnosis and Treatment

After history and clinical examination, the case was diagnosed with aural hematoma. Surgical

¹Agriculture and Forestry University, Chitwan, Nepal

²Himalayan Animal Rescue Trust, Chitwan, Nepal

*Corresponding author: drbinodvet@gmail.com

IVSA MIRROR V

treatment was favored in place of non-surgical technique. The dog was sedated with a combination of xylazine and ketamine and was induced with ketamine and diazepam (1:1). The hairs were clipped and shaved in the part to be operated and the surgical site was prepared aseptically.

The dog was kept in lateral recumbency with its affected ear dorsally appearing and on the concave side over the length of ear, a longitudinal incision about 5 cm was made parallel to the margin of ear. The hematoma was removed and the cavity was curetted with saline and povidone iodine, using sterile gauze pieces and forceps to destroy the fibrin and remove it. After proper destruction of the fibrin in the cavity, full thickness mattress suture using Nylon was placed parallel to the skin incision without opposing the cut edges for sufficient drainage. A number of mattress sutures were again placed parallel to the branches of auricular artery and first suture to obliterate all the dead space from the cavity, to prevent its recurrence. After the completion of surgery, an E-Collar was used to protect the ear from self-mutilation by dog.

Post-Operative Care

The patient was medicated with oral antibiotics (Amoxy-clav) to check bacterial infections, oral meloxicam (NSAIDs) and topical herbal spray to prevent flies from contaminating the wound.

Discussion

Violent head shaking, as a result of ear disease, irritation or injury, results in cartilaginous damage and vascular injury to branches of the great auricular artery, which is the major source of hemorrhage. In aural hematoma, there is swelling of ear in the concave side until the pressure of hematoma equalizes the pressure of feeder arteries (Lewis, Wiles, Llewellyn-Zaidi, Evans, & O'Neill, 2018). The blood supply to the external ear is mainly via branches of the posterior auricular artery. These branches run in the corium on the convex side of the ear and give



Figure SEQ Figure * ARABIC 1
Hematoma in the concave part of Ear



Figure SEQ Figure * ARABIC 2
Surgical Site for Aural Hematoma

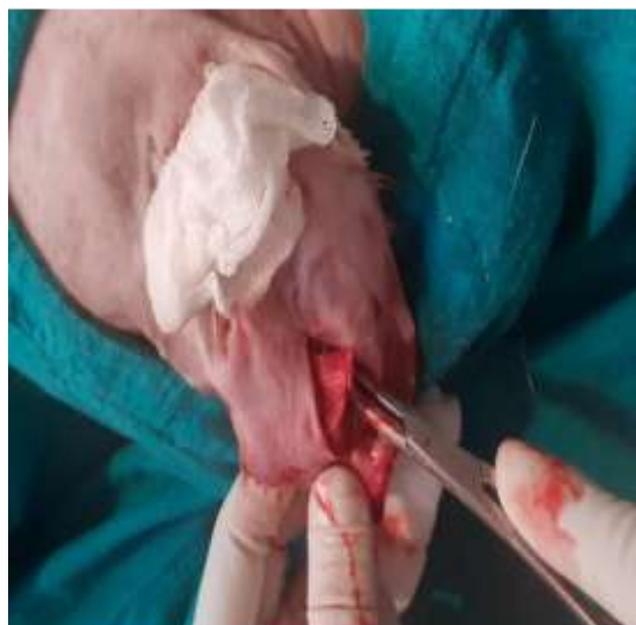


Figure SEQ Figure * ARABIC 3
Curation with Saline and PVI

off smaller vessels, which supply the concave side through the numerous foramina in the cartilage. Hemorrhage might be due to rupture of these small vessels where they pass through the foramina (Larsen, 1968).

IVSA MIRROR V

Surgical correction was favored for aural hematoma, because of its feasibility and more advantages over other methods of correction. There are many treatment options, including needle aspiration (MacPhail, 2016), tube drainage (Kagan, 1983), teat cannula drainage (Wilson, 1983), incision drainage and bandaging, but the most common and effective procedure is surgical intervention by incising over the hematoma, removing all blood, clots and fibrin and then securing the cartilage in apposition with sutures until scar tissue forms. Surgical correction also helps in relieving the pain of the ear instantly after surgery (Brown, 2010). Next surgical technique for correction of aural hematoma includes auricular pillow method, where a full length incision like above is incised and sterile pebbles are padded with cotton wool and further with gauze bandage for the absorption of hematoma fluid that act as auricular pillar on the convex side and the ear is supported by adhesive tape. The bandage should be replaced every day for the first three day and the pillow is removed after 14 days. This method has better cosmetic results than other techniques (Eyarefe, Oguntoye & Emikpe, 2013).

Another technique called as CO2 laser technique is a novel technique where the laser is used to make an incision into the hematoma to allow for evacuation of the blood, and then multiple, small incisions are made over the surface of the hematoma to stimulate adhesions between the tissue layers (Dye, Teague, Ostwald & Ferreira, 2002). Vacuum drains can also be used, where two small incisions are used to position a vacuum drain into the incised hematoma cavity, the drain exited at the base of the pinna and adjacent cervical skin, the free end of the drain attached to a vacuum reservoir for 18 to 21 days; drains and skin sutures are removed at this time along with the protective Elizabethan collar. All hematomas resolves during the minimum 6-month period by this technique (Pavletic, 2015). Other alternatives include sucking of fresh hematoma by *Hirudo medicinalis*



Figure 4: Parallel Horizontal Mattress

(Canpolat & Saglam, 2004) and using combination of systemic and local corticosteroids (Kuwahara, 1986b; Seibert & Tobias, 2013).

Several suture lines (1-3) using absorbable suture can be placed intradermally parallel to and on either side of the incision in the concave part including the cartilage. This technique is advantageous because of the minimal need for aftercare, absence of discomfort or irritation caused by external sutures, and lack of need for suture removal (MacPhail, 2016). Each suture was kept 5-10 mm apart with each other parallel to the major vessels, reducing the risk of ligating the major vessels. Direct apposition of the edges of the incised part of skin was avoided leaving 3-5 mm gap to allow proper drainage.

There are many complications that may arise after surgical remedy of aural hematoma like necrosis due to higher tension of sutures, reoccurrence of the hematoma, secondary bacterial infections and most common viz cosmetic alteration. Tight sutures cause

discomfort, pain, irritation, edema and necrosis, and also cause the cosmetic alteration. There is always a chance of recurrence or occurrence in another pinna, when underlying cause or predisposing factors are not corrected.

Conclusions

Aural hematoma is one of the frequent clinical cases around the globe, which can lead to severe damage to cosmetic ear of dogs. If the condition of aural hematoma is left untreated, it may lead to fibrosis and cauliflower like occurrence of the ear. It is very important to treat all the underlying and predisposing causes of aural hematoma by proper clinical examination and diagnosis of the condition. There are several methods for correction of aural hematoma which are listed above but inspite of having many easy and novel techniques, surgical method is favored due to its advantages, less cosmetic damage and rapid healing of the wound.

References

- Brown, C. (2010). Surgical management of canine aural hematoma. *Lab Animal*, 39(4), 104–105. DOI: 10.1038/laband0410-104
- Canpolat, I., & Saglam, N. (2004). Treatment of Aural Hematomas in Dogs with the Medicinal Leech, *Hirudo Medicinalis*. *Doğu Anadolu Bölgesi Araştırmaları*, 67-69
- Drolet, R., Hélie, P., & Allaire, S. D. (2016). Pathology of ear hematomas in swine. *Journal of Veterinary Diagnostic Investigation*, 28(3), 244-248. DOI: 10.1177/1040638716630768
- Dye, T. L., Teague, H. D., Ostwald, D. A., & Ferreira, S. D. (2002). Evaluation of a Technique Using the Carbon Dioxide Laser for the Treatment of Aural Hematomas. *Journal of the American Animal Hospital Association*, 38(4), 385-380. DOI: 10.5326/0380385.
- Eyarefe, O. D., Oguntoye, C. O., & Emikpe, B. O. (2013). A Preliminary Report on Aural Hematoma Management with Auricular Pillow Method. *Global Veterinaria*, 11(1): 44-48. DOI: 10.5829/idosi.gv.2013.11.1.73196
- Hall, J., Weir, S., & Ladlow, J. (2016). Treatment of canine aural hematoma by UK Veterinarians. *Journal of Small Animal Practice*, 57(7), 360-364. DOI: 10.1111/jsap.12524
- Kagan, K. J. (1983). Treatment of Canine Aural Hematoma With an Indwelling Drain. *J Am Vet Med Assoc.*, 183(9):972-974.
- Mikawa, K., Itoh, T., Ishikawa, K., Kushima, K., Uchida, K., & Sihi, H. (2005). Epidemiological and Etiological Studies on 59 Aural Hematomas in 49 Dogs. *Jpn J Vet Anesth Surg*, 36(4), 87-91. DOI: 10.2327/jvas.36.87
- Joyce, J. A., & Day, M. J. (1997). Immunopathogenesis of canine aural haematoma. *Journal of Small Animal Practice*, 38(4), 152–158. DOI: 10.1111/j.1748-5827.1997.tb03453.x
- Kuwahara, J. (1986a). Canine and feline aural hematoma: clinical, experimental, and clinicopathologic observations. *American Journal of Veterinary Research*, 47(10), 2300–2308.
- Kuwahara, J. (1986b). Canine and feline aural hematoma: results of treatments with corticosteroids. *J Am Vet Med Assoc*, 22, 641-7
- Larsen, S. (1968). Intrachondral Rupture and Hematoma Formation in the External Ear of Dogs. *Path. vet*, 5: 442-450.
- Lewis, T. W., Wiles, B. M., Llewellyn-Zaidi, A. M., Evans, K. M., & O'Neill, D. G. (2018). Longevity and mortality in Kennel Club registered dog breeds in the UK in 2014. *Canine Genetics and Epidemiology*, 5(1), DOI: 10.1186/s40575-018-0066-8
- MacPhail, C. (2016). Current Treatment Options for Auricular Hematomas. *Veterinary Clinics of North America. Small Animal Practice*, 46(4), 635–641. DOI: 10.1016/j.cvsm.2016.01.003
- Nandi, S. K., Halder, S., & Hoque, M. (2009). A Textbook on Veterinary Surgery and Radiology (1st ed.). Kalyani Publishers, India.
- Pavletic, M. M. (2015). Use of Laterally Placed Vacuum Drains for Management of Aural Hematomas in Five Dogs. *J Am Vet Med Assoc.*, 246(1), 112-7. DOI: 10.2460/javma.246.1.112.
- Pereira, S. (2006). Aural hematomas : Underlying Causes. *Banfield*, 24-30.
- Gyorrffy, A., & Szijarto, A. (2014). A New Operative Technique for Aural Haematoma in Dogs : A Retrospective Clinical Study, *Acta Veterinaria Hungarica*, 62(3), 340–347. DOI: 10.1556/AVet.2014.016
- Seibert, R., & Tobias, K. M. (2013). Surgical Treatment for Aural Hematoma. *Clinician's brief*, (3), 29–32.
- Wilson, J. W.(1983). Treatment of Auricular Hematoma, Using a Teat Tube. *J Am Vet Med Assoc.*, 182(10), 1081-1083.

TIBIA AND FIBULA FRACTURE IN DOG

A CASE REPORT



S.P. Bhusal*

Abstract: *A dog with clinical signs and symptoms of pain in the left hindlimb was brought to the Veterinary Hospital and Livestock Service Expert Center (VHLSEC), Bharatpur. The history brings out that the dog jumped from the roof of the owner's house causing injury to the leg. Slight dislocation was felt in the tibial portion of the bone on immediate examination of the affected part. Furthermore, the X-rays revealed that there was a breakage in the tibia of the left hind leg. The case was handled by plastering and bandaging the fractured limb using closed reduction technique. The prognosis of the animal was good following the initial treatment of the wound and fracture reduction and retention.*

Keywords: dog, fracture, reduction, tibia, X-rays

Introduction:

The tibia and fibula fractures are the most encountered case in canines which is around 20% of long bone fractures (Jain et al., 2016). The common cause of tibia fracture is due to fall from height or being stepped on (Raouf et al., 2019). Apart from this, accidents due to vehicles and injury caused by attack or hitting by other animals and humans are major causes of fractures in dogs respectively (Minar et al., 2013). In addition, some disease conditions can cause fractures of bones in dogs. There are numerous methods of (including external and internal fixation) treating fractured cases which majorly depends on the severity of cases and age of the animals as well. In the case of younger animals the prognosis is better (Raouf et al., 2019). In contrast, older animals have a low tendency to heal the fracture once they break. The animal with simple fracture requires only closed reduction and external fixation while for the complicated cases, open reduction with pinning or plating or wiring is necessary (Li et al., 2019 & Minar et al., 2013). The complicated cases require more care even after reduction for good prognosis because they always have a chance of secondary infection and definitely this could bring further complication in the healing process.

Case history and observation:

A three years old male dog was brought to the Veterinary Hospital and Livestock Service Expert Center (VHLSEC), Bharatpur which is a non-private diagnostic and treatment center for animal cases. The dog owner came with the history of jumping from the roof of his house and the animal was not walking with one left hind limb. On physical examination, crepitus, pain and swelling of the hindlimb was felt at the level of tibia and fibula bone, manifesting as the fracture of left tibia. The case was further sent for radiography to confirm the ongoing presumption. The radiograph revealed fracture of the tibia and fibula shaft.

Treatment:

The animal was sedated first using Xylazine @ 2 mg/kg b.wt. intramuscularly followed by meloxicam 0.5 mg/kg b.wt. through the same route. The animal was positioned in lateral recumbency with the affected part positioned outside. The tibial bone was approached gently and closed reduction was made manually. After reduction, the affected part hair was clipped and boric acid was sprinkled over the clipped area. A thick layer of cotton was placed over the fractured site covering the upper joint with femur



Fig 1: X-ray showing breakage of Tibia and Fibula of the left hind leg of dog.

and lower joint with carpal. Plaster of Paris was applied after dipping into the lukewarm water until the bubble emergence ceased. The material was placed with due precaution by not altering the previously reduced structure and much attention was paid to include both the upper and lower joint of the tibia and fibula. The limb was fixed with the body with the help of commercially available bandages to minimize the unwanted movements so as to achieve faster healing. The post-operative care owner was instructed to feed the dog a calcium rich diet with good composition of vitamins and minerals and also advised to restrict the movement as much as possible.

Prognosis:

Accurate reduction of fractured bone with normal anatomy by using proper fixation technique is essential for good prognosis (Glyde & Avnett, 2006). Although the normal healing time is 4 to 5 weeks for young dogs, we allowed one and half months to heal the fracture after the casting of Plaster of Paris was done. The result was appreciated with good healing of the fractured part of the bone.

Discussion:

Tibia is located superficially so that the chances of open fracture are common (Glyde & Avnett, 2006). But in our study the fracture is closed and the isolated tibia/fibula fracture is managed with closed reduction and application of plasters of Paris externally. Though mentioned by Zaal et al.,



Fig 2: X – ray showing healed tibia and fibula bone after plastering and bandaging.

1997, rigid fixation by external fixation or internal fixation is a method of choice for isolated tibial fracture especially in case of mature cats or dogs. It is documented that the traumatic events are mainly responsible for fractures in dogs (Liburdoni et al., 2016). Motor vehicle accidents, falling down from height, stuck in doors, slipping down, and bitten by the dogs were major reasons for bone injury respectively (Li et al., 2019 & Minar et al., 2013). In our study, the dog had jumped from the roof of the house. Although there is high rate of non-union of tibial fracture (25%) and the theory regarding tibia suggest that there is limited muscle attachment to the distal part of the tibia and high cortical: cancellous bone ratio of the distal third of the tibia as like in our case, result slow rate of healing compared to the proximal tibia (Glyde et al., 2006) our treatment showed complete healing of fracture part without any abnormality in radiograph after one and half month of casting.

Conclusions:

Tibia/fibula fracture is second most encountered cases in dogs after femur (Balagopalan et al., 1995, Glyde & Avnett, 2006, Liburodoni et al., 2016, Minar et al., 2013). Pointing the fracture is the major thing in treatment. Care must be taken for evaluating location, type, involvement of the joint, fragment direction and number and whether open or closed. Restitution of the normal anatomy of the fractured part is the major aim of

IVSA MIRROR V

treatment. So, accurate reduction of fractured bone with normal anatomy by using proper fixation technique is essential for good prognosis (Diamond, 2015). The present case report documents the successful management of fracture in the tibia of the dog by using reduction techniques and then applying plaster of Paris externally.

Acknowledgement:

The author is thankful to the Veterinary Hospital and Livestock Service Expert Center for the giving opportunity to handle the case and to the owners of the patient for their full cooperation during the case management. Similarly, I would like to thank my internship partners Ranjeet, Sujata, Bibhuti and all the personnel and JTA students at that period.

References:

- Balagopalan T. P., Devanand C.B., Rajankutty K., Amma T. S., Nayar S. R., Varkey C. A., Jalaluddin A. M., Nayar K. N. M., and George P. O. (1995). Fractures in dogs, a review of 208 cases. *Indian J. Vet. Surg*, 16 (1), 41-43.
- Diamond, D. (2015). Fracture of the femur (thigh bone) in dogs, 12 Sep., 2015, <https://www.petplace.com/article/dogs/pet-health/fracture-of-the-femur-thigh-bone-in-dogs/>
- Glyde M. and Avnett R. (2006). Tibial fracture in the dog and cat: options for management. *Irish Veterinary Journal*, 59(5).
- Jain R, S Parihar A., Kamble S., S Parihar Y., Ganguly S.(2016). Multiple fractures in tibia bone of dog: A case study. *International Journal of Contemporary Microbiology*, 2(1), 82-83. doi:10.5958/2395-1796.2016.00019.3
- Liburdoni R. D. N., Serafini G. M. C., Oliveria C. D., Schimites P. I., Chaves R. O., Feranti J. P. S., Costa C. A. S., Amaral A.S. D., Raiser A. G., Soares A. V. (2016). Appendicular fractures of traumatic etiology in dogs: 995 cases (2003-2013). *Ciencia Rural, Santa maria*, 46(3), 542-546.
- Li, J., Kacena, M. A., & Stocum, D. L. (2019). Fracture healing. *Basic and Applied Bone Biology*, 235–253. doi:10.1016/b978-0-12-813259-3.00012-9
- Minar M., Hwang Y., Park M., Kim S., Oh S., Choi S. and Kim G. (2013). Retrospective study on fractures in dogs. *Journal of Biomedical Research*, 14(3), 140-144. doi: [10.12729/jbr.2013.14.3.140](https://doi.org/10.12729/jbr.2013.14.3.140)
- Raouf M. Abd. EI, Ezzeldein S.A. and Eisa E. F. M. (2019). Bone fractures in dogs: A retrospective study of 129 dogs. *Iraqi Journal of Veterinary Sciences*, 33(2), 401-405.
- Zaal M. D. and Hazewinkel H. A. W. (1997). Treatment of isolated tibial fractures in cats and dogs. *Vet. Quart*, 19(4), 191-194. <https://doi.org/10.1080/01652176.1997.9694770>

LEARN FROM THE PAST: THE ZONOTIC PERSPECTIVE OF SARS, MERS, AND COVID-19



S. Annisa^{1*}, H. Jawad²

Abstract: *When COVID-19 was announced as a pandemic by the WHO, it got much everyone's attention including researchers. That is why veterinarians are also considering such pandemics, more so from the zoonotic point of view to save both animals and humans. In the past, MERS and SARS outbreaks affected humans and food animals as well. We are comparing the causative agent's structure, mode of transmission and effect on the community of SARS, MERS and COVID-1. By learning from previous outbreaks, some plans and studies must be made and conducted to overcome this pandemic. This paper aims to present and compare the animal reservoir of those three diseases by collecting information from various articles, journals and books. With this information, there will be a better understanding of how the zoonotic aspect is important in a disease outbreak. Moreover, better surveillance and vaccine development are expected to be obtained through previous experiences.*

Keywords: *pandemic, COVID-19, MERS, one health, SARS, zoonosis*

Introduction

On March 11th, 2020, COVID-19 was declared as a pandemic and became a global health crisis. This outbreak started from pneumonia patients reported in Wuhan, China in late December 2019. At first, the causative agent was unidentified. Later, Coronavirus study group of the International Committee on Taxonomy of Viruses initially named the virus as novel Coronavirus (2019-nCoV), then it was renamed as SARS Coronavirus 2 (SARS-CoV-2) and it was identified as the causative agent of COVID-19 (Gorbalenya et. al., 2020). Before that, we also faced the outbreak of severe acute respiratory syndrome (SARS) in China and the outbreak of Middle East Respiratory Syndrome (MERS) in Saudia Arabia. Those diseases were caused by the same group of viruses - Coronavirus. Alpha-CoV, beta-CoV, delta-CoV, and gamma-CoV are the four genera in which coronaviruses have been classified. Each of them has its own host such as humans, swine, dog, bird, and other animals.

To date, there are several human CoV. The alpha-coronaviruses are named as HCoV-NL63 and HCoV-229E. The beta-coronaviruses are

named as HCoV-OC43, HCoV-HKU1, SARS-CoV or SARSCoV-1 that causes SARS, MERS-CoV that is caused MERS and the latest is SARS-CoV-2 as the cause of COVID 19. The Human CoV-229E is transmitted to humans through alpacas and it originates from bats while Human CoV-OC43 is spread from rodents through cattle then infecting humans (Corman et. al., 2014).

The role of the animals in the coronavirus outbreak (eventually a COVID-19 pandemic) is proved by the emergence of three coronaviruses with a zoonotic origin. In SARS-CoV-2 cases, the virus adapts to humans and has higher virulence that causes higher transmission of the disease (Cavanagh, 2007; Pedersen, 2014). Thus, the understanding of how zoonotic aspects can affect the human disease outbreak is important due to the unpredictability of the evolution of the virus and how these family viruses can generate strains with different biological properties. This review article aims to present comprehensive current literature of the three pathogenic CoV that have already become epidemics and pandemics by showing the structural differences

¹Airlangga University, Indonesia

²The Islamia University of Bahawalpur, Bahawalpur, Pakistan

*Corresponding author: suwaibatul.annisa@gmail.com

and the zoonotic perspective, such as the animal reservoirs and potential intermediate hosts or the susceptible animals.

Methods

The data is collected from various online articles, journals, and books. Each data about SARS, MERS, and COVID-19 is presented and compared to give perspective about the structural difference and the zoonotic relation.

Structure of Sars-Cov-1, Mers-Cov, and Sars-Cov-2

SARS-CoV is a positive-strand RNA virus covered with an envelope and composed of about 30 kb nucleotides (Marra et. al., 2003). The genome of SARS-CoV-2 also consists of positive-strand RNA. The four primary structural proteins of SARS-CoV-2 are spike protein (S), an envelope protein (E), membrane protein (M) and nucleocapsid protein (N) (Wu et. al., 2020). The spike protein is a type I glycoprotein that cover the surface of the virus and helps in attachment to the host cell. Because of its ability to bind with the host receptor, S protein is important in the viral attachment. Like two other viruses, MERS-CoV also has four primary structural proteins that have already been mentioned.

The SARS CoV receptor-binding domain (RBD) will attach to the angiotensin-converting enzyme 2 (ACE2) as the receptor of SARS-CoV (Li et. al., 2003; Li, Li, Farzan, & Harrison, 2005). Due to its physiological function of blood pressure and blood volume regulation, the ACE2 is present in various organs such as the heart, lungs, kidney, gastrointestinal tract, blood vessels and some other organs (Anguiano, Riera, Pascual, & Soler, 2017). Recently, it has been found that human ACE2 contributes in the entry process of SARS-CoV-2 into the cells (Letko, Marzi, & Munster, 2020; Zhou et. al., 2020). Similar to the SARS CoV, RBD of SARS-CoV-2 also binds with ACE2 in cells. Therefore, the ACE2 is determined as the cell-surface receptor

for the causative agent of COVID-1 (Luan, Lu, Jin, & Zhang, 2020). As explained above, the ACE2 determines the infection rate of SARS-CoV-2 and SARS-CoV into the cell. The MERS-CoV however binds with dipeptidyl peptidase 4 (DPP4) to enter the cells (Raj et. al., 2013).

Zoonotic Perspective

1. The Natural Reservoir and Intermediate Host

Like pets and livestock, wild animals are also believed to be the natural reservoir and have an important role in highly pathogenic virus transmission such as Ebola, Coronavirus, Nipah and other deadly viruses. Animal markets had yielded the first clue in the SARS-CoV outbreak of 2002–2003, and the seafood and wildlife market in Wuhan, China is related to the initial outbreak of SARS-CoV-2 which has become a global crisis in recent days. It is presumed that wild animals also had a vital involvement in the emergence of SARS-CoV-2. However, the species and the spread of the virus and the species-crossing process remain unclear. With higher than 70% similarity with beta-CoV in genetic characteristics, SARS-CoV-2 is the latest member of the other six coronaviruses that can infect humans. The SARS-CoV-2 is presumed to have come from bats. Yet, further confirmation of the transmission process is needed whether it is directly transmitted from bats or it is transmitted via intermediate host (Jin et. al., 2020). The reason behind why bats are believed to be the origin of the virus is the result of whole-genome identification of SARS-CoV-2. It is shown that 96% of this virus is identical to a bat coronavirus. Thus, bats are the strongest candidate for becoming the natural host of the SARS-CoV-2 (Zhou et. al., 2020).

According to Eric M, due to their comparable biological characters, SARS-CoV-2 and SARS-CoV are two closely related groups. Moreover, both animals have a zoonotic origin that indicates their ability to infect inter-species.

SARS-CoV-2 is evidently presumed to have zoonotic origins. This hypothesis is supported by the discovery of several viral sequences that are related to SARS-CoV-2 in several animal species such as *Rhinolophus* bats and Malayan pangolins (*Manis javanica*) (Zhang, Wu, & Zhang, 2020; Zhou et. al., 2020). Nevertheless, the coronavirus of the bat *Rhinolophus affinis* can not bind well to the human cellular receptor due to its significant difference of the RBD of spike protein in RaTG13 (Anderson, Rambaut, Lipkin, Holmes, & Garry, 2020) whereas the Spike protein of SARS-CoV2 can bind well to the ACE2 of humans. Moreover, the other species that have a human ACE2 homologue cell-surface receptor can bind well with SARS-CoV-2 (Wu et. al., 2020).

Some studies showed that the coronavirus was found in *Rhinolophus* bats, in masked-palm civets (*Paguma larvata*) and in raccoon dogs (*Nyctereutes procyonoides*) was genetically similar or related to SARS-CoV (Gouilh et. al., 2018; Guan et. al., 2003). Thus, we can presume that the virus that causes SARS also came from one of the zoonotic species.

The Middle East Respiratory Syndrome (MERS) was frequently transmitted from animal to human. Due to its close-relation with coronavirus from bats, it is believed that MERS-CoV originated from bats; however there is no conclusion that bats are the natural reservoir of MERS-CoV. The first evidence that proved the role of dromedary camels in MERS transmission came from a serological study that showed a specific antibody found in dromedary camels. Some animals, such as alpaca, and non-human primates are susceptible to MERS-CoV.

To face the COVID-19 pandemic which is still rapidly growing, WHO has advised people to avoid any contact with wild animals, to process animals' products properly, and keep a distance (6 feet distance) with anyone with or without symptoms (Peeri et. al., 2020).

2. Animal Laboratory

According to Rowe's research that had been published in 2004, *Macaca mulatta* has limited utility to become SARS-CoV animal model in showing SARS pathogenesis and the evaluation therapies. Because of high titer in the respiratory tract of mice and findings in mice after SARS-CoV inoculation, mice make a good choice to be the animal model of vaccine, antiviral and immunoprophylaxis researches. The mice also can be the animal model of SARS pathogenesis. Hamsters also can be the ideal animal model because of the high titer in the respiratory tract and other susceptible animals can be ferrets, macaques and African green monkeys. Squirrel monkey and moustached tamarin are not susceptible to SARS-CoV (Subbarao & Roberts, 2006). The macaques are one of the MERS-CoV susceptible animals. Therefore, the macaques can be a suitable model for MERS-CoV pathogenesis and its vaccine development. However, ferret, mice, hamster not infected by MERS-CoV, dromedary camel and alpaca can be the animal model because of its susceptibility (Li et. al., 2005). Based on Jianzhong Shi's research, ferrets and cats have high susceptibility, whereas dogs have low susceptibility. The swine, chicken, ducks and other livestock animals are not susceptible (Shi et. al., 2020).

Besides collecting data from some animal samples (for example: swab method), Junwen and his team did sequence analysis of RBM of Spike protein from SARS-CoV-2 and SARS-CoV, and its ability to bind to some mammals' ACE2 receptors.

The specificity of between virus RBD and cellular receptors determine the probable host of the disease. This structure simulation, ACE2 and RBD binding were conducted to predict the host of COVID-19 (Luan et. al., 2020). The mammalian name list was selected from wild animals' protection of Hubei Province and Jiangxi Provinces and used in this simulation.

IVSA MIRROR V

Some of the mammals are bats, primates, dogs, and cats. From 42 mammals in the list, the virus RBD of SARS-CoV-2 and SARS-CoV could not bind to cell receptors in only 12 species. According to these data, finding the data from samples and in vivo research is still important to confirm the data.

The information about which animal is susceptible or has the ACE2 that can bind to SARS-CoV-2 is important to predict the transmission and for further epidemiological studies of COVID-19 or even predict the future outbreak. From the data and research that have been done, the animal-human transmission that occurred in SARS and MERS cases were in direct contact with the animals who had been infected. These two viruses have the same characteristic in animal-human transmission so we have a strong reason to give more attention to zoonotic transmission of COVID-19.

Conclusions

The SARS-CoV and SARS-CoV have the same cellular receptors, but the MERS-CoV has a different cellular receptor than that of SARS. This difference leads to different susceptible animals and also a different suitable animal laboratory. By knowing and understanding the structure and its zoonotic perspectives such as the natural reservoirs, intermediate hosts and the suitable animal model from these three similar viruses, the surveillance of some susceptible animals should be considered and further studies must be conducted to develop the vaccine of the COVID-19 which has become the global public health concern.

References

- Gorbalenya, A., Baker, S., Baric, R., de Groot, R., Drosten, C., Gulyaeva, A., ... & Penzar, D. (2020). Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species has severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature microbiology*, 2020, 03-04
- Corman, V. M., Kallies, R., Philipps, H., Göpner, G., Müller, M. A., Eckerle, I., ... & Drexler, J. F. (2014). Characterization of a novel betacoronavirus related to middle East respiratory syndrome coronavirus in European hedgehogs. *Journal of virology*, 88(1), 717-724.
- Cavanagh, D. (2007). Coronavirus avian infectious bronchitis virus. *Veterinary research*, 38(2), 281-297.
- Pedersen, N.C., 2014. An update on feline infectious peritonitis: virology and immunopathogenesis. *Vet. J.* 201, 123-132.
- Marra, M. A., Jones, S. J., Astell, C. R., Holt, R. A., Brooks-Wilson, A., Butterfield, Y. S., ... & Cloutier, A. (2003). The genome sequence of the SARS-associated coronavirus. *Science*, 300(5624), 1399-1404.
- Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., ... & Yuan, M. L. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265-269.
- Li, F., Li, W., Farzan, M., & Harrison, S. C. (2005). Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science*, 309(5742), 1864-1868.
- Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., ... & Choe, H. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*, 426(6965), 450-454.
- Anguiano, L., Riera, M., Pascual, J., & Soler, M. J. (2017). Circulating ACE2 in Cardiovascular and Kidney Diseases. *Current medicinal chemistry*, 24(30), 3231-3241.
- Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., ... & Chen, H. D. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270-273.
- Letko, M., Marzi, A., & Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B beta coronaviruses. *Nature microbiology*, 5(4), 562-569.
- Luan, J., Lu, Y., Jin, X., & Zhang, L. (2020). Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochemical and biophysical research communications*.
- Raj, V. S., Mou, H., Smits, S. L., Dekkers, D. H., Müller, M. A., Dijkman, R., ... & Thiel, V. (2013). Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature*, 495(7440), 251-254.
- Jin, Y. H., Cai, L., Cheng, Z. S., Cheng, H., Deng, T., Fan, Y. P., ... & Han, Y. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Military Medical Research*, 7(1), 4.
- Zhang, T., Wu, Q., & Zhang, Z. (2020). Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. *Current Biology*.
- Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The

- proximal origin of SARS-CoV-2. *Nature medicine*, 26(4), 450-452.
- Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., ... & Butt, K. M. (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science*, 302(5643), 276-278.
 - Gouilh, M. A., Puechmaille, S. J., Diancourt, L., Vandebogaert, M., Serra-Cobo, J., Roïg, M. L., ... & Manuguerra, J. C. (2018). SARS-CoV related Betacoronavirus and diverse Alphacoronavirus members found in western old-world. *Virology*, 517, 88-97.
 - Peeri, N. C., Shrestha, N., Rahman, M. S., Zaki, R., Tan, Z., Bibi, S., ... & Haque, U. (2020). The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned?. *International journal of epidemiology*.
 - Subbarao, K., & Roberts, A. (2006). Is there an ideal animal model for SARS?. *Trends in microbiology*, 14(7), 299-303.
 - Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., ... & Zhao, Y. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science*.

Vikings in the Himalayas

Daniel Hjorth Lund, IVSA Denmark

It is around noon on the 12th of October 2019, my plane from New Delhi is preparing to land in Kathmandu international airport. I look out of the cramped plane window and see what I believed to be mountains and valleys. Grass clad slopes with tiny, colourful houses making you realise the sheer scale and beauty of the country reaching for heaven. Thus began my trip with my fellow vet students visiting IVSA Nepal.

Travelling more than 6000km warrants staying a few extra days so I, along with another Danish vet student, had around two days to explore Kathmandu before joining up with the rest of the

Vikings and IVSA Nepal. We were recommended to journey around the Thamel area with every other tourist in Kathmandu, to visit the beautiful and secluded Garden of Dreams in order to forget the traffic and density of the city, and do a tour of the Narayanhiti Palace Museum to learn about the royal lineage of Nepal and mourn those lost in the Royal Massacre. All these small trips could be done in less than a day, which left plenty of time to get a feel of less touristy Kathmandu. The following day we walked, listened and smelt our way through Kathmandu. Going whichever way, we felt like



IVSA MIRROR V

and explored every tiny nook and cranny, street and courtyard discovering everything from the pristine whites of Hindu sanctuaries to the reds of the best chili momos I've had in my whole life. In the afternoon, all the Vikings met up and waited for our Nepali colleagues. When they arrived and we'd all been introduced we were told of a surprise. To our amazement the wonderful people, we had just met, pulled out a cake welcoming us to the country on behalf of IVSA Nepal. What a way to be greeted and the exchange was already off to great start with everyone laughing, smiling and learning about each other and our countries.

But alas, we could not stay, because a great programme had been made on our behalf and after a good night's rest we had to continue onwards into the country. The next stop was Pokhara and upon hearing it wasn't farther than 200km, we thought it to be a short trip. We were dead wrong. The Prithvi highway, while beautiful, is not the fastest highway we'd travelled along, but the company of friends of IVSA Nepal along with the Vikings made the trip feel like a breeze and soon Pokhara was in sight. The exploration of Pokhara took us to the World Peace Pagoda, standing as a white beacon of hope overlooking Phewa lake and Pokhara valley. We also visited both Devi's falls showcasing the awesome power and elegance of flowing water and the Mahendra Bat-covered limestone cave, wherein we got to climb into a very different world from the one we left. Just outside the cave we experienced cross-cultural knowledge sharing first-hand when all of us, Nepali and Vikings together, tried to find the best solution to a wounded horse given the circumstances. The last stop on our sightseeing was the international mountain museum, which provided the perfect background knowledge before my personal highlight of the trip...

The mountains of Himalaya were next on our trip and while they might look magnificent on TV, no photo or video will ever be able to properly convey the breath-taking beauty of the

giants. We left for the trek by public transport and while the seating wasn't exactly made for the long femurs of Vikings, the bus trip itself was worth it. The business of people entering or leaving the bus with everything from spears to livestock, the scenic view of the hills and valleys along with the fear of tipping over the edge into the aforementioned valley, all attributed to one of the most memorable bus rides of all of our lives. The goal of our trek was Poon Hill, but we started out in the mountain village of Ghandruk. After the usual lunch comprised of Dal Bhat, we were ready for the first leg of trip journeying to Tadapani. After passing through the enchanting rainforest and about to cross our first 'Mountain-top', we were informed that what we were in fact walking through hills, not mountains. As someone who's from a country, in which the tallest hill is called Sky Mountain and measures 147m above sea level, describing everything less than 5000m as a hill seemed far-fetched. Due to the thick cover of clouds we trekked the way to Tadapani believing ourselves to have climbed the mountains of Himalaya. It wasn't until after midnight that we got our first look at the jaw-dropping magnitude of the eternal giants. When we awoke next morning to view the early rays of sunshine catching in the snow-covered tips, only one thing could describe it: Breath-taking! It's a view that none of the Vikings will ever forget. Moving onwards we journeyed towards Ghorepani, the village in which we would be staying the night before trekking to Poon Hill. While getting up multiple hours before sunrise to trek in complete darkness is never fun, it proved to be well worth it when we arrived at the Poon Hill observation point shortly before first light. While the mountainous view of Tadapani will always be dearest to me, it can't quite compare to the magnitude of the view from Poon Hill. Every spot on the horizon was covered by mountain ranges or lone peaks – A truly awe-inspiring view. After the sunrise trip, we trekked down from Poon Hill and began our way back to Pokhara. If the trip had ended after Annapurna Conservation Area, I would still have

IVSA MIRROR V

had a great trip, but IVSA Nepal had more in store for us.

We left the Himalayan highlands and journeyed our way towards Sauhara and Chitwan National Park. After the previous bus-journey on the Nepali highway we knew what was in store, but the trip was memorable, nonetheless. The hotel felt like a little family, which made for a great stay. We were joined by multiple vet students from the area helping us learn about their school and why they had decided to study veterinary medicine. The vet students joined us when we visited the cultural centre to hear about the story of the region and to experience traditional dances in colourful clothing. We also took a short trip from the city to visit a national elephant breeding centre, which proved enlightening on how elephants have previously been used and how they ideally should be used going forward. The final agenda point of Sauhara was Chitwan National Park, in which the best-case scenario was to see both tigers and the Nepal's famous

one-horned rhino. We spotted crocs, deer, wild boars and a plethora of beautiful birds, but just as we had given up and were on our way out of the park, we spotted it: Rhinoceros Unicornis. Such an amazing animal and what a way to end our visit to Chitwan. Who needs tigers anyway?

With that the only thing remaining was our ride back to Kathmandu and a wonderful trip was coming to an end. Thank you IVSA Nepal and all the Nepali friends for planning the trip and showing us the beautiful country. It wouldn't have been the same without those you. Just as we were about to part ways with our Nepali friends, they had one final surprise in store: A framed group picture from Poon Hill saying "Lots of love from IVSA Nepal", which made me realise the following:

Nepal is a magnificent country, but IVSA Nepal makes it magical.

No matter where you are, IVSA will be there.



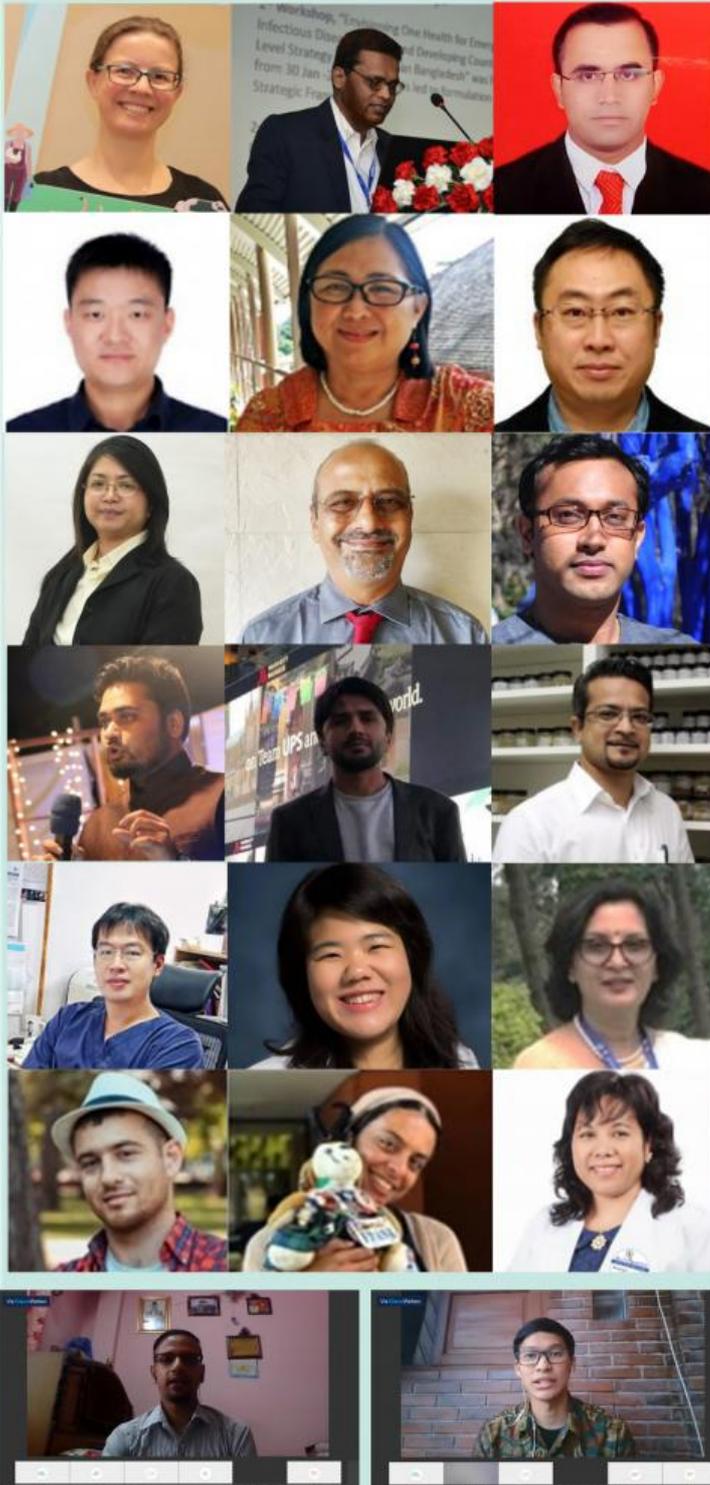
Online Asian Symposium on Zoonosis and Public Health

Organized by 15 IVSA Member Organizations in Asia

Article by Andre Firmansyah and the Organising Committee

Online Asian Symposium on Zoonosis and Public Health is an event organized by IVSA Nepal with other 14 IVSA Member Organizations in Asia as a co-organizer and a support from IVSA Regional Representative for Asia that focus on the aspects of various viral, bacterial and parasitic zoonotic diseases, their origin, types, and various diagnostic tools. The symposium provided professionals and students from various countries of Asia region to sum up their ideas in a single platform relating all the facts concerning zoonosis and public health. With this symposium, we aimed to share knowledge and holistic understanding regarding zoonosis and public health for all vet students around the globe. The 4-day program was divided into two sessions each day; i.e. morning and afternoon sessions. Each session had two presentations (except last day session we have three speakers), each presentation had one hour including question answer discussion. The presentations were recorded and uploaded directly to IVSA YouTube channel.

Online Asian Symposium on Zoonosis and Public Health was ceremonially opened by a welcoming speech from Aashirbad Porkhael, the coordinator of the organizing committee, and Andre Firmansyah, IVSA Regional Representative for Asia 2019-2020. They thanked all speakers who were willing to give their time and share their knowledge about various aspects of zoonosis and public health. They also greeted participants from very diverse backgrounds to this event and were hoping to have a memorable symposium for 4 days. In the end, they sent supports for all organizing committees for this big project, hoping for the event to go well.



Our Speakers (Above) | Welcoming speeches from the OC Coordinator (bottom left) and IVSA's Regional Representative of Asia (bottom right)

Day 1

NSA-IFMSA Joint Statement in Response to COVID-19

The first agenda after the welcoming speech was a presentation from Laure Chevalier, Chair of IVSA Standing Committee on Animal Welfare (SCAW) who presented IVSA-IFMSA Joint Statement: Response to COVID-19 – The next generation of health professionals demand immediate change to prevent future pandemics. On behalf of IVSA-IFMSA Joint Statement team, Laure explained the background story of COVID-19 outbreak and facts regarding the history of zoonosis. She also addressed some vital factors on how this disease spread so fast across the globe, from climate change, wildlife trafficking, legislation, and multidisciplinary coordination. In the end, Laure pointed out some strategies to prevent and reduce pandemic risks and also improve conservation worldwide for all relevant stakeholders. Finally, she invited all of the participants to access the joint statement document at bit.ly/covid19jointstatement

OIE: Present Situation/Scenario of Zoonosis in Asia Region



Next to the agenda was our guest speaker, Dr. Lesa Thompson, Regional Project Officer for the World Organization for Animal Health (the OIE), Regional Representative for Asia Pacific. She's hosted by Aashirbad Porkhael. She started her presentation with brief information about the OIE, its history, national focal points, its working structure, and its activities. She then moved to tripartite agreement between three general directors of FAO, OIE and WHO to address one health concept, Antimicrobial Resistance, transboundary zoonosis, and Rabies. Furthermore, she then briefly talked about the global and Asian situation of certain diseases. The topic from Dr. Lesa was the opening of our view on zoonosis, particularly in Asia.

NSA Bangladesh: Viral Zoonosis NSA China: Tuberculosis - Old Disease, New Threat NSA Mumbai: Parasitic Zoonosis

Second lecture was Prof. Dr. Abdul Ahad, a Professor of Microbiology and Veterinary Public Health and was hosted by IVSA Bangladesh. The lecture explained basic knowledge of virus and followed by various viral zoonosis, emerging diseases of the world, risk factors associating those diseases, epidemiology and so on descriptively. He also explained a brief information regarding Chikungunya, Crimean-Congo hemorrhagic virus, Influenza A Virus (IAV), Ebola, Middle-East Respiratory Syndrome (MERS), Nipah Virus, Rabies, and Zika Virus. The presentation ends with the hot topic of all. One that cannot go ignored when it comes to viral zoonosis that is COVID-19 or novel corona virus. The presentation was brought to an end with a brief conclusion and the related reasons of the persistent outbreaks that are observed throughout the world.

Later on the day 1 afternoon session, we had Dr. Lee from Department of Basic Veterinary Medicine, China Agricultural University and was hosted by IVSA China. His topic was about tuberculosis- old disease, new threat. He showed us its different forms under different conditions like observed by electrical microscope or light microscope and colony morphology. He illustrated how the tuberculosis bacteria spreads between human and animal, and its pathogenic effect. He stated that tuberculosis is indeed an old disease but with new threat. As Dr. Lee said, although Tuberculosis bacteria has been existing for a long time, it still threatens the society and public health, we still need to find out a novel and effective vaccination strategy to defend it, especially with the background of its increasing drug resistance. Dr. Lee shared the current strategies to control TB by vaccination and proposed a vision for its diagnosis and treatment in the coming years.

Moving on, we had Dr. Mukulesh Gatne, a retired university head and Professor of Veterinary Parasitology, Bombay Veterinary College, Mumbai and was hosted by IVSA Mumbai, India. He explained the topic in depth, with a briefing regarding various canine zoonotic diseases such as Taenia, Leishmania, Visceral Larva Migrant (VLM), Cutaneous Larva Migrant (CLM), and Hydatodosis. He also briefed about Ankylostoma with a specific emphasis on its zoonotic significance. Next topic was Hydatodosis caused by Echinococcus granulosus where the etiology, pathogenesis, progress, clinical signs were explained in detail. He concluded the lecture by explaining concerns about parasitology, its negligence and it's a need of the hour to begin Parasitology as a dynamic course in medical field with respect to public health concerns.

Public Health Significance of Ascarids		
Worm	Mode of Infection in dogs	Mode of Infection in Man
<i>Toxocara canis</i> (10-18 Cms)	Oral: Eggs having L ₁ Prenatal infection (L ₂) Lactogenic infection (L ₂) Through ingestion of Rodents (L ₂)	Ingestion of infective eggs containing L ₂
<i>Toxascaris leonina</i> (3-10 Cms)	Oral: Eggs having L ₂ Through ingestion of Rodents (L ₂)	
Worm	Harmful Effects in Dogs	Harmful effects in Man
<i>Toxocara canis</i>	Common in Pups/Young dogs Respiratory - Coughing/Sneezing Irregular bowel movements Pot Bellied appearance Poor growth rate Rough coat	Visceral Larva Migrants/ Ocular Larva Migrants/ Neural larva migrants
<i>Toxascaris leonina</i>	Digestive disturbances & poor Growth rate	

Next to the agenda was Dr. Daesub Song, Professor of Preclinical Science AI and a swine specialist. He was hosted by IVSA South Korea. He emphasizes the meaning of "Disease X". Disease X represents the knowledge that a serious international epidemic could be caused by a pathogen which is currently unknown to cause human disease. COVID-19 is the exact example of Disease X. WHO has predicted this disease outbreak, but still had trouble with countermeasures. He says COVID-19 won't be the only example of disease X and another one will absolutely occur. And when that day comes, we will have to prepare well. The following topic was about reverse zoonotic disease, which is the transmission from human to animal. Ringworm, influenza virus, mumps are the good examples. In one of the professor's research, he proved that although the possibility of zoonotic disease from companion animals is low, reverse zoonotic possibility is very high.



IVSA Indonesia: Wildlife Zoonosis & Management Efforts in Indonesia and Asia | IVSA South Korea: Transboundary Zoonosis

Day 2

On the opening session of day 2, we had Dr. drh. Ligaya ITA Tumbelaka, an expert in Reproduction Biotechnology of Wild Animals and was hosted by IVSA Indonesia. She brought up a topic of wildlife zoonosis management efforts in asia: A pilot project in Indonesia. She explained that for wild animals, they are in force to get contact with humans because loss of habitat, tourism, and wildlife trade which are the main source of the wildlife zoonotic disease transmission. Indonesian government have made a Wildlife Health Information System, called SEHAT SATLI, which is a communication system to report any unhealthy or dead case of wildlife animals from the workers on the habitat. The system works from wildlife investigation on site, response to any lab result, and until any measurement to control or prevent any outbreak.

IVSA Kelantan: Veterinary Occupation & Zoonosis; Risks/ Hazards | IVSA Philippines: Emerging & Re-emerging Diseases

Day 2

On the day 2 afternoon session, we had Dr. Sandie Choong Siew Shean, an expert in wildlife medicine, zoonotic diseases, and public health and was hosted by IVSA Kelantan, Malaysia. Dr. Sandie has spoken about the different types of hazards that a veterinarian would encounter in their field, this includes biological hazards such as bacteria, viruses, fungi and parasites. We could be also encounter physical hazards such as mechanical trauma and chemical hazards such as solvents, gasses and dusts. Dr. Sandie also spoke about the risk factors in Veterinary Occupation that includes a variety of factors such as age, gender, social and culture. Apart from that, Dr. Sandie also shared her experiences, the ways and methods in order to reduce the hazards and risks. Dr. Sandie emphasized on the importance of safety in the working environment at all times.

The final presentation for day 2 was from Dr. Clarissa Yvonne J. Domingo, a full-pledge professor of zoonosis and public health at Central Luzon State University, Philippines. She was hosted by IVSA Philippines. Dr. Domingo presented a topic of emerging and re-emerging zoonosis diseases. Emerging infectious diseases (EIDS) are those newly reported to infect a new host, have enhanced incidences, have new geographic range, and are caused by evolving pathogens. Factors that will lead to EIDS are global travel, urbanization, biomedical manipulation, ecological manipulation, microbial adaptation, social and cultural practices, organic farming, and disruption of health services. Re-emerging diseases is defined as diseases of the past, re-appearing in an area having a susceptible host population/(s). Some examples from re-emerging diseases are human tuberculosis, Human AID's virus (HIV), and protozoon infections. Lastly, Dr. Domingo gave stress of the One Health approach to address these problems.

NSA SCOH: How Students Spread Awareness on Zoonosis NSA Thailand: Milk-Borne Zoonosis/Diseases

Day 3

On the third day morning session, we had a presentation from IVSA Standing Committee on One Health (SCOH). The two presentators were Andreas Pappas, Chair of IVSA SCOH, and Nabeelah Imraan Rajah, Secretary and Webmaster IVSA SCOH. They talked about how students spread awareness on zoonosis. The presentation started with the importance of collaboration from all layers of organizations, ranging from local to global. They also included a brief introduction of IVSA SCOH and how they spread one health awareness from IVSA to the world. And these days, social media is a strong tool to spread all kind of information, thus IVSA SCOH suggested to make all informations as creative and communicative as possible so that it reached its maximum engagement to the society. They also shared insights about how to organize an impactful project based on their experiences and other related organizations.

After IVSA SCOH, we had Dr. Suchawan Pornsukarom, Head of Veterinary Diagnostic Department and Professor at Rajamangala University of Technology Taiwan and was hosted by IVSA Thailand. She presented a topic of milk borne zoonosis/diseases. Milk borne diseases (MBZ) are mostly acquired through consumption of infected milk and both public health and economic importance. The cycle of dairy food borne be like cow infected some pathogen, it come to milk and human infected pathogen from milk product. Sources of infection are dairy animals, human handlers and environments. One of the most popular and most severe zoonosis is Zoonotic Tuberculosis. Other MBZ are Brucellosis, Enterobacteriaceae such as Campylobacter, E. coli, Salmonella and Listeria. Viral infection also can be MBZ although it is not as frequency as bacteria. Contamination control strategies can do by Enhanced animal health, improved milking hygiene, Pasteurization, Surveillance and Public awakened.



NSA Pakistan: Epidemiological Investigation of Zoonotic Diseases NSA Nepal: Recent Diagnostic Tools and its Application in Zoonotic Disease

Day 3

On the third day we had Dr. Nasir Iqbal, a Veterinary Surgeon and Wildlife Expert and was hosted by IVSA Pakistan. He gave his presentation on the topic epidemiological investigation, zoonosis and zoonotic diseases from past 5 years. Speaker told about the zoonotic diseases and their transmission among different species. He threw a light upon the history of zoonotic diseases from 1347 when there was Black Death to COVID-19. Then, the speaker explained the 'outbreak investigation in detail. He explained zoonotic diseases thoroughly including their description, transmission, diagnosis, preventive measures and the control. Diseases which were explained includes Brucellosis, Salmonellosis, Plague, Rabies and SARS. Then speaker turned around for the debate on current global pandemic issue, COVID-19.

Final presentation on the third day was from Assoc. Prof. Dr. Ajit Kumar Karna from Department of Veterinary Public health and Epidemiology, HICAST, Nepal and was hosted by IVSA Nepal. Dr. Karna gave presentation on recent diagnostic tools and its application in zoonotic diseases. After giving a brief introduction of zoonotic diseases, Dr. Karna firstly focused on the challenges of disease control globally, that included challenges on diagnostics, epidemiology and prevention. He then talked on the sample to be taken, target product and equipment/technique to be used for diagnosis. Dr. Karna then described about some diagnostic tools such as Loop-mediated Isothermal Amplification Assay (LAMP Assay), gene targets and amplification, rapid diagnostic test, rapid screening test, rapid antibody test, RDT, PCR, and RT-PCR. Finally, Dr. Karna wrapped his presentation with dispersing knowledge on sample pooling techniques for diagnosis of zoonosis diseases.

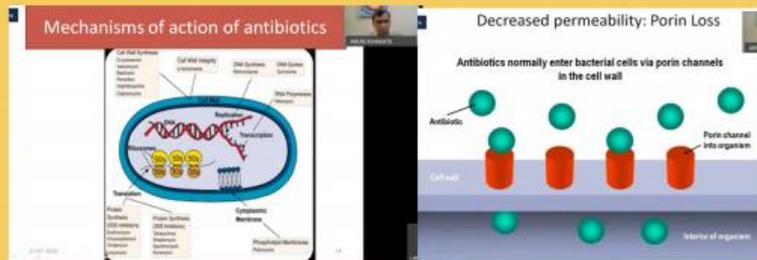
IVSA Japan: Zoonoses and Food Safety | IVSA Pakistan: Approaches towards the Investigation of Zoonotic Diseases Day4

On the fourth day morning session, we had Dr. Kohei Makita, a Veterinary Epidemiologist working in One Health and was hosted by IVSA Japan. His presentation was about zoonosis and food safety. General subtopics were impacts of zoonoses and food-borne diseases (FBD), One health and Eco health, examples of epidemiology for investigation, where and how you can contribute to controlling zoonoses and FBD, and capacity development. One example that Dr. Kohei stated is regarding the disease burden which depends on how rich the country is. In the global richest 20% countries, Non-communicative disease is at the highest rate of the total deaths in group. Whereas, communicative disease is at the highest in the global poorest 20% countries. Additionally, many of those diseases in developing country come from human food. Professor's advice for students about their learning as a vet student and become an expert of particular skill or good coordinator, students have to continue and develop capacity of others and your country.



Next after Dr. Kohei, we had Dr. Touseef Ahmed, a Veterinary Epidemiologist, Teaching Assistant at Texas Tech University and was hosted by IVSA Pakistan. He had a topic of natural barriers and ecological interventions to mitigate zoonotic disease spillovers. He explained subtopics of conventional versus ecological interventions and an explanation from a question "Are we heading toward next Pandemic?". Speaker explained about the zoonotic diseases and their transmission among different species. He threw a brief explanation upon the pathogen spillover. He continued with natural barriers versus ecological interventions in detail. After completing the subtopic, the speaker explained a subtopic of ecological interventions. He explained zoonotic diseases such as Rabies, Avian Influenza, Nipah, Hendra, Brucellosis, Primate Malaria, and Lyme. In the end, He explained novel viral sharing events coincide with population centers.

IVSA Bidar: Antimicrobial Resistance/Drug Resistance | IVSA Selangor: Vector-Borner Zoonosis in Wildlife across Asia Day4



First presentation on the fourth day afternoon session was Dr. Arun Kharate, an Assistant Professor of Public Health and Epidemiology and was hosted by IVSA Bidar, India. He explained about antimicrobial resistance/drug resistance. He stated that antibiotic resistance is defined as microorganisms that aren't inhibited by usually achievable systemic concentration of an antimicrobial agent with normal dosage schedule and/or fall in the minimum inhibitory concentration (MIC) range. Mechanism of antibiotic resistance includes intrinsic resistance, mutations, and plasmid factor. He also explained about biochemical mechanism of antibiotic resistance. Dr. Arun also explained factors of antibiotic resistance scenario in India from its environmental factors until prescriber factors. In the end, Dr. Arun addressed strategies to prevent the resistance.

Next to the agenda was Dr. Reuben Sharma, a Parasitologist from University Putra Malaysia and was hosted by IVSA Selangor, Malaysia. His topic was about vector-borne zoonoses in wildlife across Asia. He explained the epidemiology of zoonotic infections but more focused on zoonotic malaria. Epidemiology of zoonotic infection includes 4 main stakeholders (known as the epidemiological quartet) such as the agent, host, environment and vectors. Moving on to Malaria, primates are the natural reservoir for these parasitic organisms in which Dr Reuben gave further emphasis on the long-tailed macaques and leaf monkeys during his presentation. *P. knowlesi* infection can be easily treated in humans by administering proper malaria drugs as there have been no drug resistance cases among *P. knowlesi*. It is only fatal in late stages of the infection due to high parasitemia.



IVSA Nepal: Food Safety, Hygiene & HACCP; and its Importance

Day
4

Final presentation of the event was a presentation from Ms. Jiwan Prava Lama, a senior food technology advisor at Nepal Agribusiness Innovation Centre (NABIC). She's hosted by IVSA Nepal. Ms. Lama started her presentation with some case studies representing situation of food safety. The case studies included antibiotic residues in meat and milk, formalin residues in fish, arsenic contamination in water, pesticides residues in vegetables, and market situation. Then she moved to food safety, HACCP, roles and principles of HACCP. She then explained about the 'farm to fork concept of food chain' and the roles of HACCP principles and prerequisite programs in this concept. Dr. Lama then presented issues and challenge on food safety along with the roles and responsibilities of government agencies, private sectors, academic institutions, veterinarians, consumer associations, and stakeholders towards food safety. She also shared some insight regarding COVID-19. She finally concluded the presentation by focusing the food safety as a shared responsibility.

Closing

After all presentation ended, Aashirbad and Andre gave a closing speech thanking all speakers, participants, and especially the organizing committee team. It was indeed a memorable and amazing time to work with all IVSA MOs in Asia and we were hoping this will be a stepping stone for future collaborations.

Link to our YouTube streamed videos:
bit.ly/ivsasonlineasiansymposiumvideos

The Event in Numbers

1,743
Registered
Participant

+17,600
Total Viewers
from YouTube

+1,700
Total Viewers
from Webex

18 speakers
from **13** MOs in Asia,
IVSA SCAW, IVSA SCOH
and OIE Asia Pacific

Credits to:

IVSA Bangladesh, IVSA Bidar, IVSA China, IVSA Indonesia, IVSA Japan, IVSA Kelantan, IVSA Mongolia, IVSA Mumbai, IVSA Nepal, IVSA Pakistan, IVSA Philippines, IVSA Selangor, IVSA South Korea, IVSA Taiwan, IVSA Thailand

Events and Activities

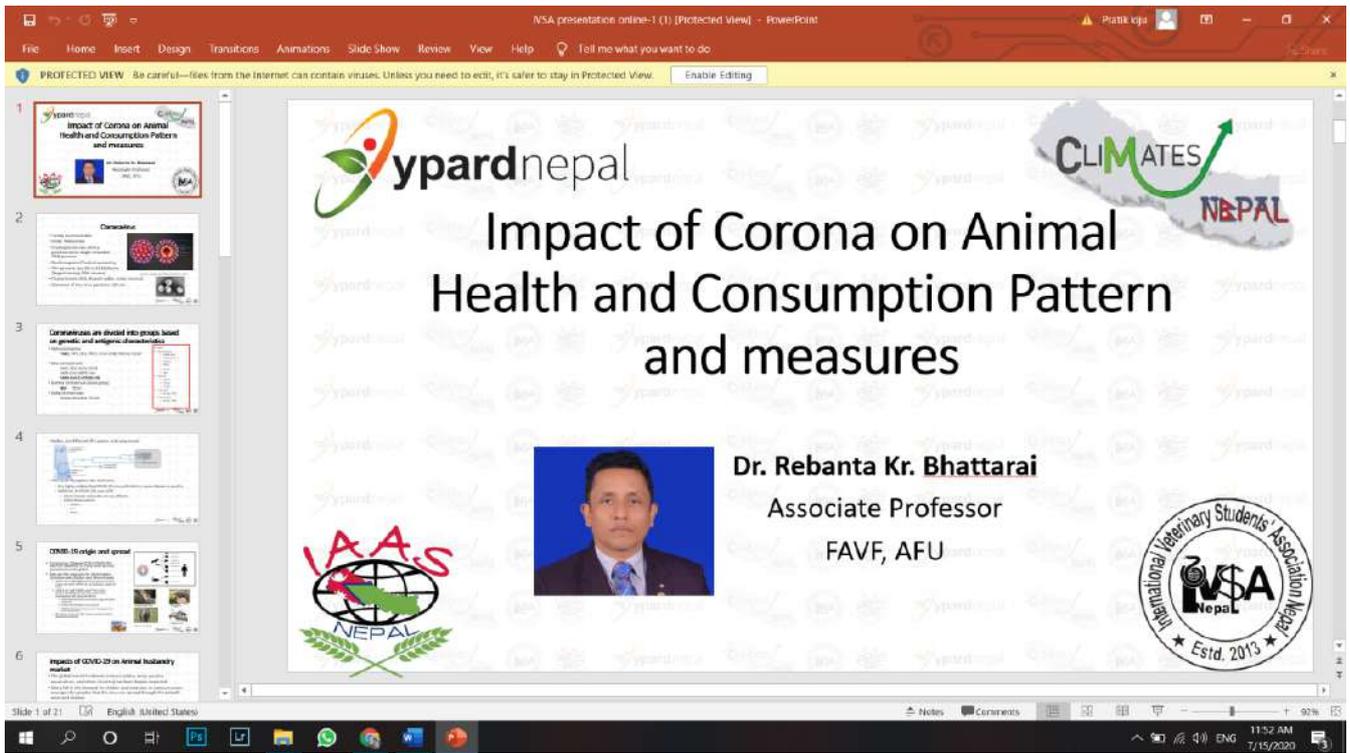
IVSA NEPAL



Online exchange with IVSA Mumbai and IVSA Selangor



IVSA Exchange with IVSA Denmark



Webinar on encompassing affairs relating to different fields

“Course on Research Design and Academic Writing”

-Biplov Sapkota

Supported By:



11th to 21st April, 2020

15



**ONLINE ASIAN SYMPOSIUM
ZONONIS AND
PUBLIC HEALTH**

Organized by IVSA Nepal with 14 other member organizations across Asia

IVSA Rampur



Animal health camp



Rabies week 2019 radio talk show (Synergy FM 91.6)



World antibiotic awareness week 2019 poster art competition



Rabies week 2019 street drama



Rabies week 2019 school awareness program



Mastitis checkup program



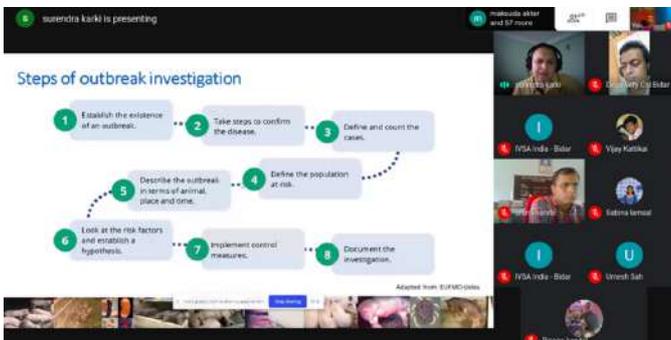
Free faecal examination
antihelminthic distribution and
awareness



Webinar on "Overview on OIE
International Standards"



Winners of Poster Competition



Panel discussion on African
Swine Fever



Webinar on food safety



Crossword Competition

IVSA Paklihawa



Caponization Training on National Poultry Day

International Veterinary Students' Association Paklihawa (IVSA Paklihawa)

Speaker

**Pandemic Psychology,
A talk show**

Upendra Raj Dhakal
Public Health Specialist
1000 Graduates Movement
Founder

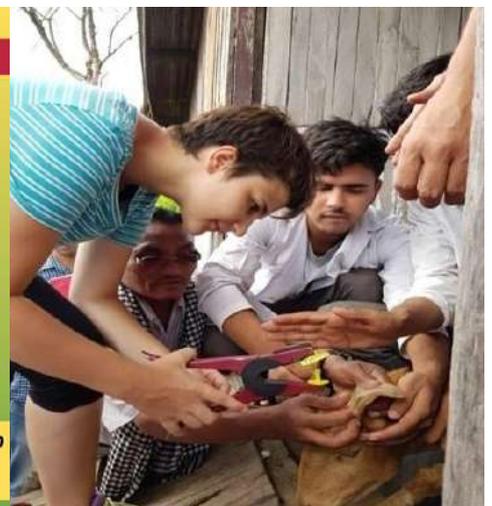
Organised by:
IVSA
Established 2016
Nepal

Harmony for Humanity
IVSA
Established 2016
Nepal

QR Code

Zoom

June 26, 2020
01:00 PM



Pandemic Psychology: A talk show

Identification, Tagging and Farm Record Keeping at IAAS Farm Animals



"Cultivating Innovation in Agriculture & Veterinary Sector"

ACCESS - EDUCON

Agrovet Exhibition-2076

सम्पूर्ण प्रतिस्पर्धी, विद्यार्थी एवम् शुभेच्छुक महातुभावहरुलाई यस कार्यक्रममा हार्दिक स्वागत गर्दछौ ।



Date :- 2076-09-04
Venue :- IAAS, Paklihawa Campus, Rupandehi

Organized by
IVSA
HAAS, Paklihawa

Access~Educon Agrovet Exhibition 2076



IAAS Collective Campaign for Rabies Awareness and Vaccination



Worldwide Digital Poster Competition on "One Health Approach on Wildlife."

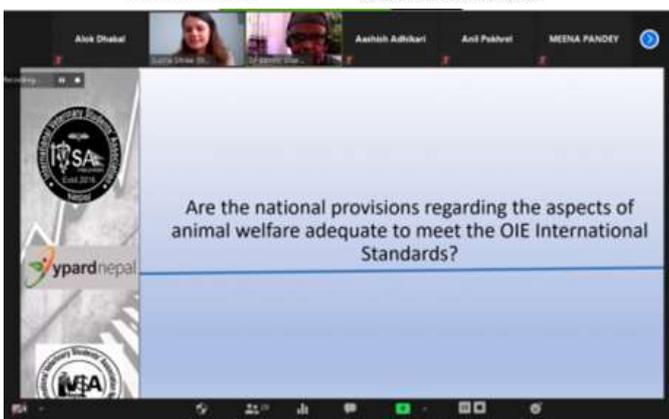
Requirements

1. Poster can be illustrated via table, charts and/or pictures.
2. Body of poster shall consist of multiple topics and subtopics.
3. Poster shall be submitted in pdf format to ivsapaklihawa@gmail.com.
4. Appropriate and standard size of poster will be entertained.

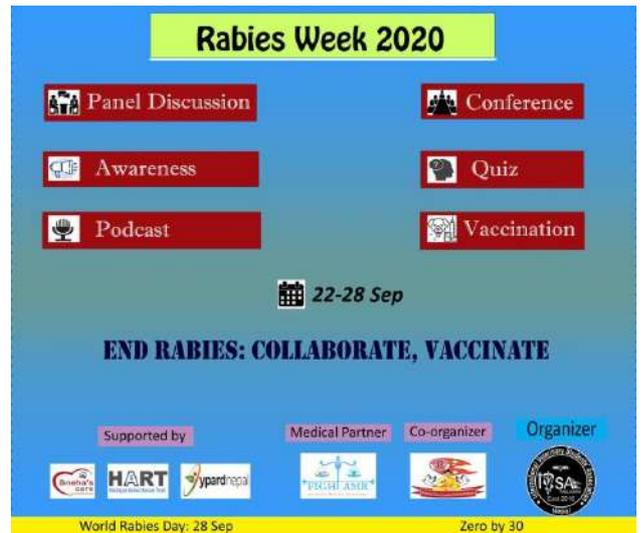


Deadline of submission
5th November, 2019

For any queries
Contact: ivsapaklihawa@gmail.com
Santosh Panta: +977 9851153439



Live Interview on "OIE International Standards in the context of Nepal."



Celebration of Rabies Week 2020 through National Conference, Live Mass Vaccination & Social media awareness



Free Medicine Distribution Program



An hour on Lumpy Skin Disease ~ Panel Discussion

IVSA HICAST



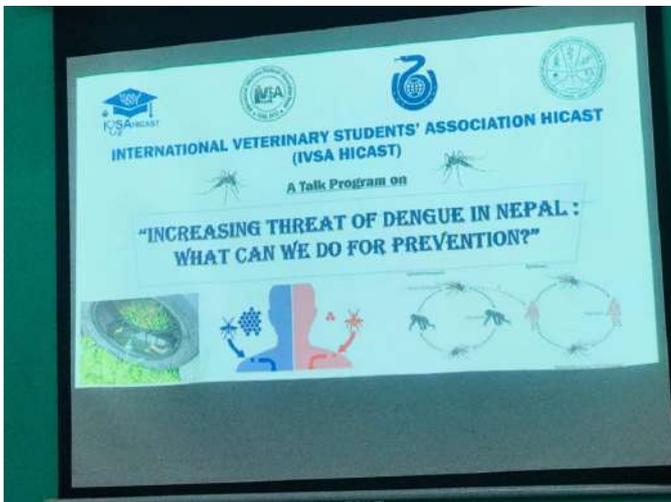
Welcoming IVSA Madrid at TIA.



Interaction program with IVSA Barcelona at HICAST Seminar Hall, Kalanki and visit to HICAST farm, Kritipur



IVSA HICAST advocating for Animal Welfare



IVSA HICAST organized the talk program on "Increasing Threat of Dengue in Nepal: What Can We Do for Prevention?" at HICAST Seminar hall on 14th September.
 Presenter: **Dr. Surendra Karki, PHD (Epidemiology)**

IVSA NPI



“खोप लगाऔं, रेबिज उन्मुलन गरौं”
"Rabies Vaccinate to eliminate"

१३ औं विश्व रेबिज दिवस-२०१९
निःशुल्क रेबिज खोप शिविर

मिति : २०७६ असोज १० र ११ गते (संयुक्त वडा कार्यालय १४ देखि १७) अमरापुरी
२०७६ असोज ११ र १२ गते (संयुक्त वडा कार्यालय १० देखि १३) हर्कपुर
२०७६ असोज १३, १४, १५ र १६ गते (मैडाकोट नगर कार्यपालिकाको कार्यालय)

आयोजक
मैडाकोट नगरपालिका
पशु सेवा शाखा
मैडाकोट बजारपारसी (वर्दीघाट-सुला पूर्व)

सह आयोजक
International Veterinary Students' Association
NPI



IVSA NPI had celebrated the Rabies Week 2019 with the theme of "Vaccinate to Eliminate " by doing above different programmes



Some glimpses of the free animal health Camp-2020 organized by IVSA NPI



Public Awareness on Meat Hygiene & Associated Health Hazards



International Veterinary Students' Association Nepal (IVSA Nepal) is a National branch of a global organization, International Veterinary Students' Association (IVSA), established in 1953. The organization was solely established for benefiting the animals and people of the world by harnessing the potential and dedication of veterinary students to promote the international application of veterinary skills, education and knowledge. IVSA Nepal as being the National member organization of the global committee works for the same purpose and was established in 2013. During the course of its growth this organization worked in several dimensions regarding Veterinary education, Veterinary Profession and issues related to health of animals and people. The organization with its devouring success efficiently established itself as a potent group of skillful and enthusiastic veterinary students and reached to the culmination of eminence for its creative and productive works. In 2016 IVSA Nepal was acknowledged as the National member organization of IVSA Global committee with four local chapters working under the guideline of National executive committee. This expansion led the organization to the next level increasing its areas of activism as well as its efficiency by establishing a strong nexus in between the veterinary students of different institutes running the veterinary program in Nepal. Currently, this organization focus on eclectic programs regarding Animal welfare and is involved in different work areas that comprises Specific programs for promoting veterinary education in Nepal . It has regularly been an important hand for conducting different programs responsible for raising knowledge as well as awareness at both student and farmers level.

WINNER OF IVSA TOP MO COMPETITION 2020

NATIONAL MO



LOCAL CHAPTERS

