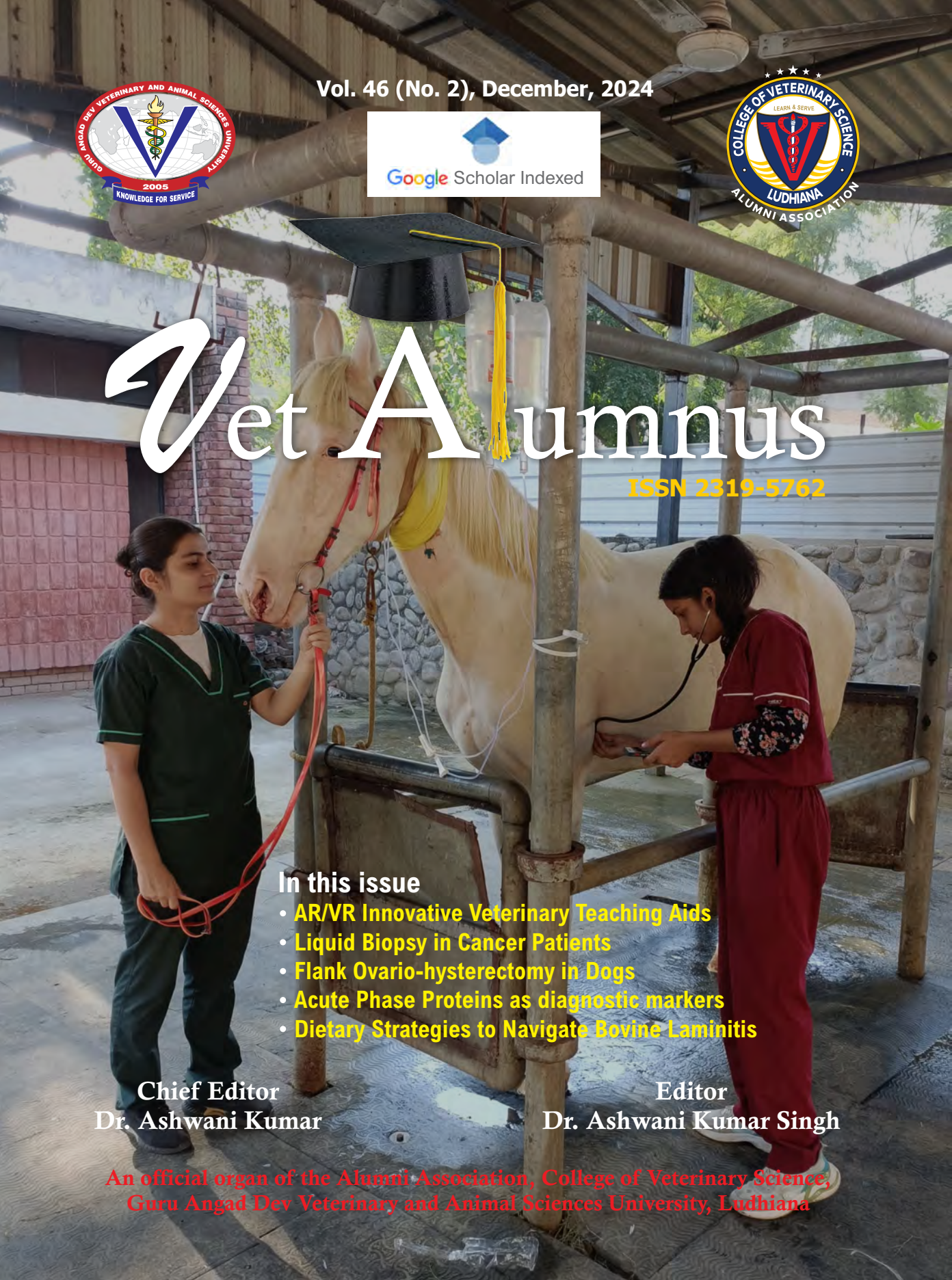
Vol. 46 (No. 2), December, 2024

Google Scholar Indexed

Vet Alumnus

ISSN 2319-5762



In this issue

Chief Editor
Dr. Ashwani Kumar

Editor
Dr. Ashwani Kumar Singh

An official organ of the Alumni Association, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Since 2015 all published issues of
the 'Vet Alumnus' are available as OPEN ACCESS (PDF)
on https://www.gadvasu.in/page/vet-alumnus

Printed at Foil Printers, 2051, Gobind Nagar, Civil Lines, Ludhiana-141001, Punjab, India E-mail: foilludhiana@gmail.com Ph.: 0161-2404093 and published on behalf of the Alumni Association of the College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana -141004, Punjab, India

VET ALUMNUS

Vol. 46 No. 2 December 2024

CONTENTS

Note: Authors are sole responsible for the contents given in their articles

EXECUTIVE COMMITTEE

ALUMNI ASSOCIATION

College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary & Animal Sciences University

Founder President	Late Dr. Balwant Singh
President	Dr. Sarvpreet Singh Ghuman
Vice-President	Dr. Swaran Singh Randhawa
Secretary	Dr. Navdeep Singh
Joint-Secretary	Dr. Jaspal Singh Hundal
Treasurer	Dr. Rajnish Sharma
Co-Treasurer	Dr. Randhir Singh
Chief Editor	Dr. Ashwani Kumar
Editor	Dr. Ashwani Kumar Singh

EXECUTIVE MEMBERS

Dr. Gurcharan Singh Dhaliwal
Dr. Gurinder Singh Walia
Dr. Kewal Arora
Dr. PS Walia
Dr. Amrik Singh
Dr. Sham Singh
Dr. Ranjeev Bali
Dr. Parvinder Kaur Lubana
Dr. Rana Preet Gill
Dr. Daljeet Singh Dhami
Dr. Ravinder Singh
Dr. Mandeep Singh
Dr. Tejbir Singh Randhawa
Dr. Kanwar Anoop Singh Kaler
Dr. Darshan Das
Dr. Gagandeep Kaushal
Dr. Gurdev Singh
Dr. Amarpreet Singh Pannu
Dr. Gurdit Singh
Dr. Raj Sukhbir Singh
Dr. Navdeep Singh Ratta

Mini Review

Liquid Biopsy: An Emerging Tool for Rapid Diagnosis of Cancer in Veterinary Practice

Sonam Sarita Bal, Geeta Devi Leishangthem and Nittin Dev Singh*

Department of Veterinary Pathology
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email; nitindevsingh@gadvasu.in

Received: 07.10.2024 Accepted: 25.10.2024

Abstract

Liquid biopsy is a non-invasive and innovative method used for the early diagnosis and monitoring of cancer. It analyzes biomarkers like cell-free nucleic acids, circulating tumor cells, tumor-educated platelets, exosomes and proteins from biological fluids. Liquid biopsy tests complement, rather than replace, gold-standard diagnostic methods like tissue biopsy, aiding in the identification and monitoring of cancer patients. In veterinary medicine, liquid biopsy serves primarily as a cancer screening tool for older dogs and breeds at high risk. Different blood based (OncoK9), Nu.QVet and urinary based (CADET BRAF, Oncotest) liquid biopsy tests are available for veterinary practice.

Keywords*: Cell-free nucleic acids, Liquid biopsy, OncoK9, Tumor-educated platelets.*

Cancer is the primary cause of death in dogs, largely because it is often diagnosed late, when symptoms have appeared and prognosis is poor. Dogs have a much higher annual cancer rate compared to humans, despite having a similar lifetime risk due to their shorter lifespan. Approximately one-quarter of dogs will develop cancer, with this rate increasing to nearly half for those over ten years old. Early cancer detection is critical for veterinarians. Recent advancements now allow for non-invasive testing using simple blood draws, known as "liquid biopsy." This method involves sampling and analyzing analytes from biological fluids like blood (and sometimes urine, cerebrospinal fluid, or other secretions) using minimally invasive techniques. Blood-based liquid biopsy specifically involves analyzing cell-free nucleic acids, circulating tumor cells, exosomes, tumor educated platelets and proteins. These components provide valuable information about molecular dynamic at genome, transcriptome and proteome level. Liquid biopsy does not replace tissue-based diagnosis but act as an aid to it. Studying these components helps to understand their relationship with tumor biology by investigating three key areas: First their origin, specifically which cells contribute to their presence in the blood. Second, accessing their clonal burden i.e. the specific tumor clones from which it is derived from; finally, in response to therapy the variation in their blood level. Understanding these aspects will enhance monitoring and treatment of cancer. These components in

plasma can originate from primary tumors, metastases sites, or blood circulating tumor cells, either by the way of apoptosis, necrosis, or as secretion. Evidence suggests that they primarily originate from dying tumor cells within the tumor itself, rather than from circulating tumor cells (Speicher and Pantel, 2014). In the tumor microenvironment, after they are released, they are cleared by lymphatic drainage and then through organs like lymph nodes, kidneys, and liver. Their clearance is influenced by factors such as its form (e.g., exosome), size, associated molecules (e.g., lipids, proteins), and release mechanism (Leung et al., 2016).

In veterinary medicine, liquid biopsy serves primarily as a cancer screening tool for older dogs and breeds at high risk. For dogs with a history of cancer, detecting tumor-derived components can indicate residual disease post-treatment or tumor recurrence. However, biomarker presence does not always correlate with treatment response. Whether for screening or monitoring, liquid biopsy results need interpretation alongside other clinical and diagnostic findings. Different liquid biopsy tests available, and it depends on the specific condition being investigated.

Advantages of Liquid Biopsy

- Minimal invasiveness leading to low risk, along with less time and cost during sampling procedure compared to tissue biopsy.
- Repeated liquid biopsy samples can be taken to monitor disease's dynamic molecular changes, especially under selective drug treatment pressure.
- Tissue biopsies may miss aggressive tumor subclones due to tumor heterogeneity, whereas liquid biopsies capture a comprehensive heterogenetic range of tumor properties, including metastases and biochemical changes from dissemination.
- Compared to routine needle biopsy which can't access remote locations, needle biopsy can be substituted in that.

Limitations of Liquid Biopsy

- Blood concentrations are minimal, necessitating specialized techniques and require collection of 7-9 milliliters of blood.
- It is less effective for detecting early or localized disease and lacks sensitivity when multiple tumor types are present.
- It does not provide insights into tumor microenvironment or structure, and there is a scarcity of targeted therapies available in veterinary medicine.

Components of Liquid Biopsy

Circulating tumor cells (CTCs), cell-free nucleic acids (CFCs), exosomes and tumor-educated platelets (TEPs). After centrifugation the top layer of plasma/serum contains circulating cell-free nucleic acids (cell-free RNA, ct-DNA), exosomes, proteins

and TEPs. The tumor cell fraction with CTCs, immune cells and the non-tumor cell fractions are present in the bottom fraction.

1. Circulating Tumor Cell (CTCs)

The CTCs shed from primary tumors can potentially form distant metastases. Studying these cells can reveal molecular mechanisms involved in metastasis, particularly through epithelial-mesenchymal transition (EMT), which enhances their ability to spread from the primary site into the bloodstream and to secondary sites (Alix-Panabières et al., 2017). Detecting both epithelial and mesenchymal fractions of CTCs helps to understand the EMT process in tumor metastasis. However, studies in both human and dog indicate that a significant percentage of metastatic cancer patients may not have detectable CTCs in typical sample volumes collected. Due to these limitations, CTCs remain primarily a research tool rather than widely used in clinical practice.

Various techniques are currently available for the enrichment and detection of CTCs that are as follow:

a) CTC Enrichment: CTC enrichment in ex-vivo uses various physical and biological properties that gives the distinctive properties of CTCs compared to normal hematopoietic cells. The techniques for analyzing physical attributes include density gradient centrifugation, filtration through specialized filters, three-dimensional microfilters, and dielectrophoretic field-flow fractionation (DEP-FFF) devices. The techniques for analyzing biological attributes comprise of expression of cell surface markers like epithelial cell adhesion molecule (EpCAM) for positive selection and CD45 for negative selection allowing identification of CTCs through surface protein expression. Recently combination of both these properties are being utilised to identify CTCs e.g. CTC-iChip.

The in-vivo nano-detector enables the direct enrichment and extraction of CTCs in the patients' arm vein, thereby enabling the collection of larger quantities of CTCs.

b) CTC identification: Most CTC assays utilize a common identification step involving fluorescent staining for cytokeratins, EpCAM (a positive marker for epithelial tumor cells), and CD45 (an exclusion marker). Cancer-specific detection methods can also be used (for e.g. PSMA or PSA for prostate cancer). EPithelial ImmunoSPOT (EPISPOT) assay, an assay for CTC analysis detects CTCs based on proteins secreted, shed, or released during short-term culture (24-48 hours), without direct contact with the target cells. EPISPOT has been applied in breast, colon, prostate cancer patients' blood, bone marrow samples (Alix-Panabières & Pantel, 2015). Ultra high-speed automated digital microscopy that uses fiber-optic-array scanning technology also being used these days.

Uses

- Prognostic Information: Estimating the risk of disease progression or any

metastatic reoccurrence.

- Classifying patients, tracking therapy effectiveness in real-time and monitoring therapy efficacy in clinical trials.
- Identification of therapeutic targets and resistance mechanisms.
- Understanding the process of metastasis in cancer patients.
- Additionally, analyzing CTCs can reveal mechanisms of drug resistance.
- CTC-derived cell lines and xenografts can be used for in vitro and in vivo drug testing.

2. Circulating Cell-Free Nucleic Acids (cfDNA)

The cfDNA have become the most promising class of circulating biomarkers for the non-invasive identification and monitoring of cancer during the last ten years. Circulating cell-free nucleic acids consist of cfDNA, cfRNA and cfmiRNA. The apoptotic and necrotic tumor cells release them into the bloodstream. There increased level is mostly found during late stage of cancer. Among these, cfRNA is least stable in blood. MicroRNAs (miRNAs) are now emerging as important markers in liquid biopsy for monitoring therapy and predicting responses to different treatments (Mader & Pantel, 2017).

Advantage

- The cfDNA could offer a comprehensive representation of the tumor and any metastatic clones.
- The cfDNA offers a personalized overview of a patient's disease. They could be applied in various areas, such as early cancer detection, monitoring minimal residual disease, assessing treatment responses, and determining recurrence risk.

Current Technologies for Investigating ctDNA include

- Technologies are categorized into either targeted or untargeted approaches. Targeted approaches focus on specific gene mutations and untargeted approaches (e.g., array-CGH, whole-genome or exome sequencing) screen for new genomic aberrations, such as resistance mutations. Targeted methods generally offer higher analytical sensitivity compared to untargeted methods.
- Next-generation sequencing can be used to examine cfDNA for methylation, rearrangements, point mutations, and aneuploidy. Point mutations typically provide the best balance among these techniques for the majority of clinical applications.
- Now-a-days highly sensitive and specific methods, such as BEAMing, Safe-SeqS, TAM-Seq (tagged-amplicon deep sequencing), CAncer Personalized Profiling by deep Sequencing (CAPP-Seq) and digital PCR, are being used to

detect cfDNA. These methods target single nucleotide mutations or use whole-genome sequencing to identify copy number changes. BEAMing, a PCR-based technique that works to detect somatic point mutations with sensitivities ranging from 1% to 0.001% on magnetic beads in water-in-oil emulsions.

3. Exosomes

Through micro vesicles like exosomes, tumors and healthy cells can discharge cellular material into the bloodstream, including DNA fragments, RNA, mRNA, miRNA, proteins, lipids etc. These exosomes are more stable than cell-free nucleic acids in blood and provide protective environment for the released cellular material. Exosomes can be isolated using ultracentrifugation, density-based separation, or immune-affinity capture with magnetic beads. Exosomes in tumors manipulate local and systemic environments, aiding cancer growth and dissemination, and at the same time they can also program the immune system to elicit an anti-tumor response through complex interactions (Kahlert and Kalluri, 2013). Exosome-derived miRNA and mRNA offer potential biomarkers and can help to classify patients into risk categories, enabling more personalized cancer therapy. Impaired exosome secretion could be a cancer therapy target. Calcium channels regulate exosome release by increasing intracellular calcium, and blocking these channels reduces exosome secretion. Moreover exosomal integrins may be able to predict organ-specific metastasis (Kahlert and Kalluri, 2013).

4. Tumor-Educated Platelets (TEPs)

The second most abundant cell type in peripheral blood are anucleated cell fragments are TEPs. Tumor cells mostly transfer any of their associated biomolecule mostly RNA into the platelets thus are educated by tumor. These platelets can be harvested by centrifugation, and their RNA is analyzed using RT-PCR. The TEP mRNA sequencing could provide a 96% accuracy rate in differentiating cancer patients from healthy individuals, and can correctly identify the location of primary tumor with 71% precision (In ‘t Veld and Wurdinger, 2019).

Current Available Liquid Biopsy Tests in Veterinary Medicine

The following tests are currently available in various developed countries of the world.

1. Blood Based Liquid Biopsy Tests

i) **OncoK9:** OncoK9 (developed by PetDx) measures cell-free DNA (cfDNA) levels, which are higher in dogs with malignant tumors. It detects common canine cancers with a 62% detection rate and 55% overall sensitivity, increasing to 85% for lymphoma, hemangiosarcoma, and osteosarcoma. The test is recommended annually for dogs aged 7 and older or younger high-risk breeds (Hannah et al., 2022).

ii) **Nu.Q Vet:** The Nu.Q Vet cancer test (developed by Volition Veterinary Diagnostics Development LLP) measures nucleosome levels in plasma. The test is for dogs aged 7 and older or high-risk breeds. It detects 7 common cancers with 50% sensitivity and 97% specificity, with the highest sensitivity for hemangiosarcoma (82%) (Wilson-Robles et al., 2021) followed by lymphoma (77%) (Wilson-Robles et al., 2020).

2. Urine Based Liquid Biopsy Tests

i) **CADET BRAF:** CADET BRAF (developed by Sentinel Biomedical) is used to diagnose bladder or prostatic tumors in dogs. It detects a BRAF gene mutation in cancer cells in the urine, especially in high-risk breeds like Scottish Terriers and Shetland Sheepdogs, potentially identifying UC before symptoms appear. The test has 85% sensitivity and over 99% specificity (Mochizuki et al., 2015).

ii) **Oncotect:** Oncotect (developed by Oncotect Inc) uses the olfactory ability of the nematode *Caenorhabditis elegans* to detect Volatile organic compounds (VOCs) in dog urine samples for detecting lymphoma, melanoma, hemangiosarcoma, and mast cell tumors. The VOCs are the metabolic by-product of cancer cells. This test can identify a patient's cancer risk rather than specific cancer types. The test has 85% sensitivity and 90% specificity. It is marketed as a cancer screening tool for dogs aged 7 years and older, or in 5 to 6 high-risk breeds, to be used at every 6 to 12 months (Hirotsu et al., 2015; Namgong et al., 2022).

Gaps in Research and Future Prospects

Although liquid biopsy tests are accessible in various developed countries, there isn't much data to support their broad use in India. In modern veterinary clinics and research facilities, some experimental techniques such as Next-generation sequencing, BEAMing, CAncer Personalized Profiling by deep Sequencing (CAPP-Seq), digital PCR and immune-fluorescent staining are being used. International collaborations, like trials involving PetDx's OncoK9, show how these tests can improve cancer outcomes by allowing earlier interventions in pets. Despite growing oncology practices and diagnostic advancements, India still lag behind in adopting these advanced tests.

References

Alix-Panabières, C., & Pantel, K. (2015). Liquid biopsy in cancer patients: advances in capturing viable CTCs for functional studies using the EPISPOT assay. *Expert Review of Molecular Diagnostics*, 15(11), 1411-17.

Alix-Panabières, C., & Pantel, K. (2021). Liquid biopsy: from discovery to clinical application. *Cancer Discovery*, 11(4), 858-73.

Alix-Panabières, C., Mader, S., & Pantel, K. (2017). Epithelial-mesenchymal plasticity in circulating tumor cells. *Journal of Molecular Medicine*, 95, 133-42.

Baccelli, I., Schneeweiss, A., Riethdorf, S., ... & Trumpp, A. (2013). Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. *Nature Biotechnology*, 31(6), 539-44.

Hannah, G., Amal T. & Alex A. (2022, May 3). Cancer Detection in Dogs: OncoK9 Liquid Biopsy and the CANDiD Study Q&A with Daniel Grosu of PetDx. https://www.decibio.com/insights/cancer-detection-in-dogs-oncok9-liquid-biopsy-and-the-candid-study-q-a-with-daniel-grosu-of-petdx

Hirotsu, T., Sonoda, H., Uozumi, T., Shinden, Y., Mimori, K., Maehara, Y. & Hamakawa, M. (2015). A highly accurate inclusive cancer screening test using Caenorhabditis elegans scent detection. *PloS One*, 10(3), e0118699.

In 't Veld, S. G., & Wurdinger, T. (2019). Tumor-educated platelets. *Blood, The Journal of the American Society of Hematology*, 133(22), 2359-64.

Kahlert, C., & Kalluri, R. (2013). Exosomes in tumor microenvironment influence cancer progression and metastasis. *Journal of Molecular Medicine*, 91, 431-37.

Leung, F., Kulasingam, V., Diamandis, E. P., Hoon, D. S., Kinzler, K., Pantel, K., & Alix-Panabières, C. (2016). Circulating tumor DNA as a cancer biomarker: fact or fiction? *Clinical Chemistry*, 62(8), 1054-60.

Mader, S., & Pantel, K. (2017). Liquid biopsy: current status and future perspectives. *Oncology Research and Treatment*, 40(7-8), 404-08.

Mochizuki, H., Shapiro, S. G. & Breen, M. (2015). Detection of BRAF mutation in urine DNA as a molecular diagnostic for canine urothelial and prostatic carcinoma. *PloS One*, 10(12), e0144170.

Namgong, C., Kim, J. H., Lee, M. H., & Midkiff, D. (2022). Non-invasive cancer detection in canine urine through Caenorhabditis elegans chemotaxis. *Frontiers in Veterinary Science*, 9, 932474.

Speicher, M. R., & Pantel, K. (2014). Tumor signatures in the blood. *Nature Biotechnology*, 32(5), 441-43.

Wilson-Robles, H., Miller, T., Jarvis, J., Terrell, J., Dewsbury, N., Kelly, T. and Michel, G. (2020). Evaluation of nucleosome concentrations in healthy dogs and dogs with cancer. *PLoS One*, *15*(8), e0236228.

Wilson-Robles, H., Miller, T., Jarvis, J., Terrell, J., Kelly, T. K., Bygott, T. and Bougoussa, M. (2021). Characterizing circulating nucleosomes in the plasma of dogs with hemangiosarcoma. *BMC Veterinary Research*, 17(1), 231.

Mini Review

Acute Phase Protein in Disease Diagnosis of Ruminants: A Mini Review

Pooja Devi and Chanchal Singh*

Department of Veterinary Physiology and Biochemistry,
College of Veterinary Science (Ludhiana),
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab- 141004.
*Corresponding author email: drchanchal833@gmail.com

Received: 29.10.2024 Accepted: 28.11.2024

Abstract

Acute phase proteins (APP) are valuable biomarkers for diagnosing and monitoring diseases in ruminants. This mini-review summarizes key studies on the role of APP including haptoglobin (Hp), serum amyloid-A (SAA), C-reactive protein, paraoxonase, fibrinogen, alpha-1 acid glycoprotein (AGP), and lipopolysaccharide-binding protein (LBP) in the diagnosis of various diseases of cattle, sheep, and goats. Hp and SAA levels rise significantly in various infectious diseases such as metritis, mastitis, brucellosis, and respiratory disorders. Increased SAA concentrations are also observed in lame cows, indicating hoof diseases like sole ulcers. The Hp, SAA, and LBP have been found useful in detecting bovine respiratory disease and subacute ruminal acidosis. In small ruminants, increased concentrations of Hp, SAA, and AGP are associated with parasitic infections and reproductive disorders. These findings emphasize the clinical relevance of APP as early and sensitive indicators for disease diagnosis and management in ruminants.

Keywords: *Acute phase proteins, Disease diagnosis, Haptoglobin, Ruminants, Serum Amyloid A*

The acute phase proteins are a large group of plasma proteins which originate mainly from the liver in response to pro-inflammatory cytokines, and released into the circulation quickly in response to inflammation, trauma, stress, infection, tumor, burns, surgery and immunological disorders (Saco & Bassols, 2023). Certain tissues, including testicular, adipose, lung, ovary, uterus, mammary glands, and digestive system can also produce APP. Animal species differ in the type and level of APP expression, and healthy animals have very low plasma concentrations of the APP. Their levels are significantly raised in the initial hours following exposure to the disease causing agents during the acute phase of inflammation. The concentrations of serum APP typically reach a peak in 24 to 48 hours. Within four to seven days post challenge, the feedback APP regulation inhibits the response if no new stimulus emerges and inflammation subsides.

Classification of Acute Phase Proteins

i) Negative Acute Phase Proteins: The concentrations of certain proteins in plasma

decreases in response to inflammation are called negative acute phase proteins. The decrease in concentration occurs very fast within a day or reduction may occur gradually over a period of days. Albumin and transferrin are two important negative acute phase proteins (Saco & Bassols, 2023).

ii) **Positive Acute Phase Proteins:** The concentrations of certain proteins in plasma increases in response to inflammation are called positive acute phase proteins. Generally, positive APP functions to trap microorganisms with their products, activate complement system, scavenge free haemoglobin and its radicals as well as modulate the host immune response (Saco & Bassols, 2023).

Positive APP are further categorized into following types

i) **Major APP:** Proteins that increases 10–1000 fold during acute phase response and reach their peak level in two to three days then begin to decline, however, the serum of normal healthy animals has a low concentration of <0.1 μg/dL. Serum amyloid A (SAA) is a major APP.

ii) **Moderate APP:** Moderate APP increases 4-10 folds only during acute phase response. An example of a moderate APP is haptoglobin (Hp).

iii) **Minor APP**: Represent those with only slight 2 to 3 fold increase during acute phase response. Fibrinogen is an example of minor APP.

Importance of Acute Phase Proteins in Ruminants

Cattle: In cattle, serum Hp and SAA are the primary APP, while albumin and paraoxonase are negative APP. Healthy ruminants exhibit low circulating Hp levels, but these levels can increase more than 100-fold when the immune system is activated. Calves due to their physiological demands during growth, present higher APP concentrations compared to adult cattle. Estimation of SAA and Hp helps to differentiate between acute and chronic inflammation in cattle. SAA1, SAA2, and SAA3 are active during the acute phase response, while chronic inflammation in cows involves seven distinct SAA isoforms. SAA1 and SAA2 are liver-derived, whereas SAA3 originates from extra hepatic tissues like adipose and mammary glands.

Acute Phase Proteins in Mastitis: In mastitis, APP assays are particularly useful for diagnosing subclinical cases. SAA concentrations increase in both serum and milk, with milk levels rising earlier and preceding increases in somatic cell count. A mammary isoform, M-SAA3, is secreted during mastitis (Molenaar et al., 2009). SAA levels rose 12 hours after experimental *E. coli* mastitis, peaking at 60 hours post-infection. Elevated levels of SAA and ceruloplasmin were also noted in subclinical infections, while Hp increased in both serum and milk during clinical mastitis (Szczubia et al., 2012). M-SAA levels are higher in milk from clinically and subclinically affected quarters. So, APP (particularly SAA) can be used as markers for tissue damage severity and early mastitis

detection.

Acute Phase Proteins in Metritis: Metritis occurs when bacteria colonize the uterus during parturition, leading to subfertility and infertility. APP increase during metritis, as in other infections and this inflammatory response occurs before the appearance of clinical symptoms. Cows with mild or severe metritis have higher Hp concentrations than healthy cows, and elevated Hp levels have been detected before the onset of abnormal uterine discharge. Cows with Hp values ≥1 g/L on day 3 postpartum had a higher risk of developing metritis. Similarly, Hp and serum amyloid-A (SAA) were associated with chronic subclinical endometritis, inflammation markers, and reproductive issues. SAA has gained attention due to its quick plasma rise, short half-life, and dynamic concentration changes during metritis and reproductive disorders like mastitis (Bazzano et al., 2022; Trela et al., 2022). CRP in uterine lavage fluid has been proposed as a local biomarker for uterine inflammation, decreasing alongside leukocyte presence during uterine involution (Tanai et al., 2020). Blood Hp levels above 80 mg/dl are markers of reproductive disorders during the first postpartum week. Schneider et al. (2013) observed reduced serum albumin and paraoxonase (PON) activity, alongside increased Hp in multiparous cows with uterine infections. These findings highlight the importance of APP for early diagnosis of uterine infections, offering potential for timely treatment and prevention of metritis.

Acute Phase Proteins in Infections: Coskun et al. (2012) reported a 10-fold increase in serum haptoglobin (Hp) and serum amyloid-A (SAA) concentrations in *Anaplasma marginale* infected cows compared to healthy controls. In cows infected with brucellosis, SAA levels significantly increased, while Hp showed no significant rise (Sharifiyazdia et al., 2012).

Ruminal Disorders: SAA concentration increased in cows fed high grain diets (Emmanuel, 2008). However, subacute ruminal acidosis induced by reduced fiber intake, does not elevate serum APP levels. Cannizzo et al. (2012) found that ruminal pH/acidosis did not trigger an acute phase response, though fatty liver development is associated with increased APP levels.

Acute Phase Proteins in Hoof Diseases and Lameness: Lame cows with sole ulcers exhibit higher SAA concentrations than healthy cows. Heifers with foot disease showed significantly elevated levels of SAA, Hp, and fibrinogen (Tothova et al., 2011). Smith et al. (2010) found that cows with claw abnormalities associated lameness had elevated Hp levels, suggesting an acute phase response.

Respiratory and Gastrointestinal Problems: Calves exposed to challenging husbandry conditions, such as mixing calves of different origins or inappropriate feeding, are more susceptible to respiratory and gastrointestinal diseases. Bovine respiratory

disease (BRD) is particularly common, often exacerbated by transportation stress. Hp, lipopolysaccharide-binding protein (LBP), and SAA have been explored as markers for BRD detection in feedlot conditions with high concentrations, and fever serving as a reliable disease indicators (Joshi et al., 2018). SAA, LBP, and Hp are sensitive markers for respiratory infections and valuable for clinical research on host response (Tothova et al., 2013).

Use of Acute Phase Proteins in Small Ruminants: Hp and SAA are the primary APPs, while alpha-1 acid glycoprotein (AGP), fibrinogen, and ceruloplasmin are also important in sheep and goats (Gomez-Laguna et al., 2011). Changes in APP concentrations occur early, before clinical signs appear, making them useful for early disease detection (Iliev & Georgieva, 2019). High Hp levels in sheep with dystocia indicate the presence of dead lambs, increased surgical risk, and poor prognosis. Elevated Hp and SAA levels in goats with mixed helminth infestations (e.g., Trichuris spp., Trichostrongylidae, Fasciola spp.) and in sheep experimentally infected with *Haemonchus contortus*, elevated levels of SAA, Hp, LBP, and AGP were observed (Zhong et al., 2014).

Table 1: Clinical uses of important acute phase proteins in ruminants

S.No	Name of the APP	Origin	Mechanism of Action	Clinical Use	Reference
1.	Haptoglobin (Hp)	Liver, locally synthesized in mammary gland	Binds free hemoglobin to prevent oxidative damage, modulates inflammation	Indicator of infections such as metritis, mastitis, respiratory and hoof diseases, reproductive disorders	Coskun et al. (2012), Smith et al. (2010), Tothova et al. (2011)
2.	Serum Amyloid-A (SAA)	Liver, mammary gland (M-SAA3)	Participates in inflammatory response, opsonizes bacteria, enhances recruitment of immune cells	Early marker for metritis, mastitis, brucellosis, ruminal disorders, respiratory issues, hoof diseases	Bazzano et al. (2022), Trela et al. (2022), Emmanuel, (2008)
3.	C-reactive protein (CRP)	Liver, present in uterine lavage fluid	Bind to dead cells and pathogens, activates complement system	Local biomarker for uterine inflammation in metritis	Tanai et al. (2020)

4.	Paraoxonase (PON)	Liver, other tissues	Antioxidant, prevents lipid peroxidation	Reduced activity in cows with uterine infections and reproductive issues	Schneider et al. (2013)
5.	Fibrinogen	Liver	Coagulation, promotes wound healing	Elevated in foot disease, useful for monitoring inflammatory conditions in ruminants	Tothova et al. (2011)
6.	Alpha-1 Acid Glycoprotein (AGP)	Liver	Anti-inflammatory, modulates immune response	Increased in gastrointestinal infections like *Haemonchus-contortus* in sheep	Zhong et al. (2014)
7.	Lipopolysaccha-ride Binding Protein (LBP)	Liver	Binds bacterial LPS, enhances immune response	Useful for detecting respiratory infections like BRD	Joshi et al. (2018), Tothova et al. (2013)

Despite their clinical importance, studies on acute phase proteins (APP) in India are limited, particularly in the context of cattle breeds and their unique physiological responses. Most research focuses on human and small animals, leaving a gap in understanding livestock-specific variations in APP expression during health and disease. Furthermore, the application of APP as routine diagnostic biomarkers is underutilized due to limited availability of standardized assays and reference ranges for Indian livestock. The influence of diverse climatic conditions, nutritional practices, and endemic diseases on APP dynamics remains poorly studied. Expanding research on APP in Indian settings could enhance early disease detection, improve herd health management, and support breeding programs for disease resistance. Collaborative efforts between veterinary institutions and animal scientists are needed to establish cost-effective diagnostic tools and promote their field application, offering significant opportunities for young researchers to contribute to this evolving field.

References

Bazzano, M., Marchegiani, A., Troisi, A., McLean, A., & Laus, F. (2022). Serum amyloid A as a promising biomarker in domestic animal's reproduction: current knowledge and future perspective. *Animal*, 12(5), 589.

Cannizzo, C., Gianesella, M., Giudice, V., Messina, G., Piccione, M., & Morgante (2012). Serum acute phase proteins in cows with SARA (SubacuteRuminal Acidosis) suspect. *Arquivo Brasileiro De Medicina Veterinaria E Zootecnia,* 64, 15-22.

Coskun,A., Ekicio, D., Güzelbektes, H., Aydoodu, U. & Sen,I. (2012). Acute phase proteins, clinical, hematological and biochemical parameters in dairy cows naturally infected with anaplasma marginale. *Kafkas Universities Veteriner Fakultesi Dergisi,* 18, 497-502.

Emmanuel, D. G. V., Dunn, S. M., & Ametaj, B. N. (2008). Feeding High Proportions of Barley Grain Stimulates an Inflammatory Response in Dairy Cows. *Journal of Dairy Science*, 91, 606-14.

Gomez-Laguna, J., Salguero, F. J., Pallarés, F. J., Rodríguez-Gómez, I. M., Barranco, I., & Carrasco, L. (2011). Acute phase proteins as biomarkers in animal health and welfare: In Book *Acute phase proteins as early non-specific biomarkers of human and veterinary diseases*, edited by F. Veas, Published by Intech Croatia, 10, 259-80.

Iliev, P. T., & Georgieva, T. M. (2019). Acute phase biomarkers of diseases in small ruminants: an overview. *Bulgarian Journal of Veterinary Medicine,* 22(1), 1–12.

Joshi, V., Gupta, V. K., Bhanuprakash, A. G., Mandal, R. S. K., Dimri, U., & Ajith, Y. (2018). Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. *Microbial Pathogenesis*, 116, 33-37.

Molenaar, A. J., Harris, D. P., Rajan, G. H., Pearson, M. L., Callaghan, M. R., Sommer, L., & Stelwagen, K. (2009). The acute-phase protein serum amyloid A3 is expressed in the bovine mammary gland and plays a role in host defence. *Biomarkers*, 14(1), 26-37.

Saco, Y., & Bassols, A. (2023). Acute phase proteins in cattle and swine: A review. *Veterinary Clinical Pathology*, 52, 50-63.

Schneider, A., Corrêa, M.N. & Butler, W.R. (2013). Acute phase proteins in Holstein cows diagnosed with uterine infection. *Research in Veterinary Science*, 95, 269-71.

Sharifiyazdia, H., Nazifi, S., Nikseresht, K. & Shahriari, R. (2012). Evaluation of Serum Amyloid A and haptoglobin in dairy cows naturally infected with Brucellosis. *Journal of Bacteriology Parasitology*, 3, 157.

Smith, B. I., Kauffold, J., & Sherman, L. (2010). Serum haptoglobin concentrations in dairy cattle with lameness due to claw disorders. *The Veterinary Journal,* 186(2), 162-65.

Szczubia, M, R., Dbrowski, M., Kankofer, M., Bochniarz, M. & Komar. (2012). Concentration of serum amyloid A and ceruloplasmin activity in milk from cows with subclinical mastitis caused by different pathogens. *Polish Journal of Veterinary Sciences,* 15, 291-96.

Tanai, S., Endo, N., & Tanaka, T. (2020). Quantifying the C-reactive protein concentrations of uterine lavage samples in postpartum dairy cows. *Animal Reproduction Science,* 217, 106455.

Tothova, C., Nagy, O., &Kovac, G. (2013). The serum protein electrophoretic pattern and acute phase proteins concentrations in calves with chronic respiratory diseases. *Acta Veterinaria*, 63(5-6), 473-86.

Tothova, C.S., Nagy, O., Seidel, H., Paulíkova, I. & Kovac, G. (2011). The influence of hoof diseases on the concentrations of some acute phase proteins and other variables of the protein profile in heifers. *Acta Veterinaria (Beograd),* 61, 141-50.

Trela, M., Domańska, D., & Witkowska-Piłaszewicz, O. (2022). Diagnostic use of serum amyloid A in dairy cattle. *Agriculture,* 12(4), 459.

Zhong, R., Yang Li H., Sun H., & Zhou, D. (2014). Effects of supplementation with dietary green teapolyphenols on parasite resistance and acute phase protein response to *Haemonchus contortus* infection in lambs. *Veterinary Parasitology,* 205, 199–207.

Mini Review

Reinforcing Flipped Learning in Veterinary Science with Knowledge Dissemination through Augmented Reality and Virtual Reality

Nirmal Singh*, HS Banga, Jaswinder Singh and Rajnish Sharma

Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding Author email: nirmal02@yahoo.co.in

Received: 14.10.2024 Accepted: 08.11.2024

Abstract

The pervasiveness of ever-evolving technological interventions has persistently intrigued and transformed the educational sector making teaching-learning activities more involving and interactive for better comprehension of the subject(s). Augmented Reality (AR) and Virtual Reality (VR) are revolutionizing education by enabling personalised, pragmatic, and experiential learning, encouraging active engagement of stakeholders. In veterinary education, use of two dimensional teaching aids for veterinary training as an alternative to in-situ cadaver exposure limits the scope for practical learning, leaving much to the conjectural vision of learners. In this situation, AR and VR offer up new vistas for honing the clinical skills of veterinary students by allowing them real like animal handling experiences, providing an immersive environment for their improved practical learning. This paper provides an overview of AR and VR technology and enlists the major global initiatives to harness the potential of this novel technology in veterinary and animal sciences education and training.

Keywords*: Augmented Reality, Immersive education, Veterinary education, Virtual Reality.*

Adaptation to and adoption of emerging technologies is imperative to make education dynamic and engaging and for providing the necessary environment to pave paths for lifelong learning by students. Furthermore, educators have to develop capabilities amongst students to prepare them for primarily technology-driven fields (Statti and Torres, 2020). The pervasiveness of powerful technologies synergised with flexible modes of delivery, smart integration strategies, and effective policies enhances access to quality education and self-directed learning (Senteni, 2014; Groff, 2013).

The emerging technologies viz. Artificial Intelligence, Internet of Things, 3D Printing, Augmented Reality (AR) and Virtual Reality (VR) are going to be the game changers, facilitating personalised, practical and more realistic learning experiences and thus stimulating the active participation of stakeholders. AR and VR technologies are an interactive learning environment giving learners better control of their learning and interaction with digital objects in a real and virtual environment, respectively (Almoosa, 2018). This article discusses about the utilization of AR and VR by various institution(s) globally for boosting veterinary education, practice and self-learning of students. The use of these technologies not only reduces the need for cadavers for education and training

but is also pivotal for prevention of zoonotic diseases and health hazards while animal handling. In addition, the user may repeat the procedure multiple times. Though still in the infancy stage, these technologies have started gaining the attention of veterinary science stakeholders, and the future of AR and VR in veterinary education seems to be optimistic and promising in supplementing teaching-learning processes in multiple subjects. However, the high cost of developing learning modules based on these technologies is a major hindrance obstructing their accessibility in economically weaker countries.

Augmented Reality (AR) and Virtual Reality (VR): AR and VR are the interactive cutting-edge digital media harvests of Information and Communication Technology novelties making inroads to various domains encompassing the education/ business sectors. Sometimes AR and VR are thought to be same, but the level of immersion/ interaction differentiates these two technologies. They are entrenched in the same concept with difference of the level of immersion being offered manipulating the 'Reality-Virtuality Continuum' (Milgram et al., 1994).

AR amplifies information contained in print and/or digital media coupling a blend of physical and digital worlds (Almoosa, 2018). This involves enhancing the real world environment and/or objects "by digital content that is tied to certain activities, places or objects" (Uluyol & Sahin, 2016), allowing the mixing of digital content into the physical environment, aimed at augmenting the users' knowledge and understanding of what is going on around him/her. In this, simulated cues are overlaid on the real-world for users interaction with the content. Thus, it generates a mixed environment allowing a conglomerate of virtual objects generated by computer(s) within a real environment for viewing with technological gadgets in real time (Redondo et al., 2014; Silva, et al., 2003). The information supported by AR is "most often visual, sometimes auditory and is rarely haptic" (Budhwar, 2017). The AR has applicability in various fields such as advertising, entertainment, sightseeing, driving assistance, tourism, professional gesture assistance, games, education etc. (Almoosa, 2018; Arnaldi et al., 2018). The affordability and universal availability of mobile phones, integration of digital media in these, and accessibility of internet over these handheld devices have increased the number of AR tools and its applications (Almoosa, 2018).

The VR comprises of construction of a fully-immersive digital environment, where the user interacts with the content using technological gadgets, viz. Head Mounted Device, visualization goggles and/or haptic gloves, supplemented with headphones. It is a process arbitrating interaction between man and machine in a simulated realistic environment (Latta & Oberg, 1994). In addition, VR may have the advantage of auditory systems. To generate the sensation of immersive environment the technology must "deceive the brain" by providing it with information identical to the information the brain would perceive in the real environment" (Arnaldi et al., 2018).

The virtual environment brings users closer to the real world leading to effective learning practices (Juanes and Ruisoto, 2016), as VR is a safe and cost-effective tool. One can repeat the procedure till his/her satisfaction without fear of causing any harm to the subject or danger of zoonosis, as the animal is in virtual mode only. VR is being used for innovative training processes in education, healthcare, and various business sdectors (Arnaldi, 2018; Thompson, 2022). Despite various potential benefits, AR and VR have not attained much attention as learning tools in educational spheres, except in the medical education sector (Blackwell et al., 2014; SpeakUp, 2016; Marks & Thomas, 2022). The prospects for the utilization of such technologies have not yet been fully realised in agriculture and livestock spheres (Kiryakova, 2018).

AR/ VR in International Veterinary Education

The livestock sector is a key component of the global food system, having a polar role in ensuring rural employment and poverty alleviation by safeguarding a stable rural economy. The expansion and prosperity of the livestock industry is highly reliant on the academic proficiency of stakeholders involved in expert extension services, high-quality veterinary education, and animal husbandry methods. The comprehension of anatomical, physiological, pathological, medicinal, surgical and other para-clinical and clinical subjects is imperative for the strong foundation of any high-quality capacity-building programme. However, an absence of precise understanding of the clinical processes/ procedures involved, and subsequent failure on part of the learners can lead to the mortality of the patient/animals. Furthermore, the animal protection regulatory bodies may impose restrictions over the use of live animals/cadaver/, leaving a few opportunities for veterinary students to learn with animals in a practical setting. Thus, it has become difficult to use animals in research and education. The use of two-dimensional graphics, texts and/or videos for veterinary training have been used as an alternative to limit the use of real animals (Xu, 2021), curtailing the scope for practical learning, leaving much to the conjectural vision/imagination of learners.

In addition to the ethical issues of using live animals in research and education, it is also possible that an outbreak of a disease may not occur during a student's academic career, leaving students handicapped with no practical exposure to such epidemics or diseases. AR and VR open up new vistas to address these problem(s) by enabling real like animal/livestock handling experiences in an immersive environment for better practical learning. These technologies allow students/stakeholders to understand intricate procedures better and may enhance the veterinarians' clinical reasoning abilities and hands on skills to support the livestock industry. By making content readily available to learners and giving them extra inputs while they interact with the subject, AR empowers them to manage their time from a remote location and develop their workplace competencies. VR replaces real-world settings and is quite useful to impart education about procedures and

processes involved in diagnosis/treatment of animals without causing any practical harm to animals due to repeated handling or even damage caused by an amateur professional, and prevents disease transmission during practical exposure. These technologies have a plethora of usages in health sciences for honing the skills of learners without risking human life. Dr. Queens opined "If it's working in human medicine and human surgical training, then there's no reason why this isn't going to work there" (in veterinary science). Talking about effectiveness of the technology, in reference to the Stanford Heart Project, he claimed, "Honestly, spending five minutes in this has given me more learning value than I ever got from reading textbooks and attempting to piece this material together from two-dimensional sources" (Peng, 2019). It is in congruence to the adage "I tell you – you forget; I show you – you remember and I involve you – you never forget".

The avenues opened up with AR and VR, have led to global initiatives to harness the potential of this novel technology for improving the competencies and practical skills of veterinary professionals to serve the livestock sector. AR has been implemented primarily to understand the anatomical systems permitting one to move inside the body of the animal using gadgets. For better understanding of the subject matter, Virginia-Maryland College of Veterinary Medicine at Virginia Tech, USA developed a virtual dog anatomy allowing students to access, explore, and learn the location of organs within the skeletal system of dogs. Hardware features help to make it easier to explore the layers of tissues, zoom in on specific organs, and let users step into sections of a virtual dog's anatomy that would otherwise be impossible. To make the software publicly available under an open licence so that other universities and veterinarians of Virginia Tech's land-grant mission may also use this technology, the project was funded by the University Libraries Open Education Faculty Initiative Grant (Virginia Tech., 2018). The Virtual Canine Anatomy programme, developed by Colorado State University's College of Veterinary Medicine and Biomedical Sciences, provides high-quality cadaver photographs as a supplement to the university's first-year anatomy classes (Stilwell, 2018). Development of such content requires meticulous and accurate inputs from the subject specialists, so that the realistic subject may be replicated in digital form for learning by the learners. A leading provider of educational technology, LlamaZOO, collaborated with the University of Missouri, the University of Saskatchewan, and the University of Pennsylvania, USA to create the EasyAnatomy for veterinary learners, accessible on tablet, laptop, and desktop computers LlamaZOO, 2017).

Cornell University (USA) has developed an augmented reality application to aid DVM students in their study of the equine musculoskeletal system. A digital image of a horse limb is superimposed onto the environment through an iPad in an application developed with funding from an internal Educational Technology Innovation Grant. This allows practise without use of numerous live animals or the risks associated with

repeated exposure to x-ray beams for both practitioners and patients. By bridging pre-clinical and clinical content, the augmented reality app helps students comprehend the relevance of the anatomy in a better way (Cornell University, 2019). In another initiative, the University of Pennsylvania, School of Veterinary Medicine developed an augmented reality interface for a difficult spinal cord surgery to give newer veterinary students the chance to interact with real anatomies and diseases well before they work/ interact with patients in clinics during their fourth year (Adorno, 2018). The School of Veterinary Science at the University of Liverpool (England) created a 3D image of an equine heart that can be seen on a user's smartphone when it is placed next to cardiac diagrams (University of Liverpool, 2014).

Some 3D models have also been made publically available through dedicated platforms. To overcome the problem of obtaining meat carcasses for teaching anatomical meat science in the United States, University of Nebraska-Lincoln (USA) created an open repository of 3D virtual animal carcass simulations that are freely accessible from Sketchfab, an online platform for publishing, sharing, and discovering 3D and VR content. This gives the students access to laboratory resources and allows them to digitally learn cattle anatomy and tissue cutting. These models were used by the developers to develop computer and mobile simulation software for studying carcasses using 3D flashcards. The platform-neutral application was developed for students who were interested in meat-judging competitions. Compared to the pricey paper-based textbooks and other reading materials, application is cost-effective (Guru et al., 2017).

Research has also been conducted to examine the effectiveness of the AR and/or VR content to supplement the learning experiences of users. Little *et al*. (2021) investigated how pre-veterinary students at Ross University School of Veterinary Medicine used and preferred the IVALA® augmented reality heart programme, providing free access via Veterinary Information Network®. Compared to textbooks and other lecture materials, students found the IVALA® AR programme to be more engaging as it enabled interactions through 360-degree rotation, zoom-in capability, overlay of the 3D model onto cadaveric tissue allowing identification of the structure of the actual heart, and further engagement with specific anatomic components. The findings depicted a positive correlation between inherent spatial ability and the learning of cardiac anatomy.

Different applications developed permit different levels of interaction of the user with the subject. The University of Georgia, College of Veterinary Medicine used software from the BodyViz firm, created by Iowa State University, during the COVID-19 epidemic. Based on clinical situations that involved the use of computed tomography and magnetic resonance imaging, the application reconstructs virtual 3D animals. Students can then visually dissect these models by making "cuts" in various planes to access, see, and recognise anatomical features (Fender, 2021).

Besides veterinary anatomy, AR/VR has witnessed application in veterinary medicine, surgery, pathology etc. An AR intravenous (IV) injection simulator was created by Konkuk University in the Republic of Korea to teach veterinarian and pre-veterinary students how to venipuncture dogs. The assessment of students' skill with IV injection technique showed that the group taught using an AR simulator was more skilled than the control group at IV injection technique when using real canines, advocating that AR simulation is an advantageous teaching tool for medical professionals (Lee et al., 2013). The quality of experience (QoE) of 360-degree immersive video in university education of fourth-year veterinary medicine students covering topics of surgical pathology and surgery related to horses was assessed at Universidad Alfonso X El Sabio (UAX), Madrid, Spain. VR technologies were integrated into the already-existing practical lessons. The majority of respondents (79%) rated the experience as excellent or good and admitted that using VR as a teaching tool had improved their learning. Students experienced a sense of spatial presence in the operating room, which caused them to perceive the scene as though they were present at the time of the surgical intervention (Guervos et al., 2019).

Experiments have also been conducted on use of technology to regulate animal movement and behaviour with different success levels. In an experiment at a farm in Pathanamthitta, Kerala, India, AR stimuli, was gauged for its potential to enhance the welfare of animals herded using virtual fencing. When utilised to mark the virtual limits of the paddock while an animal was grazing, auditory cues and/or electric shocks caused the animal to become more stressed, which in turn reduced milk production by ten to twenty percent, reflecting the adverse effects of technology (Simon and Prasad, 2017). The potential of augmented reality for human-animal interaction in terms of mental and social health has also been explored (Norouzi et al., 2020; Oxley et al., 2022).

In addition to the core subjects of veterinary and animal sciences, AR/VR technology has been applied to create awareness about the animal structures/ morphology amongst masses. For the purpose of bringing scientifically accurate models to a large audience and promoting paleoart, researchers from the Natural History Museum of Los Angeles County, the La Brea Tar Pits and Museum, and the University of Southern California created three-dimensional, animated models of some of the ice age animals discovered in the site of Rancho La Brea, Los Angeles (Jones,. 2022).

These technologies have also been used for sensitising school kids for better recognition of animals. A study was done at a kindergarten in Indonesia's Gerokgak District to help design an application that would teach kids about animal classification and explanation using 3D objects representing information about sounds, habitat, and breeding. A few of the app's intriguing features include the display of 3D models, animations, and verbal descriptions of the creatures in augmented reality. The application was tested, and the outcome was encouraging. The interface and storyboard design

could be implemented into the application with success (Marti et al., 2020). Similarly, applications for learning media that can introduce water and land animals to kindergarten children have been created to increase flexibility in teaching and learning activities (Aditama et al., 2021).

Creation of AR/VR models and applications requires collaboration of veterinarians with IT experts for producing workable models. Christ et al. (2018) presented the processes, challenges, and solutions for creation of accurate, data-based anatomical model(s) that could potentially be used in the veterinary curriculum. They also established a methodology for the creation of an AR application for basic canine head anatomy. Virtual-reality headsets have also been used by veterinarians to examine radiographs and show these to the owner to discuss the potential diagnosis (Packer, 2020).

AR/ VR in Veterinary Education in India: An observation of the studies/ AR and VR initiatives given above reflects that majority of such efforts have been concentrated on veterinary anatomy learning. There is lack of focus on the exploration of possibilities to use this novel technology in veterinary surgery, pathology, social aspects etc. Besides augmenting teaching-learning in anatomical and physiological structures, these technologies offer every possibility to digitally support para-clinical and clinical domains with the addition of desired levels of complexities to prepare students to deal with any situation. It is high time to explore the potential of AR and VR for delivering customized training in multiple veterinary domains, in a safe and realistic or near-realistic milieu. The real cadaver are preserved in formalin and during practice an exposure to formalin through inhalation and/or by skin and/or eye contact may pose health hazards to learners. The 3D models free learners from such risks. These technologies enable multiple learners to practice on the same cadaver simultaneously, depending upon the hardware devices available. The AR and VR content developed by an institution/organization with public funding can be made available globally to benefit stakeholders world-wide, or even by charging a nominal subscription fee to meet maintenance and other expenditures involved.

Furthermore, these technologies bid promising prospects to reach-out to the farmers at mass level to empower them by disseminating *au currant* and practical knowledge at their doorsteps. Guru Angad Dev Veterinary and Animal Sciences University has taken the lead in this direction. The University has developed AR and VR contents under the Rashtriya Krishi Vikas Yojana (RKVY) project funded by the Government of India. A few stills from the modules developed are given here (Figures 1-3).

The AR-enabled hybrid interactive books have also evolved, complementing textual and graphic information with mobile application-based 3D models. The ‘Cardiologia 3d En Pequeños Animales’ (3D cardiology in small animals) created by Pixeldreams permits readers to access dynamic 3D images of the animal heart and its

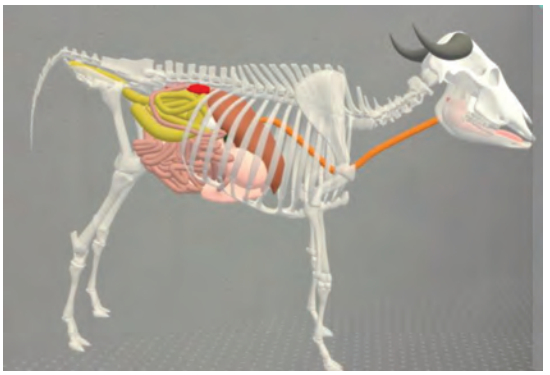


Fig. 1. AR/VR model to demonstrate anatomy of visceral organs of bovine

Fig. 2. AR/VR model to demonstrate topography and muscular anatomy in dog

pathologies by scanning the QR codes that appear in the book through a dedicated mobile application (Pixeldreams, n.d.).

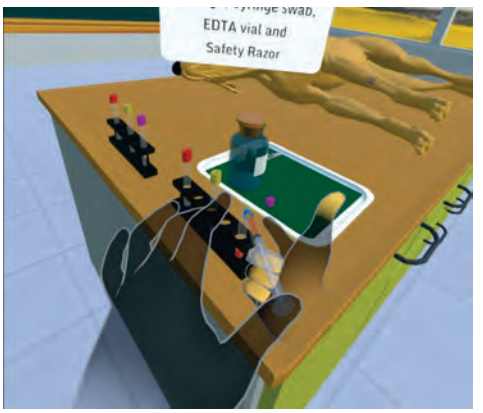


Fig. 3. A still from VR module of blood collection

Limitations of AR/VR: Despite having various advantages, the development of AR models and VR scenarios involves a new level of time, cost and expertise, making these technologies expensive. Accuracy in content development needs a continuous alliance and communication amongst veterinary peers and technical developers, particularly in the case of complex models. The 3D modelling requires thorough expertise in the use of software for this purpose. The complexity of the models is correlated to cost due to the time and effort involved. Head Mounted Displays (HMDs) are also very expensive and usually not affordable at an individual level. The user of HMDs may suffer headache and eye pain as the brain has to adjust to a completely new environment immediately upon bearing the device, and prolonged usage of such devices may turn the user as couch potatoes, due to lack of physical exercise.

Though the time, efforts and high cost involved hinders the wider acceptance of AR/VR technology, the impending benefits outweighs these factors. Complying with the Cape Town Open Education Declaration (2008) and Paris OER Declaration (2012) for sharing of public funded research can enable the accessibility of learning material benefiting users globally. It would also cut the costs and diminish the duplicity of efforts of AR and VR content development.

References

Aditama D., Damastuti F.A., Majid N.S., Mufid M.R., & Fiyanto A. (2021). "Development of animal recognition e-learning using augmented reality technology", *Proceedings of the International Conference on Applied Science and Technology on Social Science (iCAST-SS 2021), Seies: Advances in Social Science, Education and Humanities Research*, DOI: https://doi.org/10.2991/assehr.k.220301.163

Adorno S. (2018). "Augmented Reality at Penn Vet", https://www.vet.upenn.edu/about/news-room/bellwether/penn-vet-extra/penn-vet-extra-april-2018/augmented-reality-at-penn-vet

Almoosa A.S. (2018). "A qualitative case study in augmented reality applications in education: dimensions of strategic implementation". Dissertations, 485. https://digscholarship.unco.edu/dissertations/485

Arnaldi B., Guitton P., & Moreau G., ed. (2018). *Virtual reality and augmented reality: myths and realities*, London, Wiley.

Blackwell C.K., Lauricella A.R., & Wartella E. (2014). "Factors influencing digital technology use in early childhood education". *Computers & Education*, 77, 82-90. doi:10.1016/j.compedu.2014.04.013

Budhwar K. (2017). "The role of technology in education". *International Journal of Engineering Applied Sciences and Technology*, 2(8), 55-57. https://www.ijeast.com/papers/55-57,TESMA208,IJEAST.pdf

Cape Town Open Education Declaration (2008). https://www.capetowndeclaration.org/read/

Christ R., Guevar J., Poyade M., & Rea P.M. (2018). "Proof of concept of a workflow methodology for the creation of basic canine head anatomy veterinary education tool using augmented reality". *PLoS ONE*, 13(4), e0195866. https://doi.org/10.1371/journal.pone.0195866

Cornell University (2019). "College-designed AR app sparks new level of learning for veterinary students", https://www.vet.cornell.edu/news/20190508/college-designed-ar-app-sparks-new-level-learning-veterinary-students

Fender K. (2021). "How Technology Is Transforming Veterinary Education: From virtual reality to virtual dissection, technology in veterinary education is kicking into high gear, Today's Veterinary Practice, May/June 2021. https://todaysveterinarypractice.com/technology/how-technology-is-transforming-veterinary-education/

Groff J. (2013). "Technology-Rich Innovative Learning Environments", https://www.oecd.org/education/ceri/Technology-Rich%20Innovative%20Learning%20Environments%20by%20Jennifer%20Groff.pdf

Guervos E., Ruiz J.J., Perez P., Munoz J.A., D´az C., & Garc´a N (2019). "Using 360 VR video to improve the learning experience in veterinary medicine university degree", *IS&T International Symposium on Electronic Imaging 2019 Human Vision and Electronic Imaging*. https://oa.upm.es/64508/1/INVE_MEM_2019_310462.pdf

Guru H., Jones S., & Guru A. (2017). "3D Visualization and Virtual Reality in Animal Science", Monday, October 9, 2017, *Educause Review*. https://er.educause.edu/articles/2017/10/3d-visualization-and-virtual-reality-in-animal-science

Jones S. (2022) "New Augmented Reality Models Bring Ice Age Animals to Virtual Life., Scientific American", https://www.scientificamerican.com/article/ice-age-animals-come-to-life-via-augmented-reality/

Juanes JA, & Ruisoto P. (2016). "Latest technology trends in health sciences education". *TEEM '16: Proceedings of the Fourth International Conference on Technological Ecosystems for Enhancing Multiculturality*, pp. 457-60. https://doi.org/10.1145/3012430.3012556

Kiryakova G., Angelova N., & Yordanova L. (2018). "The Potential of Augmented Reality to Transform Education into Smart Education", *TEM Journal*, 7(3), 556-65. DOI: 10.18421/TEM73-11

Latta J.N., & Oberg D.J. (1994). "A conceptual virtual reality model". *IEEE Computer Graphics and Applications*, 14(1), 23-29. DOI:10.1109/38.250915

Lee S., Lee J., Lee A., Park N., Lee S., Song S., Seo A., Lee H., Kim J.I., & Eom K. (2013). "Augmented reality intravenous injection simulator based 3D medical imaging for veterinary medicine", *The Veterinary Journal*, 196(2), 197–202. https://doi.org/10.1016/j.tvjl.2012.09.015

Little W.B., Dezdrobitu C., Conan A., & Artemiou E. (2021). "Is Augmented Reality the New Way for Teaching and Learning Veterinary Cardiac Anatomy?" *Medical Science Educator*, 31(2), 723-32. DOI: 10.1007/s40670-021-01260-8

LlamaZOO. (2017). "LlamaZOO to Unveil World's First Virtual Reality Canine Dissection World's First Virtual Reality Canine Dissection at 2017 Western Veterinary Conference, Las Vegas", https://llamazoo.com/llamazoo-to-unveil-worlds-first-virtual-reality-canine-dissection-at-2017-western-veterinary-conference-las-vegas/

Marks B., & Thomas J. (2022). "Adoption of virtual reality technology in higher education: An evaluation of five teaching semesters in a purpose-designed laboratory". *Education and Information Technologies*, Vol. 27, pp. 1287-1305. https://doi.org/10.1007/s10639-021-10653-6

Marti N.W., Dewi L.J.E., Permana A.A.J., &Ariawan I.M.Y (2020). "Augmented reality (AR) based application to introduce animals for children". *Journal of Physics: Conference Series*, 1516, 012022, DOI: 10.1088/1742-6596/1516/1/012022

Milgram P., Takemura H., Utsumi A. & Kishino F. (1994). "Augmented Reality: A class of displays on the reality-virtuality continuum". *Proceedings of SPIE* 2351. Telemanipulator and Telepresence Technologies.

Norouzi N., Bruder G., Bailenson J., & Welch G (2020). "Investigating augmented reality animals as companions", https://www.stanfordvr.com/mm/2020/08/norouzi-ismar-investigating.pdf

Oxley J.A., Santa K., Meyer G., & Westgarth C. (2022). "A systematic scoping review of human-dog interactions in virtual and augmented reality: the use of virtual dog models and immersive equipment", *Frontiers in Virtual Reality*, 3, 782023. DOI: 10.3389/frvir.2022.782023

Packer R.A. (2020). "The future of virtual care in veterinary medicine", dvm360, 51(10). https://www.dvm360.com/view/the-future-of-virtual-care-in-veterinary-medicine

Paris OER Declaration (2012). https://en.unesco.org/oer/paris-declaration

Peng S. (2019). "Dr. Queen shares the possibilities of AR/VR in veterinary science", Emerging Media Lab, Vancouver, Canada. https://eml.ubc.ca/news/dr-queen-shares-the-possibilities-of-ar-vr-in-veterinary-science/

Pixeldreams (n.d.). "Pixel Dreams Projects: Creation of a book on Cardiology using augmented reality for Editorial Servet". https://www.pixeldreams.info/en/books-augmented-reality-editorial-servet

Redondo E., Puig J., Fonseca D., Villagrasa S., & Navarro I. (2014). "Augmented and Geo-Located Information in an Architectural Education Framework". *In: Shumaker, R., Lackey, S. (eds) Virtual, Augmented and Mixed Reality. Applications of Virtual and Augmented Reality*. VAMR 2014. Lecture Notes in Computer Science, vol 8526. Springer, Cham. https://doi.org/10.1007/978-3-319-07464-1_2

Senteni A. (2014). "The Role of New Technologies in Advancing Education and Learning". *ECSSR 19th Annual Conference - Technology: Impacts, Challenges and the Future*, Emirates Centre for Strategic Studies & Research, Abu Dhabi

(UAE), 2014.

Silva R., Oliveira J.C., & Giraldi G.A. (2003). "Introduction to augmented reality", https://www.lncc.br/~jauvane/papers/RelatorioTecnicoLNCC-2503.pdf

Simon S., & Prasad A. (2017). "Examining the potential of augmented reality to improve health and welfare of animals herded using virtual fencing", *1st Asian-Australasian Conference on Precision Pastures and Livestock Farming*, New Zealand: Precision Agriculture Association New Zealand, Hamilton.

SpeakUp. (2016). "Augmented and virtual reality in K-12 education: Current status and aspirations", https://tomorrow.org/speakup/speak-up-2016-augmented-and-virtual-reality-in-k12-education-april-2017.html

Statti A. & Torres K.M. (2020). 'The Advancement of Technology in Schools and Universities". *Peabody Journal of Education*, 95(2), 115-16. DOI: 10.1080/0161956X.2020.1745591

Stilwell N. (2018). "New Augmented Reality Program Teaches Canine Anatomy", https://www.dvm360.com/view/new-augmented-reality-program-teaches-canine-anatomy.

Thompson S. (2022). "VR Applications: 23 Industries using Virtual Reality", https://virtualspeech.com/blog/vr-applications

Uluyol C. & Sahin S. (2016). "Augmented Reality: A New Direction in Education", Emerging Tools and Applications of Virtual Reality in Education, DOI: 10.4018/978-1-4666-9837-6.ch011

University of Liverpool (2014). "Analysing animal anatomy using augmented reality", https://phys.org/news/2014-10-analysing-animal-anatomy-augmented-reality.html

Virginia Tech. (2018). "Virtual reality brings dog's anatomy to life for veterinary students". https://www.eurekalert.org/news-releases/472157

Xu X., Pan X., Kilroy D., Kumar A., Mangina E., & Campbell A.G. (2021). "Augmented Reality for Veterinary self-learning during the pandemic: a holistic study protocol for a remote, randomised, cross-over study", *Proceedings of the 34th British HCI Workshop and Doctoral Consortium*, DOI: 10.14236/ewic/HCI2021-W2.3

Clinical Article

Right Flank Ovario-Hysterectomy for Animal Birth Control Program in Dogs

Vandana Sangwan*, Ashwani Kumar and Maninderjit Singh

Department of Veterinary Surgery and Radiology,
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: drvandanasangwan@rediffmail.com

Received: 22.10.2024 Accepted: 25.11.2024

Abstract

Increasing street dogs population is of serious concern in urban and sub-urban regions of India. Government and many non-government organizations are working to solve the problem of street dog's population. This report describes the technique of right flank ovario-hysterectomy in dogs for the purpose of animal birth control under general anesthesia. The technique is economical, quick, requires less abdominal exposure, less complications and early recovery that ensures early release of the dog. This technique has potential to be adopted for mass Animal Birth Control Program.

Keywords*: Dog, General anesthesia, Ovario-hysterectomy, Right flank, Spay*

The present scenario on the day by day increase in the street dog's population is of great concern in urban and suburban areas. The ovario-hysterectomy (OVH) of the female dog is considered one of the nominal solutions, provided if it is done at high rate locality wise. The conventional OVH is a major surgical procedure and is usually approached through linea alba but it is associated with various postsurgical complications such as evisceration, delayed healing, wound dehiscence, and herniation (Sharda et al., 2022). The flank approach for OVH has certain advantages (Sangwan 2005; Reece et al., 2012) over linea alba approach, therefore it has a potential to be adopted practically, especially for the control of stray dog's population. This communication describes the detailed technique for the right flank OVH in female dogs.

Anesthesia, Restraint and Preparation

1. The dog can be restrained/controlled using a higher dose of xylazine @2-3mg/Kg, Intramuscularly, even if the dog is not off-feed (dog will vomit).
2. Intravenous cannulation/scalp vein set applied for intravenous fluids.
3. The right flank area (including stifle fold, tuber coxae, lumbar processes, and last thoracic rib) is shaved and prepared aseptically.

5. Xylazine @0.5mg/Kg + Ketamine @3mg/Kg can be used intravenously for induction and intubation. The anesthesia can be maintained using isoflurane (1.5-2.0% in oxygen). If inhalation anesthesia is not available, the above mixture and amount is kept filled to top up anesthesia (one third to half dose every time), whenever required.

Technique of Right flank OVH (Step wise)

Step 1. The dog is restrained in lateral recumbency with the right flank upwards, with the hind limbs tied caudally with mild stretch.

Step 2. Landmarks for incision (Fig. 1).

Step 3-6. Skin incision (Fig. 2) and all 3 abdominal muscle layers are opened to reach the abdominal cavity (Fig. 3 to Fig. 5).

Step 7. Exteriorize right uterine horn (Fig. 6) using finger or spay hook.

Step 8-9. Right ovary pulled out of the incision by breaking suspensory ligament (Fig. 7) and ligated using standard 3 artery forceps technique using Catgut No. 2 or polyglactin 910 No. 2-0. Similar procedure is repeated on the left ovarian side (Fig. 8).

Step 10. The caudal cervical stump is also ligated, transfixed and severed after check for any bleeding. The excised genitalia are checked for its complete removal, particularly both ovaries (Fig. 9).

Step 11-12. All the 3 layers of muscles (Fig. 10) and the subcutaneous tissue (Fig. 11a) are sutured in simple continuous pattern using the same thread (used for ligatures inside). Nylon is used for skin sutures (Fig. 11b). Loose 'Dynaplast bandage' can be applied around flank (Fig. 12) with a gauze piece kept on the incision line.

Post-operative Care: Single dose of long acting antibiotic and anti-inflammatory is sufficient, if proper asepsis is followed during the procedure. The bandage, if comfortable may be removed at 7-10 days and sutures are opened. If the dog is under supervision of some local resident, it may be set free on 3rd day with bandaging on. And it may be caught later on for suture removal and final release.

Advantages of Flank Approach over Linea alba (Sangwan 2005; Reece *et al*., 2012)

1. Smaller incision (Fig. 11b).
2. Faster operative procedure.
3. Faster healing since muscles are involved.
4. One thread (suture material) is sufficient.
5. Flank being non-dependent part, so less soiling and suture related complications.
6. Early release of the dog.

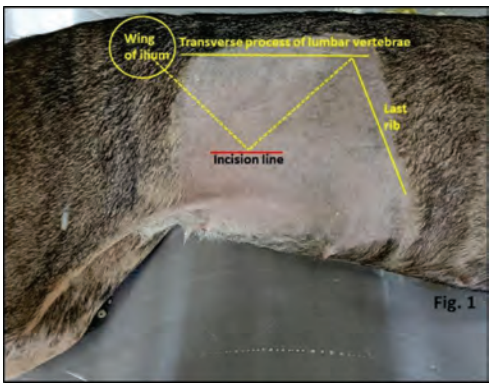


Fig. 1. Photograph showing the landmarks and site of incision (approx. 2.5 -3cm).

Fig. 2. An incision line in made with scalpel blade.

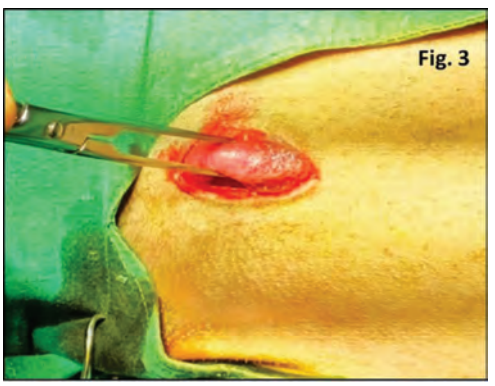


Fig. 3. The first layer of flank muscles is grasped with an Allis tissue forceps.

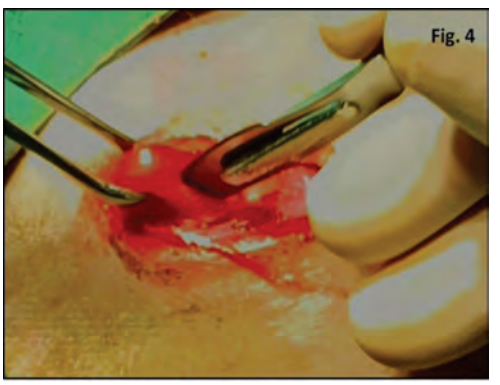


Fig. 4. A small incision is made on the muscle layer.

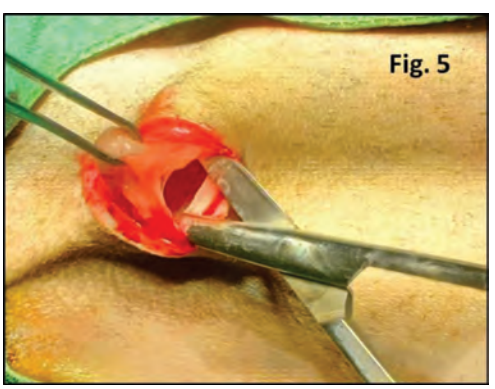


Fig. 5. The incision is opened with a scissor and extended on both sides.

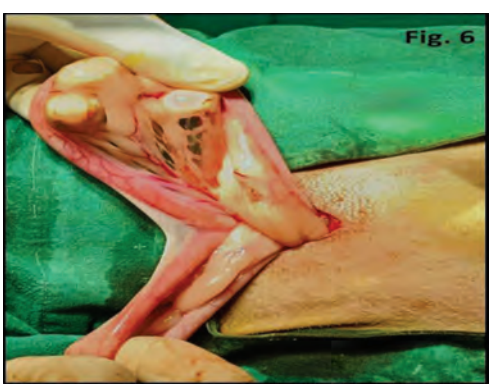


Fig. 6. The right uterine horn is taken out with the help of spay hook or curved first finger. The bifurcation can be checked to confirm uterus.

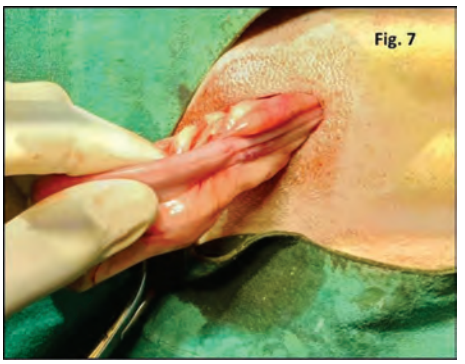


Fig. 7. The right ovary is just underneath the incision and can be pulled out easily. The suspensory ligament may be broken if required. The standard ligatures are applied, severed and left inside.

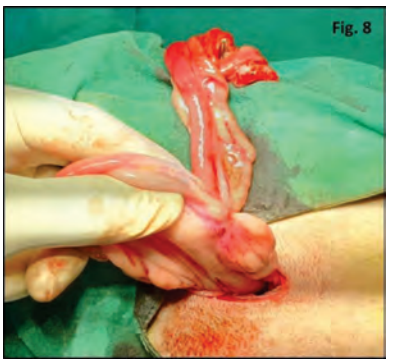


Fig. 8. The left ovary is pulled out by following the other horn from the bifurcation. The breaking of suspensory ligament is must for it.

Fig. 9. The photograph of the removed uterine horns and both ovaries.

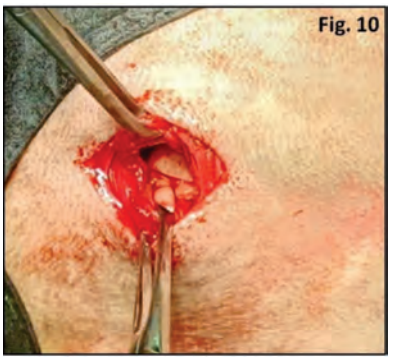


Fig. 10. The lower most muscle layer is grabbed with Allis tissue forceps.

Fig. 11. Photograph showing muscle suturing in progress (a) and the final sutured incision with scale (b).

Fig. 12. Loose dynaplast bandage can be applied around flank with a gauze piece kept on the incision line.

Limitations of Right flank OVH: If due to any reason, the stump is left bleeding, it is difficult to find it out from the small flank incision and in such cases, the linea alba may need to be opened to catch bleeding ovarian stump. The incision on flank would increase if OVH is done for pyometra or caesarian section. Besides, as it involves cutting of muscles, the procedure could be comparatively more haemorrhagic and painful as compared to the conventional mid ventral approach.

References

Reece, J. F., Nimesh, M. K., Wyllie, R. E., Jones, A. K., & Dennison, A. W. (2012). Description and evaluation of a right flank, mini-laparotomy approach to canine ovariohysterectomy. *Veterinary Record,* 171(10), 248.

Sangwan, V. (2005). Right flank ovariohysterectomy in dogs. *Indian Veterinary Journal,* 82, 1118-19.

Sharda, R., Sahu, D., Dewangan, R., Panchkhande, N., Sidar, S. & Yadav, D. (2022). Conventional ventral midline and right flank approach for ovariohysterectomy in female dogs. *Indian Journal of Animal Sciences,* 92 (5), 565-69.

Short Communication

Feline Chronic Kidney Disease: Evidence Based Approach towards Diagnosis and Therapy

Prabhjot Mangat*

College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: prabhjot41@hotmail.com

Received: 17.11.2024 Accepted: 04.12.2024

Abstract

Chronic kidney disease (CKD) is a common condition affecting felines in which symptoms do not appear until about more than 70% of kidney functions is impaired. International Renal Interest Society (IRIS) routinely updates specific recommendations concerning diagnosing and treating CKD in small animals to better facilitate therapeutic interventions and prevent renal disease progression. These universal guidelines aid practitioners in better understanding, diagnosing, and managing renal disease. This communication attempts to describe anatomy and physiology of the renal system, potential causes of the renal insufficiency, pathophysiology, diagnosis and treatment strategies for management of various stages of CKD in felines.

Keywords*: Feline, Excretory system, Renal failure*

Chronic kidney disease (CKD) is a common condition affecting felines aged 7 years and older. It often remains undetected until about 70% or more of normal kidney functioning is impaired. Geriatric felines are more susceptible because they are more likely to have concurrent diseases that can further exacerbate their presently compromised renal system.

According to IRIS guidelines, during early stages of CKD, cats may demonstrate clinical signs of disease (ex. Inappetence) a few months before the diagnosis (IRIS, 2019). Without routine screening, it is challenging to detect an ongoing disease until additional signs surface. For instance, a patient with a history of vomiting, nausea, weakness, inappetence, weight loss and overall declination in health may only be presented when disease has progressed to Stage 3 or 4. This is because after the initial episode, the animal may appear normal for the next few months before experiencing a sudden rapid decline. Unfortunately, when a patient's condition has advanced to Stage 3 or 4, the focus of treatment centers on enhancing their quality of life rather than preventing progression of disease (IRIS, 2023). The complicated nature of the disease also causes difficulty in distinguishing risk factors ultimately making it more challenging to understand the etiology of the disease (Finch et al., 2016). Therefore, it is crucial to understand renal anatomy and physiology, potential causes of the renal insufficiency, pathophysiology,

diagnosis and treatment strategies for management of various stages of the CKD in felines.

Normal Kidney Physiology: Kidneys are made up of small functional units called nephrons. A feline kidney is reported to have approximately 200,000 nephrons. Each nephron is composed of four main components known as Bowman's capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct. The major functions of the kidneys are to remove waste and toxins produced during the metabolic processes in the body, maintain acid base and electrolyte balance, maintain blood pressure, produce erythropoietin, reabsorb nutrients and activate vitamin D. (Fig. 1)

Potential Causes of CKD include: hypertension, acute kidney injury (due to ingestion of any toxic food, drink etc and if untreated can progress to CKD), glomerulonephritis, pyelonephritis, neoplasia (lymphosarcoma, adenocarcinoma), amyloidosis and periodontal disease (Finch et al., 2016).

Pathophysiology: The kidney contains specialized cells (juxtaglomerular cells, endothelium cells, peritubular cells, and podocytes) that play an important role in normal renal functioning (Ichii et al., 2011). Damage to these cells or other parts of the renal system can result in disease conditions that may eventually progress to CKD (Fig. 2 and 3).

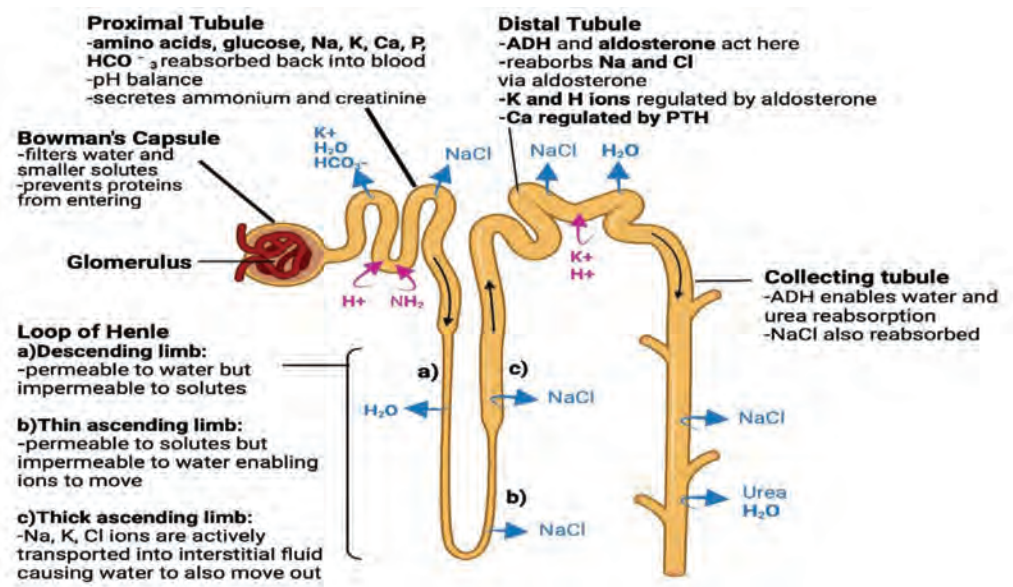


Fig. 1. Normal functioning of kidney *(Created in https://BioRender.com)*

In CKD, the loss of nephrons and reduced glomerulus filtration rate results in urea remaining in the blood instead of being excreted in the urine. The accumulation of urea in blood may lead to uremic syndrome (Reynolds & Lefebvre, 2013). Another common

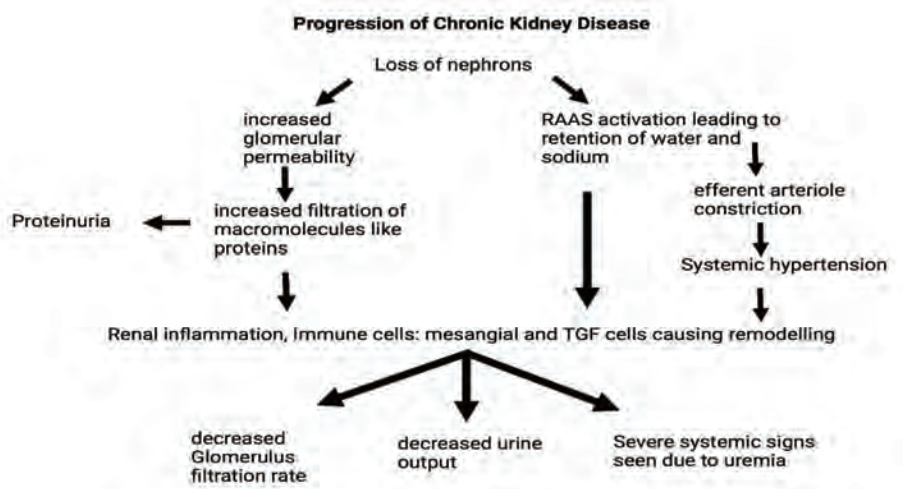


Fig. 2. Pathophysiology and progression of chronic kidney disease

finding is an increase in phosphorus levels which inhibit calcium sensing receptors. This stimulates the parathyroid gland, leading to increased calcium absorption from bones and raising risk of osteodystrophy (Chalhoub, 2018). In contrast, there are decreased levels of erythropoietin and active Vitamin D3 which may develop into anemia and hypocalcemia (Chalhoub, 2018).

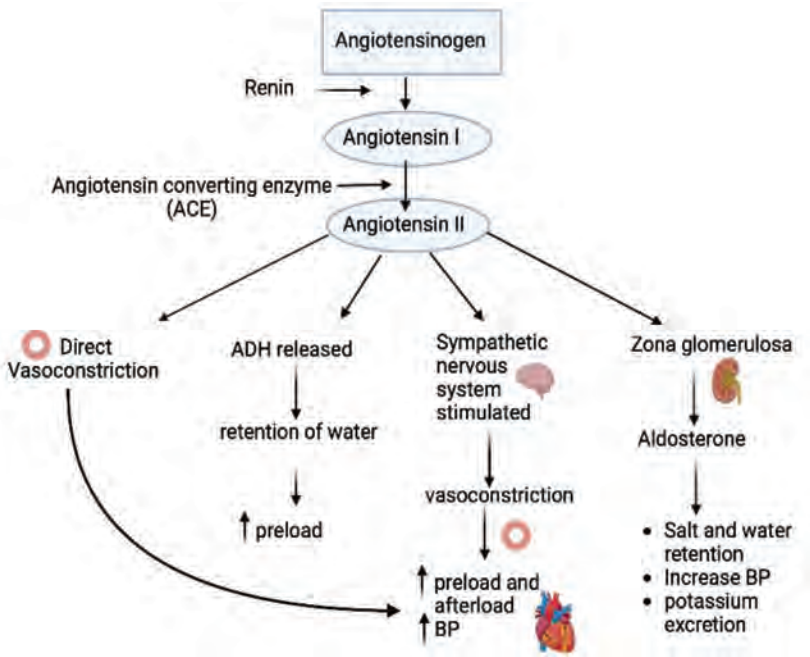


Fig. 3: Pathophysiology of CKD on cardiovascular system (Created in https://BioRender.com)

Clinical Signs: Most common signs in felines with CKD have been listed below (Paepe & Daminet, 2013):

- **Weight loss and inappetence** are one of the first signs noticed and are attributed to an ongoing uremic crisis.
- **Halitosis (Bad breath)**: It is indicative of mouth ulcers in felines suspected of CKD and is due to excessive urea in the body.
- **Polydipsia and Polyuria:** Due to renal damage, kidneys have difficulty producing concentrated urine. The body responds by increased demand for water causing polydipsia but because kidneys are unable to retain the water, animal is in a constant state of dehydration.
- **Uremia:** Kidneys are responsible for urea secretion which can unfortunately be inhibited in CKD, particularly at end stage CKD. This leads to an accumulation of urea in the blood causing uremia and can cause severe complications in body including signs of encephalopathy, neurological dysfunction, anorexia, vomiting, lethargy and more (Reynolds & Lefebvre, 2013).
- **Sudden blindness**: CKD cases with systemic hypertension may exhibit sudden onset of blindness due to retinal detachment or hyphema (pooling of blood in the eye). In these cases, recovery of eyesight is rare, irrespective of treatment (Jepson, 2011).
- **Diagnosis:** When a patient is initially suspected of CKD, a physical exam, blood work, and urinalysis should be performed. According to Cornell Feline Health Center, the following parameters should be taken into consideration when evaluating a patient:

Physical exam

- Evaluate body and muscle condition: animal may appear anorectic, prolonged skin tenting, pale gums and conjunctiva
- Kidney palpation: In AKI, kidneys may appear enlarged on palpation, while in CKD, kidneys can feel shrunken.
- Eye exam: This is done to rule out ocular changes associated with hypertension.

Complete blood cell count (CBC) and biochemistry profile to measure:

- Red blood cell count (Mild and non-regenerative anemia is an expected finding in CKD due to inability of kidney to generate erythropoietin (Winzelberg & Hohenhaus, 2019).
- Serum protein concentrations
- Elevated Creatinine and Blood urea nitrogen (BUN):

- Symmetric dimethylarginine (SDMA): Specific and early marker of kidney damage but should be repeatedly tested
- Electrolyte panel
  - **Potassium:** usually secreted by kidney but in CKD, it can accumulate and cause hyperkalemia which may lead to cardiac arrythmias. Hypokalemia is a very sensitive indicator and can also be found during the earlier stages of CKD.
  - **Calcium:** One of kidney's role is to activate Vitamin D by synthesizing alpha hydroxylase enzyme that helps in the absorption of calcium from intestine. When kidneys are impaired, this enzyme is no longer available resulting in poor absorption of calcium leading to hypocalcemia. This triggers parathyroid hormones to release calcium from bone reserves which causes bones to become weak and brittle over time, ultimately increasing risk of renal osteodystrophy.
  - **Sodium**: In early stages of CKD, there is often hypernatremia. However, over time as GFR decreases, more sodium is excreted, causing hyponatremia. A reduction in GFR will stimulate increase in water retention but if excessive retention persists, water will begin to deposit in tissues resulting in edema of affected tissues, including pulmonary edema. Since blood volume is increased, this can cause more strain on the cardiovascular system, leading to hypertension (Finch et al, 2016).
  - **Phosphate:** Hyperphosphatemia is a common finding in CKD patients due to its poor excretion and its control is instrumental in treatment (IRIS, 2023).

Urinalysis

- Urine Specific gravity: Felines diagnosed with CKD may have impaired ability to produce concentrated urine resulting in a lower urine specific gravity of <1.035 (Mortier, et al., 2023). The normal range for a hydrated cat is 1.0350 1.060 (IRIS, 2022).
- pH: Normal range of urine pH should be 6.3- 6.6. Cats diagnosed with CKD typically have urine pH of <6.0 (Kim et al., 2021).
- UPC: (urinary protein: creatinine ratio): This ratio is used to measure how much protein is being secreted in urine. Normal levels are <0.5. An increased UPC ratio is strongly linked to more advanced stages of CKD (Fidalgo et al., 2022).
- Protein concentration: It isn't atypical for feline urine to contain small traces of protein, however, elevated amounts of it could indicate an injury or damage to the glomerulus resulting in increased permeability of larger molecules. Proteinuria is associated with hypoalbuminemia, electrolyte metabolism, change in coagulation

factors and in some cases, hyperlipidemia (Harley & Langston, 2012).

- Presence of red blood cells: Hematuria and/or hemoglobinuria can be caused by possible trauma within the urinary tract or other conditions like a bacterial infection, uroliths, and more.
- Presence of other cells
- Urine culture for bacteria

Other tests

- Renal biopsy: can be used to rule out Renal lymphoma, FIP, and conditions like amyloidosis.
- Doppler ultrasound or Cuff technique to measure blood pressure
- Abdominal ultrasound: Findings include "small and irregularly outlined kidney" renal cortex appears hyperechoic.
- X-rays
- Blood gas testing: useful in determining metabolic acidosis

Early Detection (IRIS, 2023)

Treatment plans place more significance on using reliable biomarkers for early detection and protective measures to prevent progression of disease while improving patient's quality of life.

Important biomarkers for early detection include creatinine and SDMA.

- **Symmetric Dimethylarginine (SDMA)** is one of the only biomarkers that is indicative of early stages of CKD when as little as 20% of kidney function is lost. In contrast creatinine, a widely used marker for CKD detection, increases when there is 75% or more damage to the nephrons. **(**Healthy Birman cats naturally have higher serum SDMA and creatinine levels.)
- Screen for azotemia and proteinuria regularly as early detection can signify a better prognosis and prevent complications.
- Regularly record felines weight, feeding schedule, body and muscle condition to monitor any drastic increase or decrease that may occur.
- Take blood pressure readings along with lab tests for serum creatinine, UPC, SDMA. A urinalysis to screen proteinuria and other abnormalities is also recommended (IRIS, 2023).

Table 1. Staging of CKD based on blood Creatinine and SDMA levels in felines according to IRIS guidelines (2023)

Stage	Creatinine mg/dl	SDMA µg/dl	Feline's body condition	Clinical signs	Prognosis
1	<1.6	<18	Normal	No signs	Fair
2	1.6-2.8	18-25	Minor weight loss	Mild or absent	Fair-moderate
3	2.9-5.0	26-38	Very noticeable weight loss	Marked systemic signs (vomiting, polyuria, polydipsia, anorexia, poor coat quality, halitosis) appear in the later stages.	Poor
4	>5.0	>38	Anorectic, dull coat	Systemic signs can be seen due to uremia	Poor, even with treatment, median survival rate 35 day

- **Proteinuria**

Proteinuria is found to be a more accurate way of screening and is a reliable method for predicting prognosis. UPC levels of 0.2-1 are consistent findings of felines with CKD. It's crucial to determine whether cause of proteinuria is pre renal, renal or post renal (Paepe. 2016).

- In cases of pre-renal azotemia, primary issue is lack of adequate blood flow to the kidney. Once the underlying cause of hypotension is corrected, patient's health status should return to normal (Chalhoub, 2018).
- In renal azotemia, the kidney is the primary organ affected and due to this, focus should be on investigating possible toxin exposure, infection or other possible source of renal injury. Examples of exposure can be prolonged use of NSAIDs, particularly Meloxicam as its use has previously been linked to higher incidences of CKD (Wun et al., 2022).
- Post-renal azotemia is usually caused by an obstruction of urinary tract which leads to increased pressure on renal system and may pose as a source of infection in absence of medical intervention. This obstruction can be caused by urinary stones/uroliths which prevent the flow of urine and lead to the increased pressure on kidneys (Chalhoub, 2018). The depending upon level of proteinuria, the cats are classified as Non- proteinuric (<0.2), Borderline proteinuric (0.2-0.4) or Proteinuric (>0.4)

- **Systolic blood pressure**

Systolic blood pressure (SBP) should be constantly monitored in cats with CKD as it's an underlying cause as well as result of the disease. As SBP increases so does the increased risk of damage to organs such as heart, eye, brain and kidney. System Hypertension is commonly considered as SBP measuring >160 mm Hg (Table 2) (Lawson & Jepson, 2021).

Table 2. Systolic Blood Pressure (mm Hg) and Future Risk of Kidney Damage

Systolic Blood Pressure (mm Hg)	Blood pressure Substage	Risk of Future Target Organ Damage
<140	Normotensive	Minimal
140-159	Prehypertensive	Low
160-179	hypertensive	Moderate
>180	Severely hypertensive	High

Treatment

According to IRIS guidelines (2023), treatment is based upon staging of CKD patients (Table 3).

During Stage 1: Identifying and correcting any other concurrent conditions or causes is vital in prolonging patient's life. These can include primary disease, pre-renal or post renal complications as well as hypertension and renal proteinuria. Patients should be taken off all medications that may be nephrotoxic. Medications that may be introduced at this time are Angiotensin Converting Enzyme inhibitors, Calcium channel antagonists (CCA's), and Angiotensin Receptor Blockers. The CCA's play a major role in reducing hypertension in cats and can be effectively used with ACE inhibitors. No drugs should be given if patient is suspected of dehydration. Immediate efforts should be taken to correct dehydration with replacement fluid solutions such as Lactated Ringer's solution by IV or SC method. Other isotonic or polyionic solutions are also indicated. Dietary changes include reduction in sodium and protein to decrease hypertension and proteinuria. Serum creatinine levels should also be closely monitored and reassessed once in 6 months.

During Stage 2: Along with all measures taken in Stage 1, therapeutic plan must include a renal diet low in phosphorous and close monitoring for metabolic acidosis. Acidosis can be prevented by actively measuring serum bicarbonate, if found to be low, dietary supplementations of oral sodium bicarbonate or potassium bicarbonate can be given. The serum phosphorous concentrations should be maintained around 2.7-4.6 mg/dL, If hyperphosphatemia, phosphate binders can be given. It's important to note that phosphate binders need to be given with feed to increase its efficacy otherwise they will not bind to the phosphate in the diet. When giving calcitriol supplements, consider phosphate binders

without calcium in them as it can risk hypercalcemia. Any changes in diet should not be sudden but rather a gradual transition over the span of several weeks to ensure successful integration. Potassium and omega-3 fatty acids supplementation is also recommended at this stage. Due to advancement of disease, it remains crucial any complications are identified and treated. Reassessment of patient is recommended within 3-6 months.

During Stage 3: In Stage 3, all previous treatment should be continued. Owners may also opt for symptomatic treatment for various clinical signs to increase patients' quality of life. Increasing calorie intake is a desired goal at this stage as animal often appears anorectic. Phosphorus levels, in this stage, are expected around 2.7-5.0mg/dL, if elevated, phosphate binders are important in preventing further complications. In early Stage 3, monitoring can be done between 3-6 months; however, late Stage 3 should be reexamined within 1-2 months.

During Stage 4: Continue all treatments given previously. At this stage, the focus of therapy shifts from preventing and identifying complications to simply providing the highest quality of care and creating the most comfortable environment for the patient. This can be achieved by alleviating the patient's discomfort through symptomatic treatment. This can be managed by prescribing appetite stimulants, antiacids, antiemetics and more importantly, correcting any dehydration or other metabolic deficits such as hyperphosphatemia. A close monitoring of serum creatinine is also advised at this stage.

Table 3. Recommended Medications for CKD Treatment According to Iris and IDEXX Labs (Robertson, 2017)

Therapy/Drug	Dose	Frequency	Route	Indications
Renal protective diet	-	Feed 2-3x a day or ad libitum	PO	Can begin IRIS CKD stage 1 but definitely use in Stages 2-4
SQ Fluid therapy	75-125ml	Every 1-3 days	SQ	Maintain hydration
Ace Inhibitor (Benzapril)	0.5-1 mg/kg	Once a day	PO	Maintain blood pressure
Angiotensin receptor blocker: Telmisartan	1mg/kg	Once a day	PO	Maintain blood pressure
Calcium channel antagonist Amiodipine	0.625-1.25 mg/kg	Once a day	PO	Maintain blood pressure
Maropitant citrate	4mg	Once daily	PO	antiemetic

Mirtazapine 15mg tablet	1.88 mg	Every other day	PO	Appetite stimulant and antiemetic
Cyproheptadine	1-2mg	BID	PO	Stimulate appetite
Omeprazole	1mg/kg	BID	PO	antacid
Lanthanum Carbonate, Fosrenol 500mg tab	-	Dose divided and given with meals	PO	Maintain phosphorus levels below target
Lanthanum Carbonate Octahydrate (Renalzin®; 200 mg/ml)	2 mL in food	1-2 times a day	PO	Maintain phosphorus levels below target
Sevelamer hydrochloride (Renalgel) 400mg	33-54mg/kg	Dose divded and given with meals	PO	Maintain phosphorus levels below target
Chirosan and Calcium Carbonate	4.4gm/10kg	Dose divided and given with meals	PO	Maintain phosphorus levels below target
Potassium gluconate	2-6 mEq/day	BID with food	PO	To treat hypokalemia and metabolic acidosis
Potassium citrate	40-60 mg/kg	BID or TID with food	PO	To treat hypokalemia and metabolic acidosis
Sodium bicarbonate	8-12mg/kg	BID to TID	PO	To treat metabolic acidosis
Amlodipine	0.625mg/kg, if <5kg; 1.25 mg, if >5kg	Once daily	PO	To treat hypertension
Iron dextran	50mg	Every 3-4 weeks	PO	To treat anemia

Renal diseases, particularly, the chronic kidney disease is on rise in an alarmingly high speed in animals and humans worldwide. Due to multifactorial etiology, the accurate identification of major challenge for the nephrologists. It is crucial to diagnose CKD in the early stages so as to implement effective preventive and therapeutic plans.

References

Chalhoub, S. (2018). Chronic Kidney Disease, Ureteroliths, and Proteinuria. Faculty of Veterinary Medicine, University of Calgary. https://www.canadianveterinarians.net/media/5yxjorhl/ckd-ureteroliths-and-proteinuria-2018.pdf

Fidalgo, M. A., Leal, R. O., & Duarte-Correia, J. H. (2022). Urinary Protein/Creatinine Ratio in Feline Medicine: Reasons to Perform It and Its Role in Clinical Practice - A Retrospective Study. *Animals*, 12(12), 1575.

Finch, N., Syme, H., & Elliott, J. (2016). Risk factors for development of chronic kidney disease in cats. *Journal of Veterinary Internal Medicine*, *30*(2), 602–10.

Harley, L., & Langston, C. (2012). Proteinuria in dogs and cats. *The Canadian Veterinary Journal*, *53*(6), 631–38.

Ichii, O., Yabuki, A., Sasaki, N., Otsuka, S., Ohta, H., Yamasaki, M., Takiguchi, M., Namiki, Y., Hashimoto, Y., Endoh, D., & Kon, Y. (2011). Pathological correlations between podocyte injuries and renal functions in canine and feline chronic kidney diseases. *Histology and Histopathology*, *26*(10), 1243–55.

International Renal Interest Society (IRIS) Ltd. (2023). *Treatment recommendations for CKD in cats*. http://www.iris-kidney.com/pdf/IRIS_CAT_Treatment_Recommendations_2023.pdf

IRIS Kidney - Education - Risk factors. (2019). http://www.iris-kidney.com/education/risk_factors.html

Jepson, E. R. (2011). Feline systemic hypertension. *Journal of Feline Medicine and Surgery*, *13*(1), 25–34.

Kim, Y., Carrai, M., Leung, M. H. Y., Chin, J., Li, J., Lee, P. K. H., Beatty, J. A., Pfeiffer, D. U., & Barrs, V. R. (2021). Dysbiosis of the Urinary Bladder Microbiome in Cats with Chronic Kidney Disease. *mSystems*, *6*(4), e0051021.

Lawson, J. S., & Jepson, R. E. (2021). Feline comorbidities: The intermingled relationship between chronic kidney disease and hypertension. *Journal of Feline Medicine and Surgery*, *23*(9), 812–22.

Mortier, F., Daminet, S., Duchateau, L., Marynissen, S. J., & Paepe, D. (2023). Comparison of cystocentesis versus home sampling to determine urinary protein: Creatinine ratio and urine specific gravity in cats. *Journal of Veterinary Internal Medicine*, *37*(4), 1401-08.

Paepe, D. (2016). Proteinuria in Feline CKD - Assessment and Control. World Small Animal Veterinary Association Congress Proceedings.

Paepe, D., & Daminet, S. (2013). Feline CKD. Diagnosis, staging and screening – what is recommended? *Journal of Feline Medicine and Surgery*, *15*(1_suppl), 15–27.

Reynolds, B. S., & Lefebvre, H. P. (2013). Feline CKD: Pathophysiology and risk factors — what do we know? *Journal of Feline Medicine and Surgery*, *15*(1_suppl), 3–14.

Robertson, J. (2017). A practical approach to using the IRIS CKD guidelines and the IDEXX SDMA test in everyday practice. In 2017 Hill's Global Symposium. https://vetfolio.s3.amazonaws.com/db/40/e400cbcf49048a56041bdb820ed3/a-practical-approach-to-using-the-iris-ckd-guidelines-and-the-idexx-sdma-test-in-everyday-practice-proceeding.pdf

Winzelberg Olson, S., & Hohenhaus, A. E. (2019). Feline non-regenerative anemia: Diagnostic and treatment recommendations. *Journal of Feline Medicine and Surgery*, *21*(7), 615-31.

Wun, M., Leister, E., King, T., Korman, R., & Malik, R. (2022). Acute kidney injury in 18 cats after subcutaneous meloxicam and an update on non-steroidal anti-inflammatory drug usage in feline patients in Australia. *Australian Veterinary Journal*, *101*(3), 90–98.

Clinical Article

Penetrating Foreign Body in the Hoof of Two Buffaloes

Nikita Gupta*, Vandana Sangwan and Anshul

Department of Veterinary Surgery and Radiology
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: guptanikita086@gmail.com

Received: 20.11.2024 Accepted: 03.12.2024

Abstract

Sharp penetrating foreign bodies causes huge economic losses in bovines. It may also be penetrated into the hooves of animals and causes lameness refractory to medicinal therapy. This communication describes two such cases of buffaloes (7 year old she buffalo and 4 year old buffalo bull) presented with chronic lameness and were diagnosed for penetrated nails in the hooves on radiography. The nails were removed and both the cases recovered successfully. The report recommends gross and radiographic examination of hoof in bovines presented for unknown history of lameness.

Keywords: *Buffalo, Foreign body, Hoof, Lameness, Penetrating nail, Radiography*

Lameness in bovines is one of the major causes of reduced productivity and morbidity. It is a symptom of an underlying disease process, which is often localized in the hooves than other parts of the limb (Murray et al., 1996). Sharp, penetrating foreign body in hooves is among the rare causes of chronic low grade lameness and these should be ruled out during the initial diagnosis. Radiographic evaluation becomes absolutely essential as a few of them may be deeply seated, making it impossible to see grossly. This communication is about two cases of buffaloes presented for chronic lameness and were diagnosed for nail penetration in the hooves.

Case History and Presentation

A she-buffalo, aged 7 years and a buffalo bull, aged 4 years were presented to the referral Teaching Veterinary Hospital in Punjab, India with a history of chronic lameness (more than one month) in right forelimb and left hind limb (Fig. 1)., respectively. Both animals showed weight bearing on toes of the affected limb.

Fig. 1: Photograph of the buffalo bull with toe touching in left hind limb.

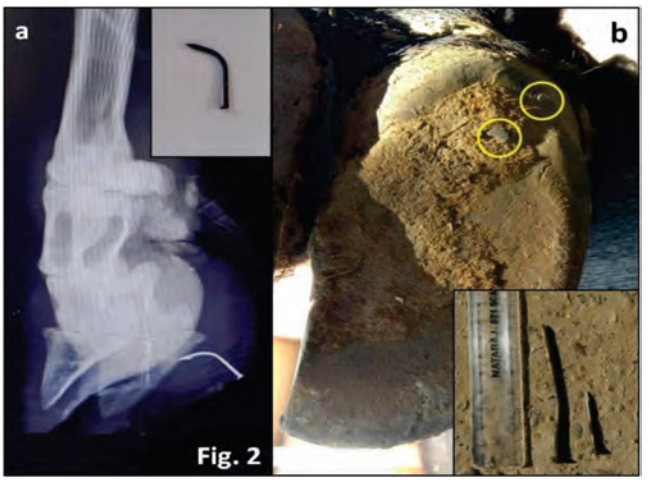


Fig. 2. Radiograph of the she-buffalo with nail visible (a) and the removed nail as inset (2a). Photograph of the hoof of the buffalo with 2 nail heads visible (yellow circles, 2b) and the removed nails (inset of 2b).

Clinical Examination, Diagnosis and Treatment

On physical examination, no apparent abnormality was found in the long bones and dorsum of hoof of the affected limb. The ventral part of the hoof could not be examined in standing position. Radiographs of the hooves were done in both the buffaloes and nails were seen (Fig. 2a). Sharp nails, one in case of buffaloes was measuring approximately 4 cm (Fig. 2a inset) and two in buffalo bull measuring approximately 7.5cm and 3cm in length (Fig. 2b, inset), were removed using a needle holder and plier.

The nail heads were grossly visible in the heel area from palmer / planter aspect when the hoof was examined in recumbent animal. Both animals were casted to remove the nails. Both the bovines were advised magnesium sulphate and copper sulphate (1%) dip/application at the site of nail penetration. Antibiotic course for 5 days with ampicillin-cloxacillin and gentamicin and analgesic meloxicam was given in standard doses. Both the buffaloes showed relief in lameness soon after the removal of the nails and complete recovery reported in next 5 days.

Discussion

Lameness in bovines, particularly originating from the hoof, is a major issue in dairy animals, which inadvertently leads to productive losses. Various causes of lameness have been identified in bovines, which may include laminitis, arthritis, fractures, interdigital phlegmon, white line disease, sole ulcer, abscess etc. (Newcomer & Chamorra, 2016). Majority of these cases can be prevented with good and hygenic management practices. Foreign body (sharp/blunt) penetration has been considered a

uncommon cause of lameness in dairy bovines; however, lack of diagnostic facilities like radiography may be a limiting factor in accurately diagnosing such cases (Browne et al., 2022).

Sharp foreign bodies like nails are a cause of various life threatening conditions (traumatic reticular peritonitis/ pericarditis, diaphragmatic hernia etc) in Indian scenario (Sharma et al., 2024), and their role in lameness cannot be ignored. This is merely a managemental ignorance which leads to a chronic low to moderate grade lameness in bovines, refractory to conservative therapy.

Radiography is the best possible aid to diagnose deeply seated foreign body and to confirm number of foreign bodies. Casting the animal helps in easy visualization and removal of sharp foreign objects. Sedation may be needed in aggressive animals. Debridement is usual treatment given to treat puncture wound along with a course of antibiotic. Such cases, if diagnosed and treated correctly, bear a favorable prognosis.

In conclusions, sharp penetrating foreign bodies in the hoof should be considered as a differential in bovines presented with unknown history of trauma for lameness and which are refractory to medicinal therapy. Radiography may be an important diagnostic aid in such cases.

References

Browne, N., Hudson, C. D., Crossley, R. E., Sugrue, K., Huxley, J. N., & Conneely, M. (2022). Hoof lesions in partly housed pasture-based dairy cows. *Journal of Dairy Science*, 105(11), 9038-53.

Murray, R. D., Downham, D. Y., Clarkson, M. J., Faull, W. B., Hughes, J. W., Manson, F. J., Merritt, J.B., Russell, W.B., Sutherst, J.E. & Ward, W. R. (1996). Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. *Veterinary Record*, 138(24), 586-91.

Newcomer, B. W., & Chamorro, M. F. (2016). Distribution of lameness lesions in beef cattle: A retrospective analysis of 745 cases. *The Canadian Veterinary Journal*, 57(4), 401-06.

Sharma, K., Sangwan, V., Kumar, A., Singh, N., Singh, T., & Mohindroo, J. (2024). Diagnosis and surgical outcome of diaphragmatic herniorrhaphy in cows. *Large Animal Review*, 31(1), 43-50.

Short Communication

An Overview on the Squamous Cell Carcinoma in Cattle

Goriya Yarmiben Mukeshbhai and Vandana Sangwan*

Department of Veterinary Surgery and Radiology,
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: drvandanasangwan@rediffmail.com

Received: 30.10.2024 Accepted: 22.11.2024

Abstract

Squamous cell carcinoma (SCC) is a malignant skin cancer mostly affecting middle aged cattle, particularly in the body areas exposed to direct sunlight or due to chronic irritation. The SCC of eye, vulva and horn is most common. Occular SCC especially occurs in Bos taurus breeds like Holstein Friesian crossbred. The SCC of horn is seen in Bos Indicus cattle. The SCC poses a significant risk to cattle, particularly in certain breeds and under specific environmental conditions. Early detection and a variety of treatment options are crucial for managing this disease effectively. Prevention and non-breeding of affected cattle and their progeny is the only method to eradicate it. This article discusses the SCC of horn and eye in cattle, highlighting their prevalence and treatment options.

Key words: *Cattle, Horn cancer, Squamous cell carcinoma.*

Squamous cell carcinoma (SCC) is a malignant type of skin cancer that primarily arises from stratified epithelial tissues and predominantly affects regions of eye ,eyelids, horn,vulva, prepuce, and muzzle in cattle and can metastasize to regional lymphnodes like, submandibular (Vegad, 2007). It lead to significant economic losses due to condemnation and a reduced productive lifespan. The population of HF cross cattle is increasing in India for their higher productive outocme, leading to high prevalance of the disease condition, SCC of eye.

The SCC of the eye is more common in Bos taurus breeds than in Bos indicus breeds, with Holstein-Friesian crossbred commonly affected in India. The disease is usually seen in middle aged cattle and rarely under the age of 3 years. However, SCC of horn is common in Bos indicus cattle and usually appears at older age of more than 10 years (Sodhi & Sangwan, 2019). Disbudding of males horns is not common in Bos Indicus breed of cattle as they are mostly used for draft purposes. However, SCC is not seen in disbudded horn.

Aetiology

i. **Ocular Squamous Cell Carcinoma (OSCC) (Fig. 1):** The OSCC can be caused by; genetics, sunlight exposure, nutrition, eyelid pigmentation, and potentially

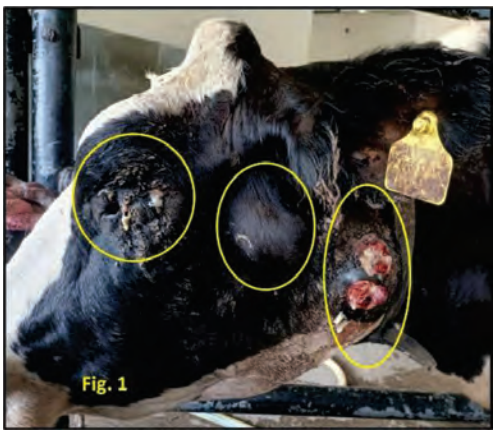


Fig. 1. Photograph of a Holstein Friesen crossbred cattle with OSCC and metastasis

Fig. 2. Photograph showing dropped left horn of a bullock due to HSCC

viral factors. Age and low pigmentation of skin are the major factors responsible for developing OSCC. The medial and lateral limbal regions, where the cornea meets the sclera, are the most commonly affected areas, along with the eyelids, conjunctiva, and nictitating membrane.

ii. **Horn Core Squamous Cell Carcinoma (HSCC) (Fig. 2):** The exact cause of HSCC is unclear; however, several predisposing factors have been identified; irritation from tying a rope to the horn, trauma by rubbing, falling against hard objects, and fighting. Chronic irritation can also result from the use of a yoke, as well as from painting the horn and exposure to solar radiation (Shastry, 2001). The HSCC affects 1% of Indian cattle population and accounts for over 80% of reported tumour in Indian cattle. The typical clinical appearance is head tilting, rubbing against hard object and bleeding from affected side nostril, however, histologic examination is required to confirm it.

Breed predisposition to OSCC and HSCC

- Horn cancer is most predominant in Bos indicus breed of cattle and is common in aged Zebu castrated male cattle throughout the world.
- Purebred cattle are more susceptible to squamous cell carcinoma of the horn than cross-bred or non-descript cattle (Kumar & Jolhe, 2024).
- Cattle with the highest incidence of eye cancer were found in Holstein Friesian crossbred, followed by jersey crossbred cows and non-descript breeds in India.
- Cattle with little to no pigment surrounding their eyes have the highest incidence of ocular squamous cell carcinoma.

Prevention of OSCC and HSCC

- Susceptible cattle should have access to shaded areas during peak sunlight hours (10 AM to 4 PM). The trees, shelters, or artificial shade structures can be utilized to minimize direct UV exposure.
- The diet which is rich in fresh green grass has more antioxidant (vitamins A, C & E) to support skin health and immune function.
- Consider breeding strategies that favour individuals with better pigmentation on face and vulvar regions and lower the susceptibility to SCC.
- Conduct routine examinations for early signs of SCC, such as lesions or abnormal growths.
- Breeding polices of the affected herds/males should be evaluated and analysed to reduce the incidence of tumours, as affected cattle and their progeny should not be bred further.

Treatment

1. **Surgical Excision**: General anaesthesia may be required if a large part is involved otherwise, nerve blocks (auriculopalpebral, retrobulbar or cornual) and sedation may serve the purpose. The horn amputation requires trimming of frontal bone at the base of the horn for successful skin closure and usually requires general anaesthesia for it (Sodhi & Sangwan, 2019). Mostly the cases are delayed in presentation and removal of healthy tissue around the tumour is not possible. The recurrence at the same site can range from 3% to 25%. Re-occurrence of OSCC can be sometimes re-attempted but skin apposition is difficult in case of horn.
2. **Cryosurgery**: This technique freezes the tumour with liquid nitrogen, leading to cell destruction and is effective for superficial lesions. It can be tried for smaller lesions (less than 5mm) on vulva or face (Farris, 1978).
3. **Use of Laser**: This method precisely targets and destroys tumour tissue while minimizing damage to adjacent areas.
4. **Topical Therapies**: For localized lesion, chemotherapy agents or immuno-modulators may be applied directly to the affected area. For eg. Cisplatin emulsified in sesame oil for post-tumour excision treatment (Parmar et al., 2024a).
5. **Immunotherapy**: Phenol extract of horn core tissue is immunogenic and is a successful treatment for the breed which is more prone to SCC of horn (Parmar et al., 2024b).

Recommendations and Scope of Research in India and Abroad

- In India, the research is focused on improving early detection, UV protection, and genetic selection for UV-resistant cattle. However, treatment options remain limited to surgical excision.
- The studies on immunotherapy and vaccines may help in better management.
- Globally, research is expanding in areas like genetic predisposition, prevention strategies, and novel therapies.
- Disbudding is recommended in all cattle breeds and gender at young age (upto 10 days) to prevent squamous cell carcinoma of horn.

References

Farris, H. E. (1978). Cryosurgery. In *American Association of Bovine Practitioners Proceedings* (pp. 68-69).

Kumar, V & Jolhe, D.K. (2024). Pathological and Diagnostic Aspects of Squamous Cell Carcinoma in Bovines. *In Bio Vet Innovator Magazine*, 1 (3): 4–11. https://doi.org/10.5281/zenodo.13701045

Parmer, P.L., Sutaria, P.T., Raval, S.H., Patel, P.B., Desai, M.J. & Patel, A.C. (2024a). Squamous cell carcinoma in HF Crossbred cattle: Clinical features and hematological considerations. *International Journal of Veterinary Scineces and Animal Husbandry*, 9(2), 889-95.

Parmer, P.L., Sutaria, P.T., Raval, S.H., Patel, P.B., Desai, M.J., Patel, A.C. & Solanki, P.G. (2024b). Exploring the impact of intralesional cisplatin on blood biochemical parameters in bovine squamous cell carcinoma. *International Journal of Advanced Biochemistry Research,* 8(3): 770-75.

Sastry, G.A. (2001). Veterinary Pathology. 7th Edn., CBS Publisher and Distributors. New Delhi. pp. 205-49

Sodhi, H.S. & Sangwan, V. (2019). Successful Horn Amputation for the Treatment of Horn Cancer in an 18 Year Old Humped Bullock. *International Journal of Current Microbiology and Applied Sciences*, 8(8): 537-41.

Vegad J.L. (2007). Textbook of Veterinary General Pathology, 2nd Revised and Enlarged Edition. International Book Distributing Co. Lucknow, pp no.: 283, 373, 376-77.

Short Communication

An Update on the Injectable Anesthesia in Dogs under Field Conditions

Maninderjit Singh and Nikita Gupta*

Department of Veterinary Surgery and Radiology
College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: guptanikita086@gmail.com

Received: 05.11.2024 Accepted: 04.12.2024

Abstract

Injectable anesthesia is frequently preferred over inhalant under field conditions because of the economics involved, ease of administration and less specialized equipment required. Multiple injectable drugs can be used to achieve balanced anesthesia in field conditions. The choice of injectable anaesthesia protocol is based on their systemic effects, age and breed of the dog, availability of drug, familiarity with the drug, type of surgical procedure, and duration of the procedure to be performed. This note, describes the available injectable anaesthetic drugs for field conditions.

Keywords: *Dog, Injectable anesthesia, Ketamine, Pre-anesthetics, Propofol*

General anesthesia is a necessary prerequisite for most surgical procedures in dogs. It can be achieved either by injectable or inhalant anesthetics or by a combination of the both, to produce adequate muscle relaxation, amnesia and analgesia. Inhalant anesthetics with adjunctive drugs even though superior as compared to injectable anesthetics, is a constraint in field conditions due to the nonavailability of specialized equipment (Boyle's Anaesthesia machine) required to deliver inhalant agents (Tranquilli et al., 2013). This makes injectable anesthetics more preferred under field conditions. Injectable anesthetics administered once, produce short-term anesthesia. It usually requires multiple doses or constant rate of infusion for longer surgical procedures, which otherwise makes it difficult to maintain a consistent level of anesthesia during a surgical procedure. Multiple doses may cause the accumulation of anesthetics, which results in adverse systemic effects on the dog's body and also leads to longer recovery time (Lin, 2022). This communication describes various recommended injectable general anaesthesia protocols for canine patients.

Pre-Operative Assessment and Fasting

Prior to any anesthetic protocol, animals should be properly evaluated physically as well as biochemically for anesthetic safety. This prevents using the same anesthetic protocol in all dogs and shifting toward the use of other protocol. In addition to this, fearful, anxious dogs may be prescribed with anxiolytics such as Gabapentin (@ 20-40

mg/ Kg orally) to be given at home 2-3 hours prior to bringing the pet to the hospital on the day of surgery (Grubb et al., 2020).

Age and breed of the dogs should also be considered, along with any co-morbidities prior to formulating the anaesthetic protocol for a particular patient. Very young and old animals require less drug dosage due to their inability to bio-transform it efficiently (Tranquilli et al., 2013)

Routinely, animals are fasted for 12 hours prior to surgery. However, recent studies suggest that lesser fasting interval leads to less gastroesophageal reflux. In summers, water may be withheld for 6 hours only.

Selection of Pre-Anesthetics

- Preanesthetic tends to calm animals prior to use of induction agent, thus reducing their dose. It includes anticholinergics, sedatives, tranquilizers, and opioid drugs.
- Atropine/gylcopyrolate are the common anticholinergic drug used to reduce secretion and prevent vagal stimulation, is the most commonly used preanesthetic in field protocols.
- Tranquilizers like benzodiazepines (e.g., diazepam, midazolam) help in causing varying degrees of sedation, anxiolysis, anticonvulsant activity, and spinal cord-mediated muscle relaxation.
- Xylazine, an alpha-2 agonist, provides sedation, immobilization, and adjuvant analgesia.
- Medetomidine and dexmedetomidine may also be used as an alternative to xylazine.
- Butorphanol, an opioid agonist, provides a mild analgesic effect (Murrel, 2016)

Induction and Maintenance Agents

- Ketamine and Propofol are the most commonly used induction agents in field conditions.
- Ketamine is a dissociative anesthetic that increases muscle tone and causes convulsions in some cases. It is used with benzodiazepine or alpha-2 agonists to reduce these side effects. For maintenance, it is usually used in combination with diazepam in a 1:1 or 2:1 ratio.
- Propofol is a non-barbiturate sedative that causes rapid, smooth induction and recovery from anesthesia. It causes significant apnea and hypotension. It is used with an optimal dose to limit cardiopulmonary depression. Its induction dose is 2-5 mg/kg, which increases to 5-8 mg/kg without any premedication. For maintenance, it is given as an intermittent bolus at 0.5-2.0 mg/kg IV or as constant

rate infusions at 0.15-0.4 mg/kg/min (Glowaski & Wetmore, 1999). CRI pumps are not available in the field, so drop-wise calculation is done via giving through the infusion set. The drip factor, which can be found printed on the IV tubing package, is the number of drops in 1ml of solution (NSS) delivered by gravity. The standard tubing set used in veterinary practice usually delivers 15 drops/ml.

- Ketofol (a ketamine/propofol combination) is used to counter propofol's adverse effect by reducing its dose, thereby limiting the cardiopulmonary depression caused by propofol alone (Lerche et al., 2000)

Table-1: Various Injectable Anesthetic Protocols for Dogs

Protocol	Preanesthetic (Dose and Route)	Induction agent (Dose and Route)	Maintenance (Dose and Route)
Protocol 1	Atropine @0.04mg/ kg IM, Xylazine@0.5-1.0mg/ kg, IM	Ketamine@ 5mg/ kg IM	Ketamine@ 2.5mg/kg IV alone or with diazepam@ 0.2 mg/kg IV (as intermittent bolus) each time movement detected
Protocol 2	Atropine@ 0.04mg/ kg IM	Diazepam@ 0.4mg/ kg IV, followed by Ketamine@ 3-mg/kg IV, one after another	Ketamine@ 2.5mg/kg with diazepam@ 0.1-0.2 mg/kg IV (as intermittent bolus) each time movement detected
Protocol 3	Atropine@ 0.04mg/ kg IM, Xylazine@0.5-1.0mg/ kg, IM	Propofol@ 2-3 mg/ kg IV, slow	Propofol@ 0.5-2.0mg/kg IV (as intermittent bolus) each time movement detected
Protocol 4	Atropine@ 0.04mg/ kg IM, Xylazine@ 0.5-1.0mg/kg IM	Ketofol @ 2mg of each drug/kg IV	Ketofol@ 1mg of each drug / kg IV (as intermittent bolus) each time movement detected
Protocol 5	Atropine@ 0.04mg/ kg IM	Zoletil @ 10mg/Kg (1ml/10 kg healthy dog), IM (Tiletamine and zolazepam)	Usually, the effect lasts for half an hour and if required may be topped @2mg/kg, slow IV once.

Local Anaesthesia as an Adjunct to Injectable Anaesthesia

Local anaesthetic agents like lignocaine and bupivacaine can also be used wherever appropriate, to reduce the dose of injectable anaesthetic agents at the site of incision. For example, injecting brachial plexus or lumbosacral plexus with local anaesthetic agents during limb amputation can reduce the requirement of injectable anaesthetic (Grubb et al., 2020). Similarly, infiltration local anaesthesia (SC injections) can be administrated at the site of incision such as at linea alba (Margeti et al., 2024)

Future Scope of Research to address Research Gaps: Despite huge research on the canine general anesthesia, most of the drugs are short-acting and did not suffice the purpose of field surgeries. Further research on the combinations of various injectable anesthesia protocols, which could increase the surgical anesthesia time, may be conducted to ease doing elective surgeries in field.

References

Glowaski, M. M., & Wetmore, L. A. (1999). Propofol: Application in veterinary sedation and anesthesia. *Clinical Techniques in Small Animal Practice*, 14(1), 1–9.

Grubb, T., Sager, J., Gaynor, J. S., Montgomery, E., Parker, J. A., Shafford, H., & Tearney, C. (2020). 2020 AAHA anesthesia and monitoring guidelines for dogs and cats. *Journal of the American Animal Hospital Association, 56*(2), 59-82.

Lerche, P., Nolan, A. M., & Reid, J. (2000). Comparative study of propofol or propofol and ketamine for the induction of anaesthesia in dogs. *The Veterinary Record*, 146(20), 571–74.

Lin, H. (2022). Injectable Anesthetics and Field Anesthesia. In Farm Animal Anesthesia (eds H. Lin, T. Passler & S. Clark-Price). https://doi.org/10.1002/9781119672661.ch4

Margeti, C., Kostakis, C., Tsiali, V., Karagianni, K. & Flouraki, E. (2024). Local anaesthesia techniques in dogs & cats: A review study. *Pets*, 1(2): 88-109.

Tranquilli, W. J., Thurmon, J. C., & Grimm, K. A. (Eds.). (2013). *Lumb and Jones' Veterinary Anesthesia and Analgesia*. John Wiley & Sons.

Murrell, J.C. (2016). Pre-anaesthetic medication and sedation. In: Duke-Novakovski, T., Vries, M., & Seymour, C.; BSAVA Manual of Canine and Feline Anaesthesia and Analgesia. Third edition. British Small Animal Veterinary Association, Gloucester, 170-89.

Short Communication

An Overview on the Dermatophytosis in Companion Animals

Ipsita Kar[1*] and Paviter Kaur[2]

[1] Department of Veterinary Microbiology, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh- 243122

[2]Department of Veterinary Microbiology, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab- 141004, India.

[*]Corresponding author email: ipsitamamunikar@gmail.com

Received: 14.10.2024 Accepted: 11.11.2024

Abstract

Dermatophytosis is a potential fungal zoonosis, affecting a wide range of animals and humans. The disease is primarily caused by the anamorphic Dermatophytic fungi, which predominantly inhabit the soil and keratinous layer of skin. Dermatophytosis, often transmitted via direct or indirect route, is characterized by localized alopecia, erythema, crusting, and skin lesions, particularly on the face, neck, and tail areas. The spread of the disease is ascribed to various factors associated with the host, pathogen, and environment. The rising prevalence and incidence of dermatophytosis have emerged as a significant public health challenge. In addition, antifungal resistance is becoming a critical issue, due to the limited availability and injudicious use of antifungal drugs. Thereby, improved diagnostics, effective therapeutics, comprehensive management of antifungal resistance, and resilient disease control strategies must be adopted to combat dermatophytosis. This article aims to highlight the epidemiology, clinical signs, pathogenesis, diagnosis, and prevention methods of dermatophytosis in companion animals.

Keywords*: Companion animals, Dermatophytosis, Keratinous layer, Zoonosis*

Dermatophytosis, commonly known as ringworm or tinea, is a superficial cutaneous infection, with significant health challenges and economic concerns. The disease is mainly associated with three major genera of keratinophilic and keratinolytic dermatophytes *viz. Microsporum*, *Trichophyton*, and *Epidermophyton*. Fungus *Microsporum* produces rough and thick wall microconidia, primarily infecting the hair. The Genus *Trichophyton* usually targets skin, hair, and nails by propagating through smooth, thin-walled cigar-shaped microconidia. However, *Epidermophyton* fungi synthesize club-shaped microconidia, exclusively invading the skin. The companion canines and felines are mostly affected by different species of *Microsporum* such as *M. canis*, *M. gypseum*, (currently known as *Nannizzia gypsea*) and *M. distortum*, as well as *Trichophyton*, while *T. mentagrophytes* may act as a carrier. The disease, transmitted through direct contact with infected animals or indirectly via contaminated fomites, is more likely to occur in summer and spring. Close association with animals significantly enhances the probability of its dissemination to humans, with 80% of human cases

being of animal origin, highlighting its considerable zoonotic potential. Therefore, comprehensive measures in disease investigation, diagnosis, treatment, and prevention should be undertaken to lessen the burden of the disease.

Epidemiology: Dermatophytosis has been reported worldwide, with a prevalence rate of 20-25% (Havlickova et al., 2008). Companion animals act as carriers of certain dermatophytes, which cannot invade the healthy skin of these animals (Gnat et al., 2019). In European countries, dermatophytosis's prevalence in cats and dogs varies from 20% to 30%. In some studies, *M. canis* has been reported as the major etiological agent of 81.8% to 97% of dermatophytosis cases in pets (Long et al., 2020). Globally, this disease afflicts 8% to 19% of dogs and 7% to 72% of the cat population.

In Indian scenario, the frequency of dermatophytosis in cats and dogs has been reported to be 37% and 20.93%, respectively (Debnath et al., 2016). Similarly, another epidemiological investigation suggested the distribution of dermatophytes among the dog population in Kolkata at 26.03% (Singh et al., 2020) and in the cat population in Madhya Pradesh at 21.1% (Gautam et al., 2024). However, 13% of dermatophytosis cases were reported in humans, who had been in close contact with pet animals, suggesting its potential zoonotic risk (Sharma et al., 2021).

Clinical Manifestations: Clinical signs of dermatophytosis vary according to the affected host. The usual symptoms involve hair loss, scaling, skin crusting, erythema, papules, hyperpigmentation, and pruritus. In dogs, the lesions can be localized to the face, legs, and tail areas (Paryuni et al., 2020). However, the disease affects the face, ears, and muzzle in felines and young puppies before expanding to other body parts. Pseudomycetomas-like nodular lesions are observed in Persian Cats, with some patients developing exudative paronychia. The clinical skin lesions such as multifocal alopecia, erythema, papule, pustule, scale, and crust altogether form distinctive lesions of ringworm and have been reported in cats. The *M. canis* infection creates alopecia in the affected area, leading to permanent alopecia, especially in chronic inflammatory conditions. Dermatophytic lesions could be mild or severe according to the species involved, virulence factors, site of infection, presence of bacterial invasion, and ecological circumstances.

Pathogenesis: The common predisposing factors for the occurrence of the disease include young or old age, sex of the animal, immunosuppression, nutritional deficiency, pregnancy, higher environmental temperature with high humidity or skin trauma (Gnat et al., 2019). Dermatophytes can enter through broken skin, following entry into the host cell, arthroconidia starts attaching to the keratin. Subsequently, the stratum corneum's invasion and germination occur after 4-6 hours of infection, resulting in various inflammatory conditions. Dermatophytes can secrete proteolytic and keratolytic enzymes that allow keratin usage as their sole nutritional source, promoting fungal growth in the stratum

corneum, and contributing to the epidermis's keratinization. The proteolytic activity of dermatophytes is attributed to the release of serine proteinase (urokinase and activator plasminogen tissue), which is responsible for damaging the external protein layer of the host cell. The severity of the disease can be affected by different factors, including the pathogenicity of microorganisms, species, age, sex, host immune response, and host-pathogen interaction. Additionally, elevated cortisol and pro-inflammatory cytokines levels have been recognized as a major component contributing to dermatophyte infection (Paryuni et al., 2020).

Diagnosis and Prevention: A rapid and confirmatory diagnosis is a definite need for limiting the disease outbreak. The Wood's lamp test is intended to detect the fluorescence produced by the dermatophytes. In case of *M. canis* infections, a characteristic green fluorescence can be observed, attributable to the presence of pteridine metabolite within the cortex and medulla of the affected hair shaft. The Dermoscopy technique has been reported as a useful tool for the detection of abnormal comma-like hair in diseased pets. However, direct microscopy followed by fungal isolation using Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) media and identification is considered as the gold standard diagnostic test. Moreover, molecular techniques like PCR are highly effective in detecting dermatophytes (Nardoni et al., 2007).

Prevention strategies include hygiene, sanitation, disinfection, isolation of infected animals, and immunoprophylaxis. Additionally, minimizing inter-species or intra-species transmission by alleviating the dissemination of carriage fomite to the environment through disinfection and sanitization, can significantly avert the risk of infection. Most effective disinfectants against dermatophyte arthrospores include 1:33 lime-sulphur, 1:10 to 1:100 household chlorine bleach, and 0.2% enilconazole (Moriello & DeBoer, 2012).

Treatment of dermatophytosis involves the administration of both topical antifungals, such as enilconazole, miconazole/chlorhexidine, and clotrimazole, and systemic antifungals like fluconazole, ketoconazole, itraconazole, and terbinafine, etc. Topical drugs are effective in killing the spores present on the hair coat; however, systemic therapy eliminates spores in the hair follicle (Frymus et al., 2013). The most effective topical drug is the combination of miconazole and chlorhexidine used biweekly (Moriello, 2017). However, itraconazole and terbinafine are found to be expeditious drugs in treating dermatophytosis. Itraconazole is commonly recommended at a dose rate of 5-10 mg/kg, orally once daily whereas terbinafine can be used @ 30-40 mg (Moriello, 2004).

Research Gaps and Future Prospective

Despite high incidence and prevalence, there has been limited research on epidemiological surveys, disease diagnosis and prophylaxis, and antifungal resistance. Vaccination against dermatophytes in cats and dogs has not been explored to date. However, the current studies on phylogenetic analysis, phenotypic and genotypic profiling of antifungal resistance, and the dynamic distribution of dermatophytes like *Microsporum* and *Trichophyton*, may be useful in understanding the epidemiology and mode of transmission of dermatophytes, aiding in mitigating the disease.

References

Debnath, C., Mitra, T., Kumar, A., & Samanta, I. (2016). Detection of dermatophytes in healthy companion dogs and cats in eastern India. *Iranian Journal of Veterinary Research*, *17*(1), 20.

Frymus, T., Gruffydd-Jones, T., Pennisi, M. G., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., Marsilio, F., Mostl, K., Radford, AD., Thiry., E., Truyen, U., & Horzinek, M. C. (2013). Dermatophytosis in cats: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*, *15*(7), 598-604.

Gautam, S., Choudhary, N. S., Gangil, R., Bagherwal, R. K., & Agrawal, V. (2024). Prevalence of Feline Dermatophytosis in and around Mhow (Indore), MP. *Indian Journal of Veterinary Sciences and Biotechnology*, *20*(4), 153-55.

Gnat, S., Nowakiewicz, A., & Zięba, P. (2019). Taxonomy of dermatophytes–the classification systems may change but the identification problems remain the same. *Postępy Mikrobiologii-Advancements of Microbiology*, *58*(1), 49-58.

Havlickova, B., Czaika, V. A., & Friedrich, M. (2008). Epidemiological trends in skin mycoses worldwide. *Mycoses*, 51, 2-15.

Lakshmipathy, D. T., & Kannabiran, K. (2010). Review on dermatomycosis: pathogenesis and treatment. *Natural Science*, *2*(07), 726.

Long, S., Carveth, H., Chang, Y. M., O'Neill, D., & Bond, R. (2020). Isolation of dermatophytes from dogs and cats in the South of England between 1991 and 2017. *Veterinary Record*, *187*(10), e87-e87.

Moriello, K. A. (2004). Treatment of dermatophytosis in dogs and cats: review of published studies. *Veterinary Dermatology*, 15(2), 99–107.

Moriello, K. A. (2017). In vitro efficacy of shampoos containing miconazole, ketoconazole, climbazole or accelerated hydrogen peroxide against Microsporum canis and Trichophyton species. *Journal of Feline Medicine and Surgery*, 19(4), 370-74.

Moriello, KA., & DeBoer, DJ. (2012) Dermatophytosis. In: Greene CE (ed). Infectious diseases of the dog and cat. 4th ed. St Louis: Elsevier, pp 588–602.

Nardoni, S., Franceschi, A., & Mancianti, F. (2007). Identification of Microsporum canis from dermatophytic pseudomycetoma in paraffin-embedded veterinary specimens using a common PCR protocol. *Mycoses, 50*(3), 215-17.

Paryuni, A. D., Indarjulianto, S., & Widyarini, S. (2020). Dermatophytosis in companion animals: A review. *Veterinary World, 13*(6), 1174-81.

Sharma, S., Verma, G., & Verma, S. (2021) Clinico-epidemiological profile of dermatophytosis. *International Journal of Dermatology Research, 3*(1), 06-09.

Singh, A. D., Debnath, C., & Banerjee, A. (2021). Epidemiological investigation, characterization and antifungal susceptibility profile of Microsporum canis isolated from pet animals. *Veterinary Archives, 91*(4), 339-47.

Mini Review

Atypical Human Trypanosomosis: A Critical Insight into an Emerging Zoonotic Protozoan Threat in India

Bhavjot Singh, Paramjit Kaur*, L.D. Singla and M. S. Bal[1]

Department of Veterinary Parasitology
[1]Animal Disease Research Centre
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University -141004, Punjab, India
*Corresponding author Email Id: paramjitkaur@gadvasu.in

Received: 30.10.2024 Accepted: 11.11.2024

Abstract

Atypical human trypanosomosis (a-HT), caused by animal-origin trypanosome species, is emerging as a significant global zoonotic threat. While regions like Africa and South America have historically dealt with Trypaniosoma brucei and T. cruzi, India is experiencing a concerning rise of a-HT cases, particularly of T. lewisi and T. evansi. This review aims to systematically analyze the current status of a-HT in India, evaluate epidemiological patterns and risk factors, assess diagnostic approaches, and propose evidence-based control strategies within a One Health framework. A comprehensive literature review was conducted using multiple scientific databases (PubMed/MEDLINE, Web of Science, Scopus, Indian Citation Index) covering the period from 1900 to 2024. Case reports, epidemiological studies, and research articles were analyzed using standardized quality assessment tools. Of the 22 globally documented a-HT cases (1933-2024), 10 originated from India, comprising 08 T. lewisi and 02 T. evansi infections. Key findings include predominant risk factors such as immunocompromised status, poor sanitation, and occupational exposure; geographic clustering in regions with high livestock density; successful treatment outcomes with suramin for T. evansi cases; variable clinical manifestations; and significant diagnostic challenges in species identification. India faces a growing challenge from a-HT, necessitating enhanced surveillance systems, improved diagnostic capabilities, standardized treatment protocols, and effective vector control strategies. Urgent implementation of coordinated One Health approach is essential for managing this emerging zoonotic threat, alongside public awareness campaigns and strengthened collaboration between veterinary and public health sectors.

Keywords: *Atypical, Human, India, Trypanosomosis, Trypanosoma evansi, Trypanosoma lewisi*

Trypanosomes are hemoflagellate kinetoplastid protozoan parasites that predominantly infect domesticated and wild vertebrate animals. Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by the salivarian group of trypanosomes: *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*. These species are predominantly endemic in Central/Western Africa and Eastern Africa, respectively, thriving within the tse-tse fly (*Glossina* spp.) vector zones.

In contrast, American human trypanosomiasis, or Chagas disease, is caused by *T. cruzi*, a stercorarian trypanosome transmitted by *Triatoma* bugs, which is prevalent in Central America, South America, and Mexico. Various trypanosome species, including *T. brucei brucei*, *T. congolense*, and *T. vivax*, primarily infect animals and are largely responsible for animal trypanosomiasis, or nagana, in Africa. Notably, *Trypanosoma evansi* is mechanically transmitted by tabanid flies and affects a wide range of domestic and wild animals across Africa, South America, and Asia, causing *'Surra'* (Table 1). In India, *T. evansi* stands out as the most prevalent pathogenic trypanosome impacting livestock and pets (Parashar et al., 2016), with diverse clinical manifestations reported in animals, especially in bovines (Singh et al., 2024). Additionally, sporadic instances of *T. theileri*, a non-pathogenic trypanosome found in cattle (Sood et al., 2011), and *T. lewisi*, a non-pathogenic rodent parasite transmitted by rat fleas, are also endemic.

Table 1: Trypanosome Species and Infection Types

Trypanosome Species	Infection Type	Key Details
Trypanosoma brucei gambiense Trypanosoma brucei rhodesiense	African Human trypanosomosis (typical)	Endemic in Central/Western and Eastern Africa, transmitted by tsetse flies
Trypanosoma cruzi	American human trypanosomosis (Chagas disease)	Endemic in Central/South America and Mexico, transmitted by *Triatoma* bugs
Trypanosoma brucei brucei Trypanosoma congolense Trypanosoma vivax	Animal trypanosomosis (Nagana)	Infect animals in Africa
Trypanosoma evansi	Surra	Infects a wide range of domestic and wild animals across Africa, South America, and Asia, transmitted by tabanid flies
Trypanosoma lewisi	Atypical human trypanosomiasis	Non-pathogenic rodent parasite, transmitted by fleas, sporadic cases in humans
Trypanosoma evansi		Mechanically transmitted by tabanid flies, 05 confirmed cases reported globally including 02 in India
Trypanosoma brucei, Trypanosoma congolense, Trypanosoma vivax		Animal-origin trypanosomes causing emerging zoonotic threat, 22 cases reported globally

Atypical human trypanosomiasis (a-HT) refers to infections in humans caused by animal-origin trypanosomes, including *T. lewisi*, *T. evansi*, *T. congolense*, *T. b. brucei*, and *T. vivax*. It is well established that human serum contains apolipoprotein L1 (APOL1), which induces lysis of trypanosomes and supports the development of innate resistance against most trypanosome species (Vanhamme et al., 2003). To differentiate the different trypanosome species by conventional microscopy can be quite challenging due to their similar morphological features. However, advancements in molecular characterization tools over the past few decades have led to a significant increase in reported cases of a-HT globally (Truc et al., 2013). The primary objectives of this comprehensive review are to systematically analyze the current status of a-HT in India, focusing on emerging cases of *T. lewisi* and *T. evansi*, evaluation of the epidemiological patterns along with associated risk factors and assess the existing diagnostic approaches and challenges. Secondary objectives include documenting all reported a-HT cases in India from 1903 to 2023 for a temporal perspective, examining the host-parasite relationship, evaluating treatment protocols, identifying gaps in surveillance and diagnostics, and proposing evidence-based recommendations for improving disease surveillance, diagnostic methods, prevention strategies, and public health interventions. This review will cover historical and current cases, clinical manifestations, treatment outcomes, and the One Health approach to prevention and control, providing a clear direction for the review, aiding reader understanding, supporting systematic organization, and guiding future research priorities.

Materials and Methods

A systematic literature review was conducted to investigate a-HT, utilizing databases such as PubMed/MEDLINE, Web of Science, Scopus, and the Indian Citation Index, along with regional and veterinary-specific databases like IndMED, covering the period from 1900 to February 2024. The search employed primary terms (e.g., "atypical human trypanosomosis," "human trypanosomiasis," "zoonotic trypanosomes"), species-specific terms (e.g. "*Trypanosoma lewisi*," "*Trypanosoma evansi*"), and geographic terms (e.g. "India," "Asia"). Inclusion criteria encompassed original research articles, case reports, epidemiological studies, and critical review articles in English with verified species identification. Exclusion criteria ruled out animal-focused studies, unconfirmed cases, conference abstracts, and duplicates. Data extraction utilized a standardized form to capture demographics, geographic location, clinical presentations, diagnostic methods, treatment outcomes, and prevention strategies. The synthesized data address global case distribution, clinical manifestations, diagnostic approaches, treatment strategies, and public health implications, particularly emphasizing cases from India.

Results

The perusal of literature depicted the first documented case of a-HT in 1933 (Malaysia) till 2023, a total of 22 cases of reported worldwide (Singh, 2024). The temporal distribution of these cases is as follows

- *T. lewisi* (11 cases): Malaysia, India, Thailand, The Gambia
- *T. evansi* (05 cases): India, Sri Lanka, Vietnam, Egypt
- *T. brucei* (04 cases): Africa (Congo, Ghana, Ethiopia)
- *T. vivax* (01 case): Africa (Ghana)
- *T. congolense* (01 case): Cote D `Ivorie

The particular concern is the increasing frequency of reported cases in recent decades especially in India, as 10 cases (08 *T. lewisi* and 02 *T. evansi*) are reported from our nation. The criteria for the *T. lewisi* and *T. evansi* case identification is given in Table 2

Table 2. Comparative overview of *Trypanosoma evansi* and *Trypanosoma lewisi*

Aspect	*Trypanosoma evansi*	*Trypanosoma lewisi*
Primary Hosts	Domestic animals (e.g., cattle, horses)	Rodents (e.g., rats)
Vectors	Tabanid flies (e.g., horse flies)	Fleas (e.g., *Xenopsyllacheopis*)
Transmission Pathway	Tabanid fly bites host animal	Flea bites a host
	Metacyclic trypomastigotes in saliva	Trypanosomes enter through the bite wound
Life Cycle Stages	1. Infective stage: Metacyclic trypomastigotes in vector saliva	1. Infective stage: Trypanosomes in flea faeces
	2. Infection: Parasite enters host bloodstream	2. Transmission: Flea bite infects rodent
	3. In host: Trypomastigotes multiply in blood	3. In host: Parasite replicates in rodent bloodstream
Clinical Manifestations	Acute and chronic forms, leading to severe illness	Generally mild, may resolve spontaneously
Human Infection	Rare, but possible via direct contact with infected blood or meat	Rare, often associated with close contact with rodents
Geographical Distribution	Primarily found in Asia, Africa, and South America	Commonly reported in areas with high rodent populations

Cases of *Trypanosoma lewisi*: The first case of a-HT due to *T. lewisi* was reported in 4-month-old infant from Malaysia who exhibited appetite loss, lassitude, and fever, but recovered spontaneously without treatment (Johnson, 1933). In India, the first documentation of an organism resembling *T. lewisi* occurred in 1974, involving an adult couple (a 40-year-old male and a 35-year-old female) residing in a rat-infested remote village in Chhattisgarh, Madhya Pradesh. Both patients displayed symptoms of fever and lassitude, which resolved without specific treatment (Shrivastava et al., 1974). Since then, numerous cases of a-HT attributed to *T. lewisi* have been reported across various states in India, including Mumbai (Kaur et al., 2007; Shah et al., 2011), Maharashtra (Doke and Kar, 2011), Gujarat (Bharodiya et al., 2018), and Uttar Pradesh (Verma et al., 2011; Jain et al., 2023). A common risk factor among these *T. lewisi*-infected individuals is their belonging to low socioeconomic strata and residing in unsanitary, rat-infested conditions (Kumar et al., 2022). The higher incidence of *T. lewisi* infections can be attributed to natural resistance to trypanolysis by human serum and limited antigenic variation in these rat trypanosomes, often resulting in asymptomatic cases and spontaneous recovery without treatment (Lun et al., 2015). Among the reported cases, most infected patients were young infants or chronically ill immunocompromised individuals, allowing *T. lewisi* to act as an opportunistic pathogen (Kumar et al., 2022).

Cases of *Trypanosoma evansi*: The first case of *Trypanosoma* reported in an adult female from West Bengal in 1903 was presumed to be *T. evansi* by microscopic examination of blood stained smear, as it was the only prevalent species found among local livestock (Parashar et al., 2016). However, the first confirmed case of *T. evansi* in India was identified in a 45-year-old cattle farmer from Seoni village in Chandrapur district, Maharashtra, in 2004. The patient was hospitalized with a 15-day history of fever, chills, perspiration, loss of sensation, and aggressive behavior. This pioneering case was confirmed through conventional microscopy, immunologically by Card agglutination test for *T. evansi* (CATT) and molecular PCR based assays (Joshi et al., 2005). The farmer likely contracted the infection through a wound on his finger from the blood of an infected cattle. *Trypanosoma evansi* is primarily transmitted through tabanid bites, oral ingestion of raw meat, or direct contact with infected blood (Joshi et al., 2005). The second confirmed case of *T. evansi* was also reported from Maharashtra, involving a 23-year-old pregnant female who presented with severe anemia, upper respiratory tract infection (Retroviral disease), ear and abdominal pain, along with pulmonary tuberculosis and HIV complications. Hepatosplenomegaly was clearly visible, and the infection was confirmed through microscopy and *in vitro* cultivation in Novy Mac Neal Nicolle (NNN) medium (Wabale et al., 2015). Both patients infected with *T. evansi* responded positively to treatment with suramin (Joshi et al., 2005; Wabale et al., 2015). Serological assays using the CATT assay on 1,806 individuals from the village of origin (Chandrapur) of

the first Indian case revealed 4.85% positivity (Shegokar et al., 2006). In a retrospective study of a-HT related to *T. evansi* in West Bengal involving 173 people, 09 (5.2%) tested positive by CATT, and 05 (2.89%) tested positive by PCR (Sengupta et al., 2022).

The anti-trypanosomal activity of human sera is believed to be notably effective against *T. evansi* (Juyal et al., 1998). However, the absence of apolipoprotein L1, as confirmed in the first reported case of *T. evansi* linked to a frameshift mutation in both APOL1 alleles (Vanhollebeke et al., 2006), along with other factors such as enhanced arginase activity and deficiencies in the complement lectin pathway, may facilitate infection (Cestari et al., 2013). Interestingly, the presence of intact, functional apolipoprotein L1 (ApoL1) in a female patient from Vietnam infected with *T. evansi* raises critical questions about the zoonotic potential of this parasite (Van Vinh et al., 2016).

Risk factors for a-HT

Atypical human trypanosomosis is influenced by various socioeconomic, environmental, and host-related risk factors. Socioeconomically, living in rat-infested, overcrowded areas with poor sanitation and limited healthcare access significantly increases risk. Occupations such as livestock farming, veterinary work, and agricultural labor also expose individuals to higher infection rates, especially in regions with high vector density and ineffective vector control. Immunocompromised individuals, those with specific genetic predispositions, and people with poor hygiene practices further heighten susceptibility to the disease.

Prevention Strategies and Economic Impact

Effective prevention strategies involve individual, occupational, and community-level measures. Individuals should utilize protective clothing and hygiene practices, while at-risk occupations need robust safety protocols and training. Community interventions should focus on vector control, animal health management, and public health measures to enhance surveillance and education. Economically, the direct costs of healthcare, including treatment and vector control, can reach millions, while indirect costs such as productivity losses and agricultural impacts compound the burden. Investing in prevention not only reduces healthcare costs but also enhances productivity and food security, highlighting the importance of a systemic approach to tackling this public health challenge.

Summary and Recommendations

Atypical human trypanosomiasis (a-HT) is rapidly emerging as a significant public health threat in India, despite its more common association with regions in Africa and South America. The clinical manifestations of this disease are diverse and can often lead to severe complications. It is imperative that healthcare providers are acutely aware of a-HT to ensure timely diagnosis and treatment. Early identification and appropriate therapeutic interventions are crucial to managing this condition and mitigating the

impacts of chronic infections.

To combat this rising concern, we must establish robust surveillance systems to effectively monitor and report cases of trypanosomiasis. This will not only enhance our understanding of the disease's epidemiology in India but will also empower public health initiatives. Furthermore, collaboration with research institutions is vital to develop rapid and accurate diagnostic tools, as well as effective therapeutics and vaccines.

Public awareness campaigns are essential in endemic regions, emphasizing the transmission routes and preventive measures. Strategies should focus on vector control and personal protection to empower communities against this zoonotic threat. Additionally, we must educate healthcare professionals about the clinical features and diagnostic methods for atypical trypanosomiasis to ensure a comprehensive response to this disease.

The activities of the Network on Atypical Human Infections by Animal Trypanosomes (NAHIAT), in collaboration with international organizations such as World Health Organization, Food and Agriculture Organization and OIE, require intensified support. This collaboration is crucial to ascertain the true status of a-HT as an emerging zoonotic disease and to mobilize the resources necessary for effective management and prevention.

The time for action is now! We must unite our efforts to address the challenge posed by atypical human trypanosomiasis and safeguard public health in India.

References

Bharodiya, D., Singhal, T., Kasodariya, G. S., Banerjee, P. S., & Garg, R. (2018). Trypanosomiasis in a young infant from rural Gujarat, India. *Indian Pediatrics, 55*, 69-70.

Cestari, I., Evans-Osses, I., Schlapbach, L. J., de Messias-Reason, I., & Ramirez, M. I. (2013). Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites. *Molecular Immunology,* 53(4), 328–34.

Doke, P. P., & Kar, A. (2011). A fatal case of *Trypanosomalewisi* in Maharashtra, India. *Annals of Tropical Medicine & Public Health,* 4(2), 91–95.

Jain, P., Goyal, V., & Agrawal, R. (2023). An atypical *Trypanosomalewisi* infection in a 22-day-old neonate from India: An emergent zoonosis. *Indian Journal of Pathology and Microbiology,* 66(1), 199-201.

Johnson, P. D. (1933). A case of infection by *Trypanosomalewisi* in a child. *Transactions of Royal Society of Tropical Medicine and Hygiene,* 26(5), 467–68.

Joshi, P. P., Shegokar, V. R., Powar, R. M., Herder, S., Katti, R., Salkar, H. R., & Truc, P. (2005). Human trypanosomiasis caused by *Trypanosomaevansi* in India: The first case report. *The American Journal of Tropical Medicine and Hygiene,* 73(3), 491-95.

Juyal, P. D., Singla, L. D., & Saxena, H. M. (1998). In vivo activity of human serum against *Trypanosomaevansi* infection in Swiss albino mice. *Journal of Parasitic Diseases,* 22, 67-68.

Kaur, R., Gupta, V. K., Dhariwal, A. C., Jain, D. C., & Shiv, L. (2007). A rare case of trypanosomiasis in a two-month-old infant in Mumbai, India. *The Journal of Communicable Diseases,* 39(2), 71–74.

Kumar, R., Gupta, S., Bhutia, W. D., Vaid, R. K., & Kumar, S. (2022). Atypical human trypanosomosis: Potentially emerging disease with lack of understanding. *Zoonoses and Public Health,* 69(4), 259-76.

Lun, Z. R., Wen, Y. Z., Uzureau, P., Lecordier, L., Lai, D. H., Lan, Y. G., & Pays, E. (2015). Resistance to normal human serum reveals *Trypanosomalewisi* as an underestimated human pathogen. *Molecular and Biochemical Parasitology,* 199(1-2), 58-61.

Parashar, R., Singla, L. D., & Kaur, P. (2016). Is atypical human trypanosomosis an emerging threat to human society? A debatable one health issue to public health experts and parasitologists. *International Journal of Veterinary Science and Research,* 2(1), 36–41.

Sengupta, P. P., Jacob, S. S., Chandu, A. G. S., & Das, S. (2022). Silent *Trypanosomaevansi* infection in humans from India revealed by serological and molecular surveys, and characterized by variable surface glycoprotein gene sequences. *Acta Tropica,* 229, 106369.

Shah, I., Ali, U., Andankar, P., & Joshi, R. (2011). Trypanosomiasis in an infant from India. *Journal of Vector Borne Diseases,* 48(2), 122-23.

Shrivastava, K. K., & Shrivastava, G. P. (1974). Two cases of *Trypanosoma* (*Herpetosoma*) species infection of man in India. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 68(2), 143-44.

Singh, B. (2024). A study on trypanosomosis in animals with indication of possible subclinical human infections. M.V.Sc. thesis, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab.

Singh, B., Singla, L., & Kaur, P. (2024). Clinical features and management of *Trypanosoma evansi* infection in buffaloes. *The Indian Journal of Animal Sciences,* 94(9), 778-81.

Sood, N. K., Singla, L. D., Singh, R. S., & Uppal, S. K. (2011). Association of *Trypanosomatheileri* with peritonitis in a pregnant cross-bred cow: A case report. *VeterinárníMedicína,* 56(2), 82-84.

Truc, P., Büscher, P., Cuny, G., Gonzatti, M. I., Jannin, J., Joshi, P., &Desquesnes, M. (2013). Atypical human infections by animal trypanosomes. *PLoS Neglected Tropical Diseases,* 7(9), e2256.

Van Vinh, C. N., Buu Chau, L., Desquesnes, M., Herder, S., Phu HuongLan, N., Campbell, J. I., Van Cuong, N., Yimming, B., et al. (2016). A clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosomaevansi* in Southeast Asia. *Clinical Infectious Diseases,* 62, 1002–08.

Vanhamme, L., Paturiaux-Hanocq, F., Poelvoorde, P., Nolan, D. P., Lins, L., Van Den Abbeele, J., Pays, A., Tebabi, P., Van Xong, H., Jacquet, A., Moguilevsky, N., Dieu, M., Kane, J. P., De Baetselier, P., Brasseur, R., & Pays, E. (2003). Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature,* 422(6927), 83–87.

Vanhollebeke, B., Truc, P., Poelvoorde, P., Pays, A., Joshi, P. P., Katti, R., & Pays, E. (2006). Human *Trypanosomaevansi* infection linked to a lack of apolipoprotein L1. *New England Journal of Medicine,* 355(26), 2752-56.

Verma, A., Manchanda, S., Kumar, N., Sharma, A., Goel, M., Banerjee, P. S., Garg, R., Singh, B. P., Balharbi, F., & Lejon, V. (2011). *Trypanosoma lewisi or T.lewisi*-like infection in a 37-day-old Indian infant. *American Journal of Tropical Medicine and Hygiene,* 85, 221-24.

Wabale, V., Nalage, P., Joshi, A., Bharadwaj, R., Deshpande, K., & Chowdhary, A. (2015). Human Asian Trypanosomiasis due to *Trypanosomaevansi*: A rare case. *Journal of Advance Parasitology,* 2(3), 65-68.

Mini Review

Prospects of Essential Oils as an Eco-Friendly Alternative for Tick Control in Livestock

Jyoti*, Manisha, Harkirat Singh and Nirbhay K. Singh

Department of Veterinary Parasitology
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: jyoti76vet@gmail.com

Received: 07.10.2024 Accepted: 05.11.2014

Abstract

Ticks and tick-borne diseases cause significant economic loss to livestock industry particularly in tropical and subtropical regions. Presently, tick control is primarily achieved by use of chemical acaricides but their indiscriminate and continuous use is associated with serious inherited disadvantages such as development of resistant tick population, chemical residues in milk and meat, and environmental pollution. However, development and use of non-chemical alternate eco-friendly plant extracts and essential oils (EOs) for suppressing tick populations and vectored diseases is the need of the hour. Use of essential oils to control ticks is an innovative approach to address drug resistance and to extend the effectiveness and lifespan of current chemical acaricides. Modifying the structure of essential oil compounds and combining them with synthetic acaricides will enable the creation of new technologies for tick control in animals. Use of nanotechnology in formulation development can maintain their efficacy for a longer duration. The future of EOs for tick control looks promising, particularly as people shift towards natural and non-toxic solutions for effective tick control.

Keywords*: Essential oils, Integrated tick management, Phyto-acaricides, Tick control.*

Ticks are obligate blood-sucking arachnids, widely distributed in tropical and sub-tropical countries. Approximately 80% of the cattle population is expected to be vulnerable to tick infestation. Ticks cause direct losses to the cattle industry through blood sucking, injection of toxins, responsible for paralysis and damage to leather resulting in depreciation in market value. They are also indirectly responsible for transmission of many important diseases in livestock and companion animals. According to a recent report, the total economic losses due to tick and tick-borne diseases in India were estimated to be 787.63 million USD (Singh et al., 2022a).

Tick control mainly relies on the usage of synthetic chemical acaricides. However, the indiscriminate use of these acaricides has led to the development of acaricide resistance. It is therefore essential to develop novel control strategies as sustainable alternatives to reduce the tick populations in animals. Herbal acaricides contain multiple active constituents, rapidly biodegradable residues and show low toxicity towards the

non-target organisms; therefore, they can serve as a suitable substitute. By incorporating herbal acaricides into integrated tick management strategies, the reliance on a single type of acaricide can be reduced and thereby risk of resistance can be minimized. Among various suitable plant-derived products, essential oils are being investigated as a viable option and have been shown to possess high anti-tick efficacy.

Essential Oils (EOs)

The EOs are natural volatile compounds that are a blend of different (about 20–60) plant metabolites. They usually contain two or three major terpene or terpenoid components, which constitute up to 30% of the oil. The EOs represent a safer alternative in many fields such as food preservation, biomedicine, cosmetics and agriculture. Numerous studies have reported that EOs also exert ovicidal, larvicidal, adulticidal and repellent effects on ectoparasites (Abbas et al., 2018). Due to their low toxicities and solubility in water, these compounds can contribute to the production of chemically-free milk and meat, which are safe to humans, animals and the environment. The active components of EOs are reported to act as synergists when used in combination. Composition of EOs varies to a large extent depending on the isolation method used. Steam distillation is most frequently used procedure for isolating EOs along with other methods viz. solvent extraction, simultaneous distillation extraction and by microwave ovens.

Mechanisms of Action against Ticks

Acaricidal and insecticidal effects of EOs are associated with their active ingredients. Because of presence of large number of constituents, EOs seem to have no specific mechanism of action. They have various effects against ticks *viz*. feeding inhibition, inhibition of chitin synthesis, decrease in growth, development or reproduction and affect tick behaviour. Recent studies have reported following modes of EOs:

Neurotoxic effects: Most of the EOs target monoamine oxidase, transient receptor potential (TRP) ion channels, gamma-aminobutyric acid (GABA) receptors and the acetylcholinesterase, causing effect on the nervous system (Vangchhia et al., 2023).

Cytotoxic effects: The cellular targets of EOs are mainly octopamine and tyramine receptors resulting in lethal effects on ticks. The constituents present in EOs may cause ticks energy deficit leading to death.

Mechanical effects: The hydrophobic nature of EOs is responsible for mechanical effect. Death from water stress or suffocation is the result of the disruption of cuticular waxes and blockage of respiratory spiracles.

Repellent effects: Many EOs have a repellent effect against ticks by producing a vapour barrier that prevents the ticks from coming into contact with the skin.

Assessment of Acaricidal and Repellent Activity of EOs

Various *in-vitro* assays have been utilized for estimating the acaricidal/larvicidal and repellent property of EOs against ticks. Bioassays like Adult Immersion Test (AIT), Larval Packet Test (LPT) and Larval Immersion Test (LIT) have been used to assess the acaricidal activity of EOs in adults or larval ticks. There are many reports on the use of these assays to determine the acaricidal activity of different EOs and their combinations from all over the world (Coulibaly et al., 2023). Tick climbing repellency bioassay and petridish bioassay are used to assess the repellent effects of EOs.

Acaricidal Activities of EOs

Since the initial study in 1990, there has been extensive research into the repellent and acaricidal effects of various EOs against ticks from all over the world. The majority studies have focused on genus *Rhipicephalus* and *Ixodes* ticks. However, reports on the effects of EOs as tick treatments or repellents *in vivo* are limited. From India, there are reports of assessment of efficacy of several EOs against ticks with favourable results (Table 1). There are also several reports on the synergistic effects of EOs against ticks

Table 1: Acaricidal activity of various EOs against cattle tick from India

Essential oil	Common name	Assay	Reference
Ageratum conyzoides	Goat weed	AIT	Kumar et al., 2016
Allium sativum	Garlic	AIT, LPT	Singh et al., 2022b; Vangchhia et al., 2024
Azadirachta indica	Neem	Syringe Test, AIT	Nawaz et al., 2015 Singh et al., 2022b
Cedrus sp.	Cedarwood	LPT	Vangchhia et al, 2024
Cinnamomum zeylanicum	Cinnamon bark	LPT	Jyoti et al., 2019
Cinnamomum camphora	Camphor	LPT	Kapoor et al., 2023
Citrus limetta	Sweet lemon	AIT, LPT	Jain et al., 2021
Cymbopogon citratus	Lemon grass	LPT	Jyoti et al., 2019
Eucalyptus globulus	Eucalyptus	*In vivo*, AIT	Goswami et al., 2022; Singh et al., 2022b
Murraya koenigii	Curry leaves	AIT	Singh et al., 2022b
Mentha piperita	Peppermint oil	LPT	Vangchhia et al., 2024
Rosmarinus officinalis	Rosemary	AIT	Singh et al., 2022b
Ricinus communis	Castor	AIT	Singh et al., 2022b
Syzygium aromaticum	Clove	LPT	Jyoti et al., 2019
Salvia officinalis	Sage	AIT	Singh et al., 2022b

of veterinary importance (Jyoti et al., 2019; Singh et al., 2022b; Coulibaly et al., 2023; Vangchhia et al., 2024). The synergistic combinations are used to reduce the dose of EOs, decrease the risk of resistance and create a dynamic product with multiple modes of action. Positive and negative synergism can occur between EOs or their components and other ingredients in the formulation.

Application on Host and Environment

The formulations and administration routes of EOs or their combinations (EOCs), most commonly used on hosts are topical sprays, pour-on, drop-on, soap foam and oral spray (Gonzaga et al., 2023). It is recommended to treat the whole body of bovines using sprays, whereas, for pour-on formulations, the dosage varies according to the animal's body weight. There are only a few studies using EOs/EOCs for tick control in the environment targeting ticks of public health importance in either naturally or experimentally infested areas covering different plot sizes using spray formulations.

Limitations of the Use of EOs

The EOs have a wide potential for tick control because they have promising acaricidal and repellent activities, but their use remains limited due to some undesirable effects. They deteriorate the cell membranes and organelles of cells, affect ATP synthesis, cause lethality in intestinal cells and allergic reactions (Abbas et al., 2018). There are certain limitations to the commercial use of EOs as repellents and/or acaricides as these are produced in small amounts, thus, the scarcity of these natural resources makes EOs expensive and unaffordable, limiting their use in the field (Gonzaga et al., 2023). Most of research work performed is limited to laboratory and scanty data on trials conducted in the field are available. The *in vitro* acaricidal efficacy of EOs and EOCs is often promising; however, the actual *in vivo* acaricidal activity yet to be proved. The concentrations of EOs and EOCs that demonstrate efficacy are often quite high, making their production for use as a tick control product unfeasible. As the EOs are volatile, their efficacy diminishes relatively quickly over time. Therefore, availability of EOs and EOCs based products in the veterinary market is currently limited.

Strategies to Increase the Efficacy of EOs and EOCs

Utilizing nanotechnology in the formulation development of EOs and EOCs has been shown to enhance efficacy even on lower concentrations. This approach also improves the economic feasibility of developing these biopesticides. The association of EOs/EOCs with synthetic acaricides is another potential strategy for improving the efficacy. Brazil already has formulations of commercial acaricides containing pyrethroids and organophosphates associated with terpenes (citronellal and geraniol EOCs) or piperonyl butoxide, a semisynthetic derivative of safrole EO. Initial data from *R. microplus* indicate

no cross-resistance between synthetic acaricides and EOs/EOCs (Gonzaga et al., 2023).

References

Abbas, A., Abbas, R. Z., Masood, S., ... , & Sindhu, Z. D. (2018). Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. *Boletín Latinoamericano Y Del Caribe De Plantas Medicinales Y Aromáticas*, 17(5), 441-52.

Coulibaly, A., Biguezoton, A. S., Hema, D. M., ... & Nebie, R. C. (2023). Evaluation of synergism in essential oils against the cattle tick *Rhipicephalus microplus* in Burkina Faso. *Experimental Parasitology*, 255, 108643.

Gonzaga, B. C. F., Barrozo, M. M., Coutinho, A. L., ... & Monteiro, C. (2023). Essential oils and isolated compounds for tick control: advances beyond the laboratory. *Parasites & Vectors*, 16(1), 415.

Goswami, R., Arora, N., Mrigesh, M., Verma, P., & Rajora, V. (2022). Effect of eucalyptus oil against tick infestation in cattle. *The Pharma Innovation Journal*, 11(6), 804-07.

Jain, P., Satapathy, T., & Pandey, R. K. (2021). Acaricidal activity and biochemical analysis of *Citrus limetta* seed oil for controlling Ixodid tick *Rhipicephalus microplus* infesting cattle. *Systematic and Applied Acarology*, 26(7), 1350-60.

Jyoti, Singh, N. K., Singh, H., Mehta, N., & Rath, S. S. (2019). *In vitro* assessment of synergistic combinations of essential oils against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). *Experimental Parasitology*, 201, 42–48.

Kapoor, A., & Preet, S. (2023). Evaluation of acaricidal activity of *Cinnamomum camphora* (F. Lauraceae) essential oil nanoemulsion against cattle tick *Rhipicephalus microplus*. *Advances in Zoology and Botany*, 11(2), 121–28.

Kumar, K. G., Tayade, A. B., Kumar, R., ... & Ghosh, S. (2016). Chemo-profiling and bioassay of phytoextracts from *Ageratum conyzoides* for acaricidal properties against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) infesting cattle and buffaloes in India. *Ticks and Tick-Borne Diseases*, 7, 342-349.

Nawaz, M., Sajid, S. M., Zulfiqar, A., ... & Imran, K. (2015). Anti-tick activity of leaves of *Azadirachta indica*, *Dalbergia sisso* and *Morus alba* against *Rhipicephalus microplus*. *Acta Parasitologica Globalis*, 6, 60-64.

Singh, K., Kumar, S., Sharma, A. K., ... & Ghosh, S. (2022a). Economic impact of predominant ticks and tick-borne diseases on Indian dairy production systems. *Experimental Parasitology*, 243, 108408.

Singh, K., Sharma, A. K., Kumar, S., Sankar, M., & Ghosh, S. (2022b). Synergistic properties of essential oils of eucalyptus (*Eucalyptus globulus*) and lemon grass (*Cymbopogon flexuosus*) against cattle tick, *Rhipicephalus microplus*. *International Journal of Acarology*, 48(4–5), 338–43.

Vangchhia, L., Jyoti., Singh, H., Dumka V. K. & Singh, N. K. (2024). Assessment of synergism in combinations of essential oils against cattle tick *Rhipicephalus microplus* (Acari: Ixodidae). *Experimental and Applied Acarology*, 93, 473–84.

Vangchhia, L., Jyoti, Singh, H. & Singh, N. K. (2023). Development of acaricide resistance in ticks and strategies for mitigation. *Vet Alumnus*, 45(2), 85–90.

Mini Review

Integrated Management of Arthropods of Veterinary Importance: Pathway to Sustainable Ectoparasite Parasite Management

Alveena Ganai* and Paramjit Kaur

Department of Veterinary Parasitology
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India.
*Corresponding author Email: dralveenaganai@gmail.com

Received: 02.11.2024 Accepted: 22.11.2024

Abstract

Arthropod-borne diseases have a major impact on animal health and welfare which leads to poor growth, development and ends with severe economic losses to the farmers. The common arthropods infesting livestock are: flies (biting and non-biting), fleas, lice (biting and sucking), ticks (soft and hard), and mites (burrowing, non-burrowing, and follicular) which are the potent vectors for many bacterial, viral, rickettsial, and protozoan diseases. In order to control these vectors, recent concept of integrated management (IM) has been acknowledged against acaricide resistance and provides protection of livestock from harmful chemicals. This article highlights the importance of efficient and sustainable IM for ectoparasites of livestock with an alternative approach via implementation of various managemental practices including mechanical, biological, herbal and chemical control measures in order to minimize the economic losses to the livestock, improves the resilience of production systems and creates new approaches for addressing the arthropods at different levels and thus maximize the effectiveness of pest control strategies and minimize the use of harmful chemical.

Keywords: *Arthropods, Economic loss, Integrated pest management, Vectors*

Livestock and pet animals are susceptible to many ectoparasites, which lead to severe economic losses to hide industry due to the direct damage to the skin through their bites and indirect losses because of annoyance, worries and psychological disturbances produced during feeding and their disease transmission potential. The common arthropods of veterinary importance found in the livestock are categorized into two categories insects (egs. flies and mosquitoes) and arachnidsegs. ticks, lice and fleas). Among these, the important ectoparasites found in India are: Flies (*culicoides, simulium, musca, stomoxys, haematobiairritans, phlebotomus sergenti* and *tabanus*), ticks (*Rhipicephalus (Boophilus) microplus, hyalomma anatolicum, Dermacentor variabilis* and *amblyomma americanum*), lice (*haematopinus eurysternus, linognathus vituli* and *bovicola bovis*), flea in poultry (sticktight flea and *echidnophaga gallinacean*) and mites (*Psoroptes, sarcoptes* and *demodex*). These vectors are responsible for transmitting various diseases like - cutaneous myiasis, anthrax, tularaemia, lyme disease and ocular

disease, leishmaniosis, rift valley fever and filariosis, louping-ill, tick-borne encephalitis and lyme disease, rocky mountain spotted fever, bartonellosis, tick-borne relapsing fever including various protozoan diseases like babesiosis, theileriosis and anaplasmosis. Feline leukaemia, typhymurium, rickettsiosis and bartonellosis transmitted by fleas and common sheep scab by mites.

The control of ectoparasites is mostly done by the use of insecticides and acaricides as the major curative and preventive strategy preferred by the veterinarians that results into the development of resistance against the chemicals especially against the flies and ticks worldwide. Now-a-days the growing interest toward the development of alternative and effective integrated control approaches against the ectoparasites that depend upon the characteristics of the pathogens they transmit, mode and type of transmission of infection, environmental factors. Integrated management (IM) of arthropods includes the effective, economical and sustainable combination of mechanical, biological, herbal and chemical control measures. In IM programme, chemical treatment would be the last resort due to their increased application cost, drug resistance and various hazards associated with their use.

INSECTS: Organic livestock production systems promote an integration of mechanical, biological and chemical practices that usually helps in cycling of resources, conserve biological diversity and promote an ecological balance.

Mechanical: The use of door and window screens prevents flies to enter the livestock production facilities, especially sensitive area such as the milk parlour room. Smoke generated by burning wood or plants should be adopted in an open area to make this technique more effective one tablespoon full of fennel seeds or neem leaves which act as fly repellents that can reduce the number of flies. Fly traps, dust bags, back rubbers (oilers) and fly tags can be placed near water tanks in order to control flies throughout the summer months. In order to control mosquitoes, general control measures such as, filling of pits especially the low-lying areas and ditches with coal ash, construction of stand post and proper drainage, mosquito proofing of overhead tanks with proper sewage system, application of expanded polystyrene beads to underground tanks, leaking sluice valve chambers and blocking of sewage manholes helps in controlling the breeding of immature mosquitoes' stages.

Biological and Herbal: Certain parasitoids like Spalangia and Muscidifurax spp. have high potential for control of *M. domestica* and *S. calcitrans*. Entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) naturally present in farms can be used for controlling flies. Several plant essential oils have been used such as basil (*Ocimum basilicum*), geranium (*Pelargonium graveolens*), lavender (*Lavandula angustifolia*), lemongrass (*Cymbopogon citratus*), peppermint and pine (*Pinus sylvestris*) repel whose

effectiveness mostly depends on their volatile bioactive constituents, the formulation and the application method. Even, mites of the family Macrochelidae naturally present in cow manure act as a prey on the eggs and larvae of dung-breeding flies. On the other hand, beetles such as histerids and staphylinids prey on eggs, larvae and Philonthus spp. are frequent inhabitants of cattle dung and effective predators of the horn fly. There are reports of some white birds like egret (*Bubulcus ibis*) which catches horseflies when they land on livestock to bite. Few species of bacteria like *Bacillus thuringiensis* produces insecticidal proteins potentially active against various species of mosquitoes. Now-a-days, wolbachia-based mosquito control has emerged as another novel and promising tool as they are maternally inherited endosymbiotic bacteria which are naturally occurre in many arthropods. Although application of bio-larvicides to water accumulated bodies and larviparous fishes like, *Poecilia reticulata* (guppy fishes) and *Gambusia affine* helps in controlling the flies as well as mosquitoes (Singh et al., 2022).

Chemical: Sprays and pour-ons on net sheds, animals and breeding places leads to the management of the flies. Examples of screw worm eradication is implemented by sterile insect technique within the context of IM principles. Larvicides can be applied directly to cow manure to control fly immature stages and several insect growth regulators such as pyriproxyfen, cyromazine, diflubenzuron, novaluron and methoprene are effective against the house fly, stable fly and horn fly. Whereas different compounds which act as adulticides against flies are: synthetic pyrethroids (permethrin, cypermethrin, deltamethrin and fenvalerate), organophosphate (diazinon), phenylpyrazole (fipronil), macrocyclic lactones (ivermectin), pyrrole derivatives (chlorfenapy), neonicotinoids (imidacloprid), carbamate (methomyl) and spinosad. Although, insecticide-treated nets have also been proposed as a novel tool as integrated management measure in livestock providing a long-lasting protection against flies. Limited number of growth regulators such as methoprene and chitin synthesis inhibitors like diflubenzuron and triflumuron are used against mosquitoe control by WHO Pesticide Evaluation Scheme. Some chemical compounds such as N, N-diethyl-3-methyl benzamide or pyrethroids (metofluthrin and transfluthrin) are also used. The most commonly used synthetic repellent is DEET (diethyltoluamide; not to be confused with DDT) against mosquitoes which remain effective up to 4 h after repellent application. (Hinkle et al., 2021).

ARACHNIDS/TICKS & MITES: The various tick control measures can be directed against both the free-living and parasitic stages against ticks and mites. The free-living stages of most tick species, both ixodid and argasidae, have specific requirements in terms of microclimate and are restricted to particular microhabitats within the ecosystems inhabited by their hosts. Destruction of these microhabitats reduces the abundance of ticks by various methods.

Mechanical: The physical control of ticks and mites may involve the smoking of sheds with raw leaves in order to destroy the breeding places which is to be undertaken in three consecutive time weekly interval within a season and ceiling of cracks and crevices where they lay eggs. Various measures should be taken under consideration like pasture rotation, vegetation management, time, type and duration of acaricide applications to pastures and on the livestock which acts as a component of multiple-factor of IM strategies. Burning of pasture land twice a year can also help in eradication of the larval stages of these ectoparasites. Example: rotation of pastures or pasture spelling has been used against one-host tick *Rhipicephalus microplus.*

Biological Control: Biological methods include the use of predators, birds, rodents, shrews, ants and spiders play important role in reducing the numbers of free-living ticks. In the New World, fire ants (*Pheidole megacephala*) are noteworthy tick predators. Whereas, engorged ticks may also become parasitized by the larvae of some wasps like Hymenoptera.

Rearing of Tick Resistant Breeds: Various breeds of tick resistant cattle breeds like Zebu (*Bos indicus*) and Sanga (a *B taurus*, *B indicus* crossbreed) which are the indigenous breeds of Asia and Africa usually become very resistant to ixodid ticks after initial exposure to the various stages of ticks.

Phytochemical: The herbal products like synthetic pesticides, botanical extracts or entomopathogenic organisms such as the recently introduced entomopathogenic fungus, *Metarhizium brunneum*, Strain 52 formulated against ticks and mite control. Many bio insectide containing the white muscardine fungus like *Verticillium lecanii* and *Beauveria bassiana* are usually applied on cattle body. The use of most common three herbal oils—neem oil (*Azadirachta indica*), karanj oil (*Pongamia pinata*) and Nilgiri oil (*Eucalyptus globulus*) can be used on animal body, whereas pheromones used by ticks for aggregation and mating can be artificially used in combination with acaricides. There are many active compounds in the medicinal plant that can serve as insecticides, acaricides, growth inhibitors, anti-molting agents, and repellents, interrupting insect's biological processes during their life cycle. Some of the important herbal plants with their common names used in India having acaricidal activity are: *Aegle marmelos* (bael), *allium sativum* (garlic), *azadirachta indica* (neem), *anisomeles malabarica* (kala bhangra), *annona squamosa* (custard apple), *carica papayaL*. (papaya), *curcuma longa* (turmeric), *cymbopogon winterianus* (lemon grass), *datura stramonium* (datura), *ocimum basilicum* (basil), *ricinus communis* (castor), *solanum trilobatum* (red pea eggplant), *withania somnifera* (ashwagandha), *vitex negundo* (Nirgundi) and many more. On the other hand, *psoroptes* mites can be controlled with the application of fungus like *Metarhizium anisopliae.* Fleas and lice infestation in the livestock can also be controlled by removal of dung, manure and providing proper provision of drainage system targeting their breeding places. Flea

control needs to be carried out on the animal and in its environment simultaneously and the agents must be used therapeutically, prophylactically and regularly on a long-term basis and consistently for all the animals in a household like (S)-Methoprene (growth regulator) which has a very low level of toxicity and does not irritate skin or mucous membrane. Whereas, lice infestation can be controlled biologically by recent use of fungi like- *Trenomyces histophtorus* against *Haematopinus eurysternus, Linognathus vituli* and *Bovicola bovis*. Also, sheep wool infected with fleas and lice can be treated by one of the bacterial strains like *Bacillus thuringiensis* (Eads et al., 2021).

Chemical: Treatment with various chemical products available in the practices of livestock production systems is still under scrutiny because of the impact acaricides and endectocides like ivermectin have on public health, the environment, and the international trade of livestock and animal products. Pyrethroids, including fipronil, permethrin and permethrin combination products are effective ectoparasiticides because of rapid penetration of tick cuticle and gets accumulated in the tissues. Flumethrin is one of the commonly used ectoparasiticide against flea and lice infecting the livestock (Rajput et al., 2006). However, topically applied cutaneous ectoparasiticides may not achieve a uniform distribution with some body parts not being covered to the same extent and concentration and thus leads to high resistance against these ectoparasites. Thus, tick resistance is mainly due to reduced susceptibility of ticks on the recommended dose or infrequent and uncontrolled use of acaricidal agents.

Vaccines: The application of vaccines for controlling tick population can limits the transmission of pathogens. The commonly used vaccines against ticks are: Tickgard, Tickgard plus and Gavac which are the recombinant vaccines available in the market against *R. microplus*. These vaccines are based on concealed tick midgut protein, Bm86 and BM86+91 strain. Vitellin and GP80 based vaccines is found to be useful for vaccination in sheep by reducing the number of attached female ticks and reduction in tick oviposition. However, tick control based on vaccine has not yet reach its optimum performance because of logistic constraints and impact of tick genetic diversity on vaccine efficacy (Abbas et al., 2023).

Future Research Perspectives in India

The present article highlights the importance of integrated management of arthropods infecting the livestock and an alternative approach for approximate estimation of economic losses. The lack of appropriate knowledge on mapping of arthropods born diseases in India because of poor network of extension activities have failed to understand livestock owners and farmers about the different methods of implementation and application of IM efficiently in the livestock sector. The research gaps related to the potential application of integrated management can be compensated by quantitative

approach and identifying the existing trends, bias, and knowledge gaps. This can help reduce reliance on traditional insecticides and contribute to improved livestock well-being and ecosystem health by use of various methods of integrated management system including phytochemical control which can provide a plausible answer to side effects of conventional drugs. It can limit any unnecessary use of chemical or synthetic drugs to overcome residue problems, toxicity and the growing resistance in the next generation. Future response related to control of arthropods via implementation of integrated management can be improved by the researchers' potential experimentations on cultivation context of herbal plant genotype, extract preparation, and the pattern of usage of phytochemicals against the arthropods in combination with all the principles of IM, focusing on treatment of animals, environmental control at breeding sites, disease management and drug resistance.

References

Abbas, M. N., Jmel, M. A., Mekki, I., Dijkgraaf, I., & Kotsyfakis, M. (2023). Recent Advances in Tick Antigen Discovery and Anti-Tick Vaccine Development. *International Journal of Molecular Sciences*, 24(5), 4969.

Eads, D.A., Jaronski, S.T, Biggins, D.E and Wimsatt, J. (2021). Insect Pathogenic Fungi for Biocontrol of Plague Vector Fleas: A Review. *Journal of Integrated Pest Management*, 12 (1). 30.

Hinkle, N.C and Hogsette, J.A. (2022). A Review of Alternative Controls for House Flies. *Insects*, 12(11), 1042.

Rajput, Z. I., Hu, S., Chen, W., Arijo, A.G and Xiao, C. (2006). Importance of ticks and their chemical and immunological control in livestock, *Journal of Zhejiang University Science B*, 7(11), 912–21.

Singh, H., Gupta, S.K., Vikram, K., Saxena, R., Srivastava. A and Nagpal, B.N. (2022). Sustainable control of malaria employing Gambusia fishes as biological control in Jalore and Barmer districts of Western Rajasthan. *Journal of Vector Borne Diseases*, 59, 91–97.

Mini Review

Probiotic Feeding: A Promising Strategy for Enhancing Ruminant Health and Productivity

Srishti Dhuria and Jasmine Kaur*

Department of Animal Nutrition
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: sachdeva_jasmine@rediffmail.com

Received: 17.05.2024 Accepted: 30.05.2024

Abstract

Farmers increasingly prioritize animal health, production and feed efficiency by incorporating growth promoters into animal feed. However, the use of antibiotics as both treatment and growth promoters has led to antibiotic-resistant microbes, posing risks to humans and the environment. In response, researchers are exploring probiotic feed additives as an alternative. Probiotics, administered in adequate amounts, confer health benefits by inhibiting pathogenic adhesion, enhancing barrier function, and modulating the immune system. Understanding probiotic functioning in ruminant health and productivity is crucial for tailoring probiotics to specific animal needs. Synergistic combinations of probiotic strains may yield greater improvements than single-strain probiotics.

Keywords: *Blood composition, Immunity, Methane mitigation, Milk yield, Probiotic*

Ruminants are an essential part of the global agricultural industry. Their unique digestive system, featuring a multi-chambered stomach called the rumen; allow them to efficiently convert plant matter into usable nutrients. A radical change in favor of sustainable and health conscious agricultural practices are occurring, especially in the area of animal nutrition. However, maintaining a healthy balance of microbes within the rumen is crucial for optimal digestion, growth, and overall health. This is where probiotic feeding comes in as a promising strategy for enhancing ruminant health and productivity.

Mode of Action of Probiotics

Probiotics acts through several modes of action (Fig. 1).

- *Inhibition of Pathogen Adhesion***:** Probiotics inhibit pathogenic adhesion in the gastrointestinal tract and suppress pathogenic growth.
- *Secretion of Defensins/Bacteriocins*: Probiotics can enhance the release of antimicrobial proteins, such as defensins and bacteriocins to help rid of infectious organisms.

- *Competitive Exclusion of Pathogenic Microorganisms*: Probiotics can compete with detrimental intestinal microorganisms for nutrients, sites of attachment, substrate and prohibit their colonization by binding with receptors in the intestinal epithelial cell or mucus layer and lead to the elimination of the pathogens.
- *Enhancement of Barrier Function*: Probiotics can enhance production of mucin glycoproteins by mucus-producing cells, resulting in a thick coating of mucus that reduces the permeability of cells to pathogens.
- *Reduction of Luminal pH*: Probiotics promote the synthesis of acetic acid, which is lethal to other pathogens and lowers the pH level of the lumen.
- *Modulation of Immune System*: Probiotics affect both innate and adaptive immunity by activating T and B cells via the mucosal dendritic cell.

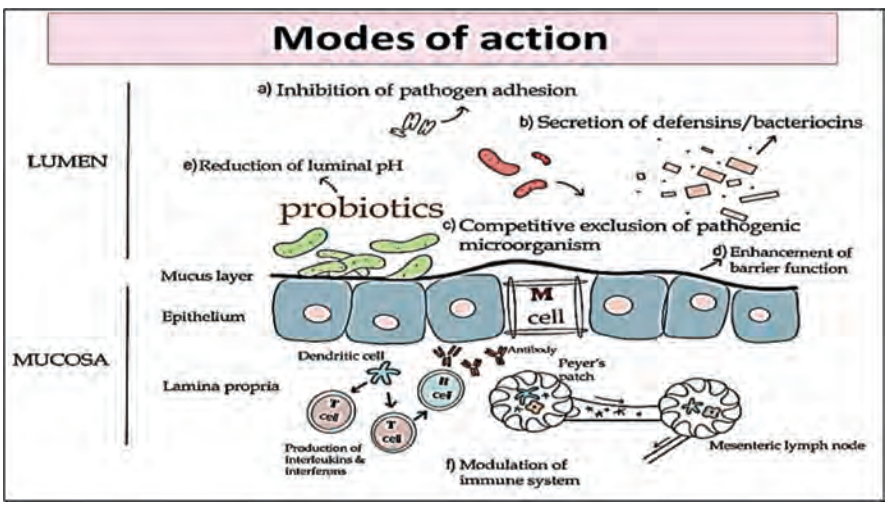


Fig. 1. Modes of action of probiotics (Anee et al., 2021)

Sources and Routes of Probiotic Administration in Ruminants

Probiotics can typically come from food sources like raw milk or fermented foods, as well as from other animal origin sources (Shewale et al., 2014). Neonatal animals' microflora has been shown to reflect the bacterial makeup of the fresh mother milk that they receive. Therefore, the milk of ruminants may serve as a natural microbiota depot and be predominantly utilized for the isolation of probiotics. According to the official list of Association of American Feed Control Officials (AAFCO), 47 micro-organisms used as probiotics in ruminant diets have been identified (Pendleton et al., 1998).

Among multiple routes of administering probiotics, the oral (Worku et al., 2016) and vaginal (Deng et al., 2016) methods are common. Deng et al. (2016) infused a combination of probiotics called lactic acid bacteria intravaginally into periparturient cows to modulate metabolic status and improved milk production and composition.

Effect on Nutrient Digestibility

Supplementing ruminant diets with probiotics has been shown to improve the digestion of fiber and other nutrients by collaborating with the ruminal microbiota. They may increase enzymatic activity in the digestive tract, which promotes nutrient and fibre digestibility. In a study carried out by Peng et al. (2020), beef cattle were supplemented with live yeast in their diets at 1 g and 2 g/ head /day (2× live cell/ g) for 70 days. Probiotic supplemented groups showed higher apparent nutrient digestibilities with significant increase of 10 and 12% in acid detergent fibre and neutral detergent fibre digestibility, respectively for the group supplemented at higher concentration. Wafa et al. (2020) supplemented the yeast culture containing inactivated strains of *Saccharomyces cerevisiae* containing 1x colony forming unit (CFU/g) in the diet of Friesian cows at pre and post-partum periods at the rate of 20 g and 40 g/cattle/day. They found increase in digestibility coefficients of nutrients as well as nutritive value of diet in treatment groups, with crude fibre digestibility being significantly higher in the yeast supplemented groups. However, the beneficial response to probiotic supplementation was not consistent and depends on factors such as microbial strain selection, combination of strains, dose, feeding frequency, diet, animal breed, physiological stage, and farm management. Addressing these gaps is required to enhance the understanding of probiotics' impact on nutrient utilization in ruminants.

Effect of Probiotics on Weight Gain

It has been documented that supplementing ruminants with particular single or multi strain probiotics can greatly improve their growth and performance, including daily body weight, total body weight gain and feed conversion efficiency. Studies suggested that the main modes of action exhibited by probiotics which aimed at promoting growth and performance include an increase and modulation of the intestinal microflora as well as enhancement of cellulose degradation that aid digestion and absorption of nutrients (Amin & Mao, 2021). Saleem et al. (2017) conducted a study on post-weaning lambs fed with the probiotic having combination of strains of *Pediococcus acidilactici* (1× CFU/ g) and *Pediococcus pentosaceus* (1.3× CFU/ g), at the rate of 0.5 g and 1 g/ lamb/ day. The probiotic supplementation led to improved weight gain and feed conversion ratio. Wafa et al. (2020) incorporated inactivated strains of *Saccharomyces cerevisiae* (1x CFU/ g) to the diets of primi-parous Friesian cows at post-partum period at the dose rates of 20 g and 40 g/ cattle/ day and found significant increase in live body weight and body condition scores. In another study, Cai et al. (2021) fed heat stressed crossbred goats with *Saccharomyces cerevisiae* (2.0 × CFU/ g) and *Clostridium butyricum* (1.0 × CFU/ g). They found increased intake of dry matter (DM), enhanced rumen fermentation activity through significant improvement in digestibility of dry matter, neutral detergent fiber, and acid detergent fiber, and therefore improved growth performance in the supplemented

groups. Furthermore, the detrimental impacts of heat stress were minimized. However, future studies should focus on understanding optimal probiotic strains, dosage, and long-term effects of probiotics on body weight gain to maximize the benefits.

Effect on Immunity

Several studies have documented immune-stimulatory effects by Probiotics by enhancing both adaptive and innate immune responses. As stated by El-Nagar et al. (2021), feeding of *Saccharomyces cerevisiae* (@ 20 g/animal/ day, 1x CFU) and *Lactobacillus acidophilus* (@ 20 g/ animal/ day, 1x CFU) to lactating buffaloes separately, resulted in noticeably higher IgA, IgG, and IgM concentrations in the colostrum of both treatment groups. Ojha et al. (2020) explored the effects of dietary supplementation of *Lactobacillus acidophilus* on antioxidant activity of Murrah buffalo calves and found substantial increase in the level of total antioxidants and activity of super oxide dismutase, thus, boosting the antioxidant capacity of the animals. While existing research provides insights into probiotic effects on immunity in ruminants, more studies are required to fully elucidate the intricate mechanisms and optimize probiotic use for enhanced immunity.

Effect on Milk Yield and Composition

Probiotic supplementation has a positive impact on milk production and composition in ruminants. Research studies have shown that cows fed inactive strain of *Saccharomyces cerevisiae* (1xCFU/ g) along with their basal diet exhibited significant increase in daily actual milk yield and 4% fat-corrected milk yield (Wafa et al., 2020). Similarly, in Sannan dairy goats, administering *Saccharomyces cerevisiae* (SC), *Enterococcus faecalis* and *Bacillus subtilis* (BS) resulted in higher milk yield. Further, feeding combination of probiotics resulted in increased milk fat and total solids, while specific probiotics (such as SC, BS, and combinations) led to higher protein percentages and lactose levels compared to the control group (Ma et al., 2020). These findings highlight the potential benefits of probiotics in enhancing milk production and quality. Further studies are needed to explore probiotics' effects on fatty acid profile of milk at different doses and viability levels in various species of the ruminants.

Effect on Blood Composition

The inclusion of probiotics in the diet of ruminants has shown positive impact on their blood composition. Lactating buffaloes were given dietary probiotics including *Lactobacillus* or yeast @20 g/head/day, by El-Nagar et al. (2021). They reported a substantial increase in blood metabolites viz., total protein, albumin, globulin, glucose and dramatically lower levels of creatinine in the *Lactobacillus* treated group as compared to the yeast supplemented group. Ojha et al. (2020) tested dietary supplementation of *Lactobacillus acidophilus* (CFU/ ml) as a fermented milk at 0 ml, 100 ml, 200 ml and 300 ml/calf/day, the plasma level of glucose was found notably higher in treatment groups

receiving 200 ml and 300 ml of fermented milk/ calf/ day as compared to the control group. The elevated glucose levels reflect the body's efficient utilization of carbohydrates, which are essential for providing energy to cells.

Effect on Reproductive Performance

Improvement in the reproductive performance of high yielders mostly depends on their ability to properly utilize energy and protein of feed, which is made possible by the use of probiotics in their diets. In a research study, lactating buffaloes were given dietary supplements of yeast (20 g/head/day) and *Lactobacillus* (20 g/head/day), and found a considerable increase in improved reproductive parameters, such as drop fetal membranes, cervical closure, postpartum first estrus interval, and calving to conception interval, in *Lactobacillus* supplemented group followed by yeast and the control group (El-Nagar et al., 2021).

Similarly, in a study by Wafa et al. (2020), primiparous cows were supplemented with yeast cultures at 20 g and 40 g/ head/ day (G2 and G3, respectively, with G1 as control group). The results showed that G3 had a 7.6-day earlier uterine involution compared to G1 group. Yeast treatment considerably reduced postpartum 1st estrus interval, days open, and progesterone level during estrus, service period length and number of services per conception. Furthermore, cows in yeast-treated groups conceived at a higher rate than control group and showed improved calf performance parameters, including birth and weaning weights, total and average daily weight gain. However, there is need to identify the most effective probiotic strains specifically for reproductive benefits, elucidating the precise molecular mechanisms by which probiotics influence reproductive processes, and assessing the long-term effects of probiotic supplementation on reproductive health which is critical for sustainable livestock production.

Effect on Methane Emission

Methane emissions from livestock sector remarkably account to the greenhouse gas emissions worldwide, hence, curbing their effects is needed to reach climate goals. Probiotics have shown effectiveness in modifying the gut flora, improving dry matter digestibility and lowering methane emissions. Asediya et al. (2024) investigated the influence of probiotics on *in vitro* methane production in Kankrej calves using consortium of the bacterial strains composed of *Lactobacillus lactis, Bacillus coagulans, Lacticaseibacillus rhamnosus, Lacticaseibacillus paracasei, Lactobacillus bifermentans, Lactobacillus acidophilus, Pediococcus acidilactici.* They noted 19.24% reduction in methane emissions across 2% probiotic concentrations, demonstrating the potential of microbial intervention as an effective technique for reducing methane emission in ruminants. Chen et al. (2020) studied the effect of different strains of *Propionibacterium* on the synthesis of volatile fatty acids and methane *in vitro* and found an increase in propionic

acid synthesis, which, therefore, led to a decrease in emission of methane upto 20%.

References

Amin, A. B., & Mao, S. (2021). Influence of yeast on rumen fermentation, growth performance, and quality of products in ruminants: A review. *Animal Nutrition*, 7, 31–41.

Anee, I. J., Alam, S., Begum, R. A., Shahjahan, R. M., & Khandaker, A. M. (2021). The role of probiotics on animal health and nutrition. *The Journal of Basic and Applied Zoology*, 82, 1-16.

Asediya, V.S., Sorathiya, K.K., Shekh, M.A., & Pandya, P.R. (2024). Exploring the efficacy of direct-fed microbials on in-vitro digestibility and methane emissions in Kankrej calves: Implications for ruminant health and environmental sustainability. *Biological Forum - An International Journal*, 16(3), 45-49.

Cai, L., Yu, J., Hartanto, R., & Qi, D. (2021). Dietary supplementation with Saccharomyces cerevisiae, Clostridium butyricum and their combination ameliorate rumen fermentation and growth performance of heat-stressed goats. *Animals*, 11(7), 2116.

Chen, J., Harstad, O. M., McAllister, T., Dörsch, P., & Holo, H. (2020). Propionic acid bacteria enhance ruminal feed degradation and reduce methane production in vitro. *Acta Agriculturae Scandinavica, Section A—Animal Science*, 69(3), 169-75.

Deng, Q., Odhiambo, J. F., Farooq, U., Lam, T., Dunn, S. M., & Ametaj, B. N. (2016). Intravaginal probiotics modulated metabolic status and improved milk production and composition of transition dairy cows. *Journal of Animal Science*, 94(2), 760–70

El-Nagar, H. A., El-Hais, A. M., & Mandouh, M. S. (2021). Influence of yeast and Lactobacillus products as feed supplements on blood parameters and reproductive performance of lactating Egyptian buffaloes. *Egyptian Journal of Animal Production*, 58(1), 1-8.

Ma, Z. Z., Cheng, Y. Y., Wang, S. Q., Ge, J. Z., Shi, H. P., & Kou, J. C. (2020). Positive effects of dietary supplementation of three probiotics on milk yield, milk composition and intestinal flora in Sannan dairy goats varied in kind of probiotics. *Journal of Animal Physiology and Animal Nutrition*, 104(1), 44-55.

Ojha, L., Kumar, S., Kewalramani, N., Sarkar, S., Singh, A. K., & Tyagi, A. K. (2020). Effect of dietary supplementation of Lactobacillus acidophilus on blood biochemical profile, antioxidant activity and plasma immunoglobulin level in

neonatal Murrah buffalo calves. *The Indian Journal of Animal Sciences*, 90(1), 48–54

Pendleton, B. (1998). The regulatory environment. *Direct-Fed Microbial, Enzyme and Forage Additive Compendium*, 4, 47–52.

Peng, Q. H., Cheng, L., Kang, K. A. N. G., Gang, T., Mohammad, A. M., Bai, X. U. E., Wang, L. Z., Zou, H. W., Gicheha, M. G., & Wang, Z. S. (2020). Effects of yeast and yeast cell wall polysaccharides supplementation on beef cattle growth performance, rumen microbial populations, and lipopolysaccharides production. *Journal of Integrative Agriculture*, 19(3), 810-19.

Shewale, R. N., Sawale, P. D., Khedkar, C. D., & Singh, A. (2014). Selection criteria for probiotics: A review. *International Journal of Probiotics & Prebiotics*, 9(1/2), 17.

Saleem, A. M., Zanouny, A. I., & Singer, A. M. (2017). Growth performance, nutrients digestibility, and blood metabolites of lambs fed diets supplemented with probiotics during pre-and post-weaning period. *Asian-Australasian Journal of Animal Sciences*, 30(4), 523.

Wafa, W. M., Farag, M. A., & El-Kishk, M. A. (2020). Productive and Reproductive Performances of Primi-parous Friesian Cows Treated with Yeast Culture. *Journal of Animal and Poultry Production*, 11(9), 331-37.

Worku, M., Adjei-Fremah, S., Ekwemalor, K., Asiamah, E., & Ismail, H. (2016). Growth and transcriptional profile analysis following oral probiotic supplementation in dairy cows. *Journal of Animal Science*, 94, 61-61.

Mini Review

Navigating Laminitis in Dairy Cows: Causes, Signs and Dietary Strategies

Jashandeep Singh, Nilakshi Prakash Patil and JS Hundal*

Department of Animal Nutrition
College of Veterinary Science (Ludhiana),
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: drjshundal@gmail.com

Received: 07.10.2024 Accepted: 05.11.2024

Abstract

Laminitis, a complicated inflammatory disease, has a major effect on dairy cows, causing discomfort, decreased output, and more management difficulties. Because of the combination of dietary variables and mechanical forces that contribute to its etiology, preventative and therapeutic efforts are essential. Early identification of clinical indications, such as altered gait and hoof appearance, is crucial for prompt intervention. Preventing laminitis mostly involves nutritional control, with a focus on balanced diets that minimize excessive energy consumption and include enough fiber. Frequent foot care and monitoring, along with ensuring the right balance of vitamins and minerals, are crucial for maintaining the health of cows. A comprehensive approach that incorporates controlled feeding, regular feeding schedules, and periodic health assessments is necessary to reduce the risk of laminitis and maximize dairy herd output. With an emphasis on enhancing herd health and lowering losses associated with lameness, this review aims to investigate the origins, clinical symptoms, and practical nutritional approaches for managing and preventing laminitis in dairy cows.

Keywords*: Buffers, Hoof health, Laminitis, Nutrition, Productivity, Rumen acidosis.*

Though its precise causes are still unknown, laminitis is an inflammatory disorder that affects the laminae of dairy cows' hooves, causing degeneration and pain, sometimes caused by a disrupted blood flow (Stokka et al., 2001). This condition can result in the rotation or sinking of the claw bone, bruising of the sole, and serious complications like sole ulcers and white line lesions, negatively impacting milk production and economics. Laminitis frequently occurs around calving and is influenced by factors such as nutrition, housing, and claw overloading. The incidence of acute laminitis is rare in cattle and often occurs when animals accidentally consume large amounts of grain, with incidence rates in dairy cattle ranging from 0.6% to 1.2%. Sub-acute laminitis is observed in young beef bulls on high-

Fig. 1 Hoof of subclinical laminitis cows (Zhang et al., 2020)

carbohydrate diets and in feeder calves. Crossbred cattle (8.92%) have been reported to suffer more likely than buffaloes (2.26%) to show lameness, including laminitis, according to a study done in the central Punjab region. Organized dairy farms had a larger incidence (9.31%) than unstructured (backyard) farms (1.99%) (Singh et al., 2016).

Causes of Laminitis

The etiology of laminitis is often linked to high carbohydrate intake. Excessive carbohydrates lead to increased lactic acid production in the rumen, lowering pH and causing an overgrowth of gram-positive bacteria like *Streptococcus bovis* and *Lactobacillus spp.* This rapid fermentation results in acidosis, which kills gram-negative bacteria and releases endotoxins (Greenough, 2012; Smith et al., 2021). When absorbed through the rumen wall, these toxins disrupt blood supply to the hoof's corium, leading to inflammation and poor hoof quality, ultimately causing lameness. Additionally, toxins from conditions like mastitis and metritis can contribute to laminitis (Lee et al., 2023). Nutritional deficiencies of zinc and biotin, essential for keratin synthesis and tissue repair, may increase risk. Mechanical factors, such as excessive walking on uneven surfaces, further exacerbate the risk of laminitis, particularly around calving or other illnesses like acetonemia. Other factors such as heat stress, transportation, hard flooring, overcrowding, genetic predisposition, overweight or underweight cattle also suffer from laminitis.

Signs of Laminitis

Laminitis presents in three forms: acute, sub-acute, and chronic (Kloosterman, 2007). Detection can be challenging unless one hoof is notably affected. While acute laminitis is rare in dairy cows, those on high-concentrate diets may experience sub-acute forms. Chronic laminitis results in a distinct ridged, slipper-like appearance and can lead to repeated episodes. In acute cases, affected cows exhibit reluctance to walk, with swollen and warm hind limbs and tenderness detectable through hoof testers (Vorster, 2015). They may walk slowly and deliberately, with elevated body temperature, respiration rate, and oedema around the coronary band. Freshly calved cows if exposed to concentrated diets too soon, exhibits skin inflammation leading to subacute ruminal acidosis, which could also increase their risk of developing laminitis. Cows with laminitis often prefer to lie down, hesitating to rise and frequently kneeling. Subclinical laminitis often goes unnoticed, but affected cows may show unusual gaits, as well as signs such as sole haemorrhages and irregularities in the hoof wall structure (Adams et al., 2022). If over 10% of a herd is affected, subclinical laminitis should be suspected. Chronic laminitis is

Fig. 2. Hoof of chronic laminitis cows (Zhang et al., 2020)

characterized by bent, flat, and square toes, resulting from repeated episodes that alter gait. The normal dorsal angles of the hind and front claws decrease due to forward hoof wall growth, leading to a flattened appearance (Vorster, 2015).

Nutritional Care and Preventive Strategies

When acute laminitis arises, it is crucial to identify the underlying cause (diet, other inflammatory conditions in the body such as mastitis, metritis, etc) and emergent veterinary consultation may be needed to ensure a balanced diet. Acute laminitis requires emergency treatment with anti-inflammatory agents to relieve pain, improve blood flow to the hoof, and addressing the root cause (Vorster, 2015). Non-steroidal anti-inflammatory drugs, along with antihistamines and prostaglandins, may be helpful (Stokka et al., 2001). It is commonly advised to use walk-through footbaths that contain 3 to 5 percent formalin, 5–10% copper sulphate, 10% zinc sulphate, or 1–10 grams per liter of antibiotics (oxytetracycline, tetracycline, lincomycin, or lincomycin/spectinomycin) (Shearer and Sagues, 2005). Addressing subclinical laminitis is important, even though diagnosis is difficult without detecting the causative factor, as lameness may not appear until weeks or months after an episode. Subclinical laminitis can be managed to prevent associated issues like sole bruising and abscesses by providing adequate fiber content in the diet, while high-starch feeds should be avoided (Parker et al., 2024). Chronic laminitis often leads to the lengthening of the claw and flattening of the sole, known as "slipper foot."

Good management and nutritional practices can control the incidence of lameness and laminitis. Precautions during feeding include using feed with enough functional fiber to promote rumination and gradually transitioning to lactation diets. By neutralizing the excess acid, buffers like calcium carbonate or sodium bicarbonate keep the pH from falling too low. Buffers assist lower the danger of acidity and, in turn, laminitis by maintaining a more stable rumen environment (Kumar et al., 2024). Alfalfa hay, silage, and alfalfa hay are examples of fibre rich carbohydrates that offer beneficial fiber that supports rumen health (Bergsten, 2003). Compared to corn and wheat, oats and barley ferment more slowly, making them safer choices. Other good energy sources that are less prone to acidosis are soy hulls and beet pulp. Cows are at risk for lameness, metabolic diseases if they are fed a diet that contains less than 27% NDF and 21% ADF of total dry matter intake (Stokka et al., 2011). Feeding mature hay such as alfalfa hay and grass hay is beneficial as it is a good fiber source, while poorly conserved silage can contribute to laminitis (Kloosterman, 2007). The NRC (2001) recommends dietary supplementation of 20 mg biotin and 200-300 mg zinc per cow per day in cases of laminitis. Trimming hooves approximately two months before calving can help prevent or minimize lameness post-calving, with hoof trimming advised 2-3 times per year. Laminitis is highly heritable. In cows, feeding of grains (less than 40-50%), well-balanced diet fortified with minerals and vitamins, etc aid the hoof health and general well-being.

Current studies on laminitis nutritional therapy in cows highlight diet's role in reducing hoof health issues. Antioxidants, omega-3 fatty acids, and trace minerals like copper and zinc have all been studied for their ability to strengthen hooves and lessen inflammation. However, research gaps exist on whole-diet formulation efficacy, interactions with management techniques, and specific variables like breed, stage of production, and geographic variations affecting response to nutritional therapies. Long-term research evaluating productivity and hoof health are also required in order to develop sensible, evidence-based farming practices.

In conclusion, controlling laminitis in cows requires a comprehensive strategy focusing on nutrient composition, balanced feeding methods, and close dietary monitoring. This includes providing fiber, limiting energy, and ensuring balanced vitamins and minerals. Regular feeding schedules and regular health evaluations can boost productivity.

References

Adams, L., Johnson, M., & Roberts, T. (2022). Signs of subclinical laminitis in dairy cattle. *Journal of Dairy Science*, 105(4), 1234-41.

Bergsten, C. (2003). Causes, risk factors, and prevention of laminitis and related claw lesions. *Acta Veterinaria Scandinavica*, 44(Suppl 1), S157.

Greenough, P. R. (2012). Laminitis in cattle. In *The Merck Veterinary Manual* (10th ed., pp. 982-985). Merck & Co., Inc.

Kloosterman, P. (2007). Laminitis: Prevention, diagnosis, and treatment. *WCDS Advances in Dairy Technology*, 19, 157-66.

Kumar, B. B., Tariq, H., Mohanta, R. K., Yaqoob, M. U., Nampoothiri, V. M., Mahesh, M. S., Kumar, D., Kumar, B., & Datt, C. (2024). Rumen Buffers to Harness Nutrition, Health and Productivity of Ruminants. In *Feed Additives and Supplements for Ruminants* (pp. 495-518). Singapore: Springer Nature Singapore.

Lee, A., Martinez, J., & Thompson, L. (2023). Nutritional deficiencies and laminitis. *Journal of Animal Science*, 101(1), 45-53. https://doi.org/10.1093/jas/skacXXXX

NRC. (2001). *Nutrient Requirements of Dairy Cattle*, 7th ed. National Academy Press, Washington, DC.

Parker, T., Smith, A., & Johnson, B. (2024). Dietary strategies for preventing laminitis. *Animal Nutrition*, 10(1), 17-24.

Shearer, J. K., & Sagues, A. G. (2005). Footbaths for the management of lameness disorders in cattle. The North American Veterinary Conference - 2005 proceedings, 25-27.

Singh, S., Dua, K., Singh, S. S., & Dhand, N. (2016). Prevalence of common claw lesions of lameness in cattle and buffaloes of central Punjab region of India. *The Indian Journal of Animal Sciences,* 86 (10), 1103-10.

Smith, J., Brown, R., & Garcia, M. (2021). Rumen acidosis and laminitis risk. *Veterinary Journal*, 288, 15-21.

Stokka, G. L., Lechtenberg, K., Edwards, T., & Dunham, J. R. (2001). Lameness in feedlot cattle. *Veterinary Clinics of North America: Food Animal Practice*, 17, 1-11.

Stokka, G. L., Smith, J. F., Dunham, J. R., & Van Anne, T. (2011). Lameness in dairy cattle. Dairy Day 1996, 46-50.

Vorster J.H. (2015). Bovine laminitis. Available online: https://vet360.vetlink.co.za/bovine-laminitis/

Zhang, X., Ding, J., Li, Y., Song, Q., Li, S., Hayat, M. A., Zhang, J., & Wang, H. (2020). The changes of inflammatory mediators and vasoactive substances in dairy cows' plasma with pasture-associated laminitis. *BMC Veterinary Research*, 16, 1-10.

Mini Review

Unconventional Feeds in Poultry Ration to Combat Antimicrobial Resistance

Neeti Lakhani*, Shwetambri Jamwal[1] and Gurslamat Singh

Department of Animal Nutrition
[1]Department of Livestock Farm Complex
College of Veterinary Science (Rampura Phul)
Guru Angad Dev Veterinary and Animal Sciences, Punjab-141004, India
*Corresponding author email: lakhani10aishi@gmail.com

Received: 13.05.2024 Accepted: 04.06.2024

Abstract

Non-conventional feeds, rich in secondary metabolites like essential oils, saponins, and tannins, have emerged as promising additives in poultry diets. These compounds positively impact the gastrointestinal ecosystem by inhibiting the growth of pathogenic microorganisms and improving the health of the digestive system. The active ingredients present in these nonconventional feeds have potential role in lowering dependency on the existing antimicrobial substances and are considered potent antimicrobials to fight against antimicrobial resistance.

Keywords: *Additives, Antimicrobial resistance, Avian, GUT immunity, Productivity, Unconventional*

Antimicrobial resistance continues to pose an escalating threat to the poultry health, diminishing the ability to effectively treat bacterial infections and heightening the risks of morbidity and mortality. Recently, certain non-conventional feed resources have emerged as promising, non-toxic additives that can enhance the balance of beneficial bacteria in the poultry gut. These unconventional feeds have rich content of secondary metabolites like essential oils, saponins, and tannins (Lakhani et al., 2019) and play a crucial role in modulating the gut environment and influencing the morphology of the intestine in poultry by neutralizing free radicals in the intestine which are responsible for increased mitosis of intestinal cells and the increased depth of the intestinal crypts (Abd El-Hack et al., 2022). They slow down the stress, improve the length and width of villi, inhibit inflammation and reduce intestinal permeability. This article explains the antimicrobial role of certain unconventional feeds used in poultry diet.

Common Unconventional Feeds

Ginger: Ginger is a medicinal plant used as a natural antibiotic alternative with various pharmacological effects on the animal body, including anti-inflammatory, gastrointestinal modulation, and antimicrobial properties. It contains compounds such as gingerol, gingerdiol, and ginerdione, which enhance the activation of digestive enzymes, leading

to improved feed utilization (Abou-Kassem et al., 2022). As a nutraceutical, ginger suppresses harmful organisms by promoting mucus secretion from intestinal epithelial tissues, reducing pathogenic adhesion and enhancing gut immunity. Its active derivatives stimulate digestion and nutrient absorption through the intestinal villi, improving nutrient digestibility in poultry. The rise in egg laying % may be due to antioxidant, antimicrobial and activities like increased blood circulation and secretion of digestive enzymes with use of ginger in diet. Additionally, its tannin and flavonoid content provide anti-inflammatory and wound-healing properties. The dose for ginger powder is @ 0.6% of diet and Ginger oil is @ 0.4% of diet.

Marigold: Marigold a natural antioxidant has yellow and orange flowers containing vitamin A and beta carotene act as antioxidants. Carotenoids are major pigments in marigold flowers, and the differing carotene (orange pigments) and xanthophyll (yellow pigments) contents in different genotypes largely contribute to the diversity of their flower colors. Marigold petals and flowers have antioxidant effects comparable to vitamin E supplementation. The active ingredients i.e. alkalonoids, terpenes, flavonoids inhibit the growth of pathogenic microorganisms and enhance the health of digestive system reducing the exposure of birds to microbiological toxins. Moreover, supplementation of marigold extract at 200mg/kg feed enhanced the antibody titre against Newcastle disease indicating the immunomodulatory potential of carotenoids (Rajput et al., 2012). Dose of Marigold leaf extract is @5% in diet.

Aloe Vera: Aloe vera possesses anti-inflammatory, immunomodulatory and antioxidant properties. It exhibits its antibacterial property because it has properties similar to prebiotics increasing the lactobacillus population. The active metabolites include Arthroquinones, Acemannan and Polysaccharides. Alteration in intestinal microflora with an increase in Lactobacillus count and Bifidobacteria count as well as a reduction in E. coli count was noticed when acemannan, polysaccharide and Aloe vera gel were added to broiler feed. The polysaccharide "acemannan" present in aloe vera gel is responsible for alteration in intestinal microflora as the polysaccharide imparts properties similar to that of the prebiotics (Guo et al., 2003). Moreover, the presence of fumaric acid content attributes antibacterial property in aloe vera. Dose of Aloe vera is @ 1.5 - 2.5% of diet.

Cabbage: Cabbage rich source of vitamin C and fibre can enhance overall vitality and digestive health among poultry birds. The active metabolites include sulforaphane and flavonoids. The active ingredients and various sulphur containing compounds in cabbage makes it a good alternative in poultry ration imparting antibacterial activity against gram negative bacteria. High fructose content in cabbage causes a reduction in clostridium species in the gut and promotes growth of lactobacilli species (Mustafa and Baurhoo, 2018). Dose is 3 to 6% in diet.

Moringa oleifera: The effect as coccidiostat, antibiotic growth promoter and high biological value makes the Moringa leaves as a potent feed additive to be used in poultry ration. The antimicrobial activities of the *Moringa oleifera* leaves may be due to the presence of lipophilic compounds and carboxylic acid and chitinases metabolites (Abd El-Hack et al., 2022). Saponins present in moringa modify cell membrane structure and cellular integrity. Dose of *Moringa* leaves is 4-6% of diet.

The lack of extensive research and need for standard protocols to evaluate the efficacy and safety of these unconventional feeds limits its use in poultry industry. Knowledge on nutritional profiling and economic viability also needs to be assessed. Addressing these gaps require a multidisciplinary approach involving animal nutritionists, veterinarians and microbiologists.

References

Abd El-Hack, M. E., Alqhtani, A. H., Swelum, A. A., El-Saadony, M. T., Salem, H. M., Babalghith, A. O., Taha, A. E., Ahmed, O., Abdo, M., & El-Tarabily, K. A. (2022). Pharmacological, nutritional and antimicrobial uses of Moringa oleifera Lam leaves in poultry nutrition: an updated knowledge. *Poultry Science*, 101(9), 102031.

Guo, F., Williams, B., Kwakkel, R., & Verstegen, M. (2003). In vitro fermentation characteristics of two mushroom species, an herb and their polysaccharide fractions, using chicken cecal contents as inoculum. *Poultry Science*, 82, 1608-15.

Lakhani, N., Kamra, D. N and Lakhani, P. (2019). Immune status and haemato-biochemical profile of buffalo calves supplemented with phytogenic feed additives rich in tannins, saponins and essential oils. *Tropical Animal Health Production.* 51, 565–73.

Mustafa, A. F and Baurhoo, B. (2018). Evaluation of dried vegetable residues for poultry: III Effects of feeding cabbage leaf residues on laying performance, egg quality, and apparent total tract digestibility. *Journal of Applied Poultry Research,* 27(2). 145-51.

Rajput, N., Naeem, M., Ali, S., Rui, Y., & Tian, W. (2012). Effect of dietary supplementation of marigold pigment on immunity, skin and meat color, and growth performance of broiler chickens. *Brazilian Journal of Poultry Science*, 14, 291-95.

Short Communication

Importance of Cow Comfort and Methods for its Measurement

Shwetambri Jamwal, Neeti Lakhani* and Yashwant Singh

College of Veterinary Science (Rampura Phul)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: lakhani10aishi@gmail.com

Received: 19.09.2024 Accepted: 08.11.2024

Abstract

In modern dairy farming, ensuring the comfort and well-being of cows has become increasingly crucial, not only for ethical considerations but also for maximizing productivity. Cow comfort encompasses various elements such as housing conditions, ventilation, cleanliness, and social interactions. Contented cows yield more milk, experience better reproductive success, and enjoy overall improved health. To enhance cow comfort, it's essential to grasp the unique dynamics of each herd and consider environmental factors. This involves creating comfortable, well-ventilated housing, ensuring appropriate flooring, and optimizing feeding and watering systems. By prioritizing cow comfort and employing effective measurement techniques, dairy farmers can not only boost production efficiency and profitability but also adhere to ethical and sustainable farming principles. This approach acknowledges the significance of animal welfare alongside economic viability, fostering a more harmonious and responsible relationship between farmers and their livestock.

Keywords*: Comfort cow, Environmental, Sustainable, Ventilated*

The concept of a cow's comfort zone refers to a temperature range where their physiological responses remain unaffected. Essentially, cow comfort is an emotional state that reflects how the animal feels, encompassing both psychological and physiological needs (Marino and Allen, 2017). Achieving this state means the cow is at peace with its environment. Ideally, cows should only be standing for essential activities such as milking, eating, and drinking, while the rest of their time should be remainlying down. There is no necessity for them to stand idly; moreover, standing for prolonged periods can cause many musculoskeletal ailments. The article aims to highlight the significance of providing a comfortable environment for dairy cows, as it directly impacts their health, welfare, and productivity. It focuses on understanding how cow comfort affects factors such as milk production, behavior, and overall well-being.

Ensuring cow comfort involves six key aspects in their housing: providing clean and palatable water with access for at least 21 hours a day, proper lighting with a minimum of six hours of darkness, fresh and clean air, a dry and comfortable resting place for at least 12 hours a day, adequate space for walking to feed and water troughs without fear, and well-formulated, palatable feed available for at least 21 hours a day.

Factors Influencing Cow Comfort (Tucker et al., 2009)

Cow comfort can be categorized into physical, social, and nutritional factors, as detailed below:

Physical Factors

i. Ensure natural ventilation and access to clean air- The number of ventilators needed in a cow shed depends on several factors, including the size of shed, number of cows, local climate, and type of ventilation system used. A general guideline might suggest a minimum of 500-1,000 cubic feet per minute (CFM) per cow to maintain comfort, though exact numbers vary based on above conditions

ii. Provide cooling through fans and sprinklers.

iii. Allow cows to rest in clean, dry areas offering soft bedding or kaccha floor.

iv. Grant access to outdoor yards at night for cooling and estrus detection- Design barns with gates or passageways that can be easily opened to grant night time access to the outdoor areas. Ensure that outdoor yards are securely fenced to prevent cows from wandering off or being exposed to potential predators.

v. Maintain clean alleyways.

vi. Provide comfortable, non-slippery surfaces for walking so that cows can stand undisturbed.

vii. Allow cows to rest for 12 to 14 hours per day. This can be achieved by providing comfortable lying surface, ensuring adequate stall availability and minimizing stress.

Social Factors

i. Allow cows to use stalls to escape from dominant individuals. Ensure there are enough stalls for every cow, ideally a 1:1 cow-to-stall ratio or better. Other strategies include designing stall having proper entry and exit, and grouping cows based on age, size or behaviour.

ii. Provide sufficient space for cows to exhibit signs of estrus. An area of at least 100 to 150 square feet per cow is often recommended, but requirements can vary based on herd size and housing type.

iii. Ensure good handling by staff to reduce flight zones, or the personal space around cows where they feel the need to move away from people. Good training and education of staff as well as use of gentle handling techniques should be exercised.

Nutritional Factors

i. Water is essential for cows, as it supports numerous bodily functions, including digestion, milk production, and temperature regulation. On an average, a lactating

cow may drink 30-50 gallons (110-190 liters) of water per day, depending on body size, milk yield, feed type, and environmental conditions

ii. Forages promote cow's natural chewing and ruminating behavior. Cows typically spend about 8-10 hours per day eating and chewing cud, which helps produce saliva and maintain optimal rumen pH.

iii. Offer a nutritionally balanced, formulated milking ration. Properly formulated rations reduce the risk of metabolic disorders (e.g., ketosis, milk fever), enhancing the overall health and comfort of the cow.

Assessment of Cow Comfort

i. The air should be free of stale smells or strong ammonia odours. Observe cows for signs of off feeding or nasal discharge.

ii. Inspect the shed for cobwebs; their presence indicates lack of continuous airflow.

iii. Look for signs of condensation and moisture damage, such as rusty nails, stalls, and dry rot in walls and roof trusses.

iv. The barn temperature should be monitored to avoid stressful conditions on the health of cow.

v. Run your fingers through the cows' coats; they should feel dry specially during humid season.

vi. Check cows for dirty udders, tails, switches, and hindquarters, as these can indicate dirty stalls or improper stall use.

vii. Look for signs of mastitis like swelling of udder, sore feet, rubbed necks, and swollen or rubbed hocks, which question improper comfort issues. If cows walk slowly or timidly with their rear feet spread wide, it could signal poor traction or laminitis.

Indexes for Measuring Cow Comfort (Cook et al., 2010)

A common approach to assess cow comfort involves walking through the barn and counting the following:

a) The number of cows lying down in the stalls.

b) The number of cows standing, either fully inside the stall or with only their front feet in the stall.

c) The number of cows feeding.

d) The total number of cows in the pen.

These counts can be used to calculate indexes such as the Cow Comfort Index (CCI), the Stall Use Index (SUI), and the Stall Standing Index (SSI) which are explained as below.

1. **Cow Comfort Index (CCI)**: The CCI is the proportion of cows in stalls that are lying down. It is calculated by dividing the number of cows lying down by the total number of cows in a stall, whether they are standing or lying. A CCI of 0% means all cows are standing, while a CCI of 100% means all cows are lying down. The recommended target for CCI is greater than 0.85. This measurement should ideally be taken after midnight, particularly around 3 A.M.
2. **Stall Use Index (SUI)**: The SUI measures the proportion of cows not eating that are lying down. It is calculated by dividing the number of cows lying down by the total number of cows in the pen that are not feeding. If every cow in the pen is either lying down in a stall or feeding, the SUI would be 100%. The recommended target for SUI is greater than 0.75, typically measured one hour after the cows return from milking.
3. **Stall Standing Index (SSI)** The SSI is the proportion of cows in stalls that are standing, calculated as 1 minus the CCI (SSI = 1 - CCI). This index measures the proportion of cows standing with all four feet on the stall platform or perching with their front two feet in the stall and rear two feet in the alley. SSI is usually collected one to two hours before morning or afternoon milking and is a predictor of standing behaviour.

Gaps in Knowledge to Ensure Cow Comfort

- **Limited Studies on Cow Comfort and Welfare**: In comparison to global research, there are fewer studies focusing on cow comfort.
- **Geographical Disparities**: Focus on specific regions, neglecting the unique challenges faced by other regions with different climates, agricultural practices, and socioeconomic conditions.
- **Lack of Long-Term Studies**: There may be a lack of longitudinal studies that assess the long-term impact of interventions or management changes.
- **Adoption of Technology**: Limited research on the integration of modern technologies (like precision dairy farming tools) and their impact on dairy systems.

References

Cook, N. B., Bennett, T. B., & Nordlund, K. V. (2004). Using indices of cow comfort to predict stall use and lameness. In *Proceedings of 13th International Ruminant Lameness Symposium, Maribor, Slovenia*.

Marino, L., & Allen, K. (2017). The psychology of cows. *Animal Behavior and Cognition*, 4(4), 474-98.

Tucker, C.B., Weary, D.M., & Beauchemin, K.A.(2009). Cow comfort in tie-stalls: Increased depth of shavings or straw bedding increases lying time. *Journal of Dairy Science*, 92 (6), 2684-90.

Short Communication

Macroscopic Anatomy of Oropharyngeal Region in Fowl (*Gallus gallus domesticus*)

Dipanwita Ghosh, Kritima Kapoor*, Opinder Singh and Anuradha Gupta

Department of Veterinary Anatomy
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: kritimakapoor89@gmail.com

Received: 12.11.2024 Accepted: 22.11.2024

Abstract

In domestic fowl, oropharynx is anatomically different from that of mammals; therefore, an extensive understanding of anatomy of avian oropharyngeal cavity is essential to appreciate its morphological changes. This study was conducted to explicate the macroscopic anatomy of oropharyngeal region in fowl. The samples were collected from head region of six adult male domestic fowls, fixed in 10% NBF and dissected along oral commissure to expose the oropharyngeal region. Macroscopic anatomy of various structures present in the roof of oropharyngeal cavity was observed and measurements were recorded. It was observed that a cartilaginous hard palate formed the roof of oropharyngeal cavity with a median swelling surrounded by two lateral palatine ridges, and well-developed caudally directed papilla at posterior part of infundibular cleft. This study concluded that the presence of papillae played a significant role in assisting swallowing by facilitating movement of food in only one direction.

Keywords: *Fowl, macroscopic anatomy, Oropharynx, Papillae*

The feeding mechanism in fowl varies in structure and function because the basic structures of this apparatus are modified expansively and reflected by the attainment of survival in a wide range of habitats. After prehension of the feed, it reaches the oral cavity of fowl which is guarded by the upper and lower beaks. The oropharynx in birds is a common cavity formed together by pharynx and the oral cavity, connected to nasal cavity which is structurally different from that in mammals. The connecting orifice of the pharynx and the nasal cavity lies in the same plane as hard palate whereas in mammals they are perpendicular to each other. The upper beak is a pointed, narrow structure that covers the fused pre-maxillary bones and extends to the maxillary bones caudolaterally (McLelland, 1990). A vivid knowledge of the anatomy of avian oropharyngeal cavity is important in considering the variations that may affect feeding pattern, breathing, vocalization and to comprehend the morphological changes in a bird oral cavity. The oropharyngeal cavity has lingual apparatus constituting of many the salivary glands, muscles, bony skeleton and cartilage influencing one another mechanically (Homberger & Meyers, 1989). The present study aims at elucidating the roof of oropharynx, a critical

region in swallowing and a vital element of both avian respiratory and digestive systems.

Materials and Methods

The samples of six adult male domestic fowls (n=6) from head region were procured for the present study from the university post mortem hall. These were thoroughly washed in running tap water and fixed in 10% Neutral Buffered Formalin (NBF). The heads were isolated and kept intact and get fixed for 48 hours; the excess formalin was removed by washing. After fixation, the dissection was performed and incision was made along the commissure of mouth using a scalpel to expose the oropharyngeal region. Further a mid-sagittal section was made on the roof of the oropharynx to study each side distinctly. The gross anatomy of various structures present in the roof of oropharyngeal cavity was observed under magnascope magnifier (5X optical lens) and the anatomical details were recorded. The biometrical parameters were recorded with the help of a non-stretchable thread and meter scale (Fig. 1). A statistical analysis was carried on the data before interpreting the results.

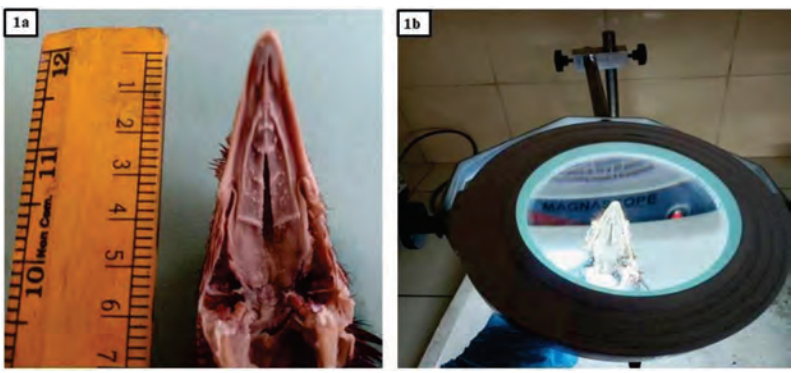


Fig 1: Photograph representing **(a)** gross parameters recorded from oropharynx of fowl with the help of meter scale and a non-stretchable thread **(b)** dissected head observed under magnascope (5X magnified) for observation of oropharynx and counting of papillae.

Results and Discussions

Upon gross observation, no distinct line of demarcation between the oral and pharyngeal cavities was observed as the roof was devoid of soft palate. Palatine cleft was the communication between the oral and the nasal cavities in birds (McLelland, 1979). A cartilaginous hard palate formed the roof of oropharyngeal cavity and well-developed caudally directed papillae were found on its mucous membrane (Fig. 2). Similar caudally directed papillae were also reported in African pied crows (Igwebuike & Eze, 2010) and turkeys (Gupta et al., 2018). These papillae were found absent in the mucosal surface of hard palate in Rhea, Ostrich and Muscovy Ducks (Gussekloo & Bout, 2005; Tivane et al., 2011; Igwebuike et al., 2013). Instead, there was presence of numerous orderly arranged rows of notches called lamellae on the lateral borders of the

hard palate in Ducks (Igwebuike et al., 2013). The oropharyngeal region was observed to be pinkish white in colour which gradually faded towards the caudal end of the cavity (Fig. 2). However, some black patches were observed instead in oropharynx of Ducks by Igwebuike et al. (2013). The infundibular slit continues with choanal slit anteriorly and laryngeal opening posteriorly (Fig. 2). It was similarly observed in fowls of this study as a median longitudinal fissure on middle of the roof of the oropharyngeal area with common opening of two eustachian tubes (King & McLelland, 1984). The choanal slit connected nasal passages to mouth and throat which thereby facilitated air to pass directly into the trachea. The length of choanal slit length was recorded as 3.1 ± 0.01 cm in fowl. However, in 60 days old Duck, the slit was measured as 0.804 cm (Madkour, 2011) and 1.97 cm in Ostrich (Tadjalli et al., 2008). The choanal slit was observed as a thin pointed arrow head shaped depression in fowls whereas in Ducks, an oval choanal slit in caudal 1/3rd of the oropharyngeal roof (Igwebuike et al., 2013). However, in Emu two slits separated by a wide raised ridge with a groove running down its midline and continuing to the infundibular cleft have been recorded (Crole & Soley, 2010). There was presence of posteriorly directed transverse row of cornified papillae as two rostral rows, two middle rows and a posterior row on either side of the infundibular cleft (Fig. 3). The margins of the choanal slit had posteriorly directed papillae and similar have been reported in Crows (Igwebuike & Eze, 2010) whereas it disappeared in Hoopoe and Common Moorhen (Mahmoud et al., 2018). The average number of papillae in

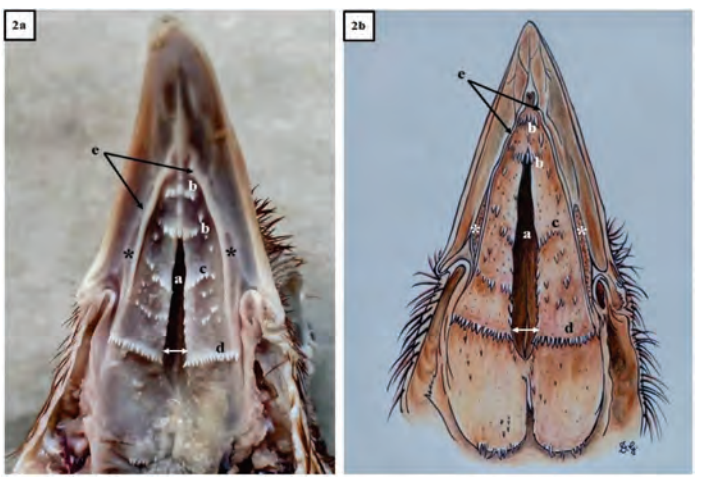


Fig 2: (a) Picture of upper jaw of fowl showing oropharynx with presence of infundibular slit **a** broadening caudally into choanal slit (double headed arrow), rostral rows of papillae **b**, middle rows of papillae **c**, caudal row of papillae **d**, lateral palatine ridge **e** (arrows) and lateral palatine groove (*) **(b)** Diagrammatic representation of oropharynx in fowl depicting similar labelled gross macroscopic features (hand-painted diagram of oropharaynx sample; drawn by first author).

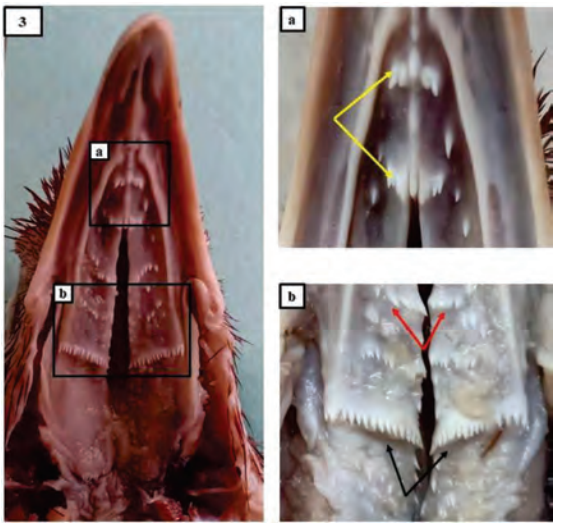


Fig 3: Oropharynx of fowl depicting **(a)** two rostral rows of cornified papillae (yellow arrows) **(b**) 2-3 middle rows (red arrows) and a posterior row of caudally directed papillae (black arrows).

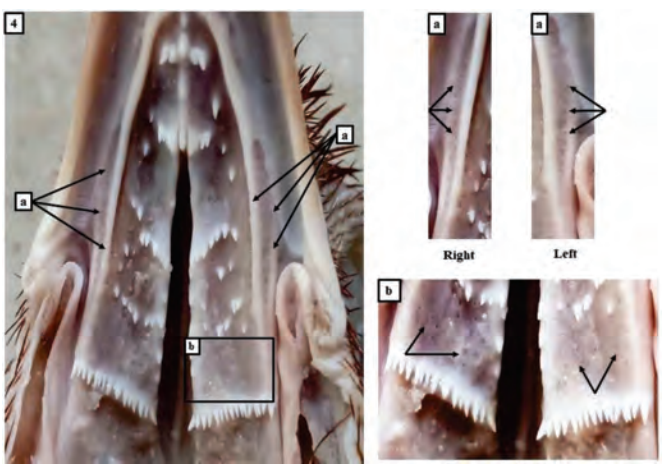


Fig 4: Oropharynx of fowl showing lateral palatine grooves (arrows) **(a)** as right and left palatine grooves with openings of palatine salivary glands (arrows) **(b)** magnified image of caudal part of oropharynx showing openings of maxillary salivary glands observed throughout its surface (arrows).

rostral, middle and posterior rows was observed to be 17, 28 and 34, respectively (Fig. 3). These papillae play a significant role to assist swallowing by ensuring that the bolus of food is moved in only one direction, towards the oesophagus, and thereby preventing regurgitation (McLelland, 1979). These papillae might serve principally as mechanical obstacles to involuntary return of food that has passed over them as stated by Gupta et al. (2014). The hard palate of fowl had a median swelling and two lateral palatine ridges.

A palatine ridge was observed on either side of this infundibular slit that enclosed lateral palatine grooves on both sides (Fig. 2 & 4). The palate and pharynx were observed with vents for glandular structures like salivary glands arranged asymmetrically on either side of the choanal slit. This groove and caudal part of oropharynx were occupied by probable openings of maxillary salivary glands arranged close to the midline in the rostral and caudal part of the palate (Fig. 4). Similar openings of maxillary gland were also reported in adult chicken by McLelland (1990). Therefore, the structural variation of oropharynx in birds affects their nutrition, food intake, and ingestion (Jayachitra et al., 2015).

Conclusively, this study established the presence of papillae in three rows which appear to play a significant role to assist swallowing by facilitating movement of food in only one direction i.e., towards the oesophagus and acted as mechanical obstacles to prevent regurgitation. Moreover, the exceptional presence of wide choanal slit, which is in contrast to mammals; thereby enabled air to pass directly into the trachea and thus assisted in breathing.

References

Crole, M.R. & Soley, J.T. (2010). Gross Morphology of the Intra-Oral *rhamphotheca*, Oropharynx and Proximal Oesophagus of the Emu (*Dromaius novaehollandiae*). *Anatomia Histologia Embryologia*, 39, 207-18. https://doi.org/10.1111/j.1439-0264.2010.00998.x

Gupta, S., Pathak, A. & Farooqui, M.M. (2014). Anatomy of Oropharyngeal Cavity of Fowl (*Gallus domesticus*). *Indian Journal of Veterinary Anatomy*, 27(1), 12-14.

Gupta, V., Pathak, A., Farooqui, M.M. & Prakash A. (2018). Anatomy of Oro-pharyngeal cavity of turkey (*Meleagris gallopavo*). *Haryana Veterinrian*, 57(2), 178-82.

Gussekloo, S.W.S. & Bout, G. R. (2005). The kinetics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *Journal of Experimental Biology*, 208, 3395-3405.

Homberger, D. G. & Meyers, R. A. (1989). Morphology of the lingual apparatus of the domestic chicken, Gallus gallus, with special attention to the structure of the fasciae. *The American Journal of Anatomy*, 186(3), 217–57. https://doi.org/10.1002/aja.1001860302

Igwebuike, U. & Ukamaka, E (2010). Anatomy of the oropharynx and tongue of the African pied crow Anatomy of the oropharynx and tongue of the African pied crow (Corvus albus Corvus albus). *Veterinarski Arhives*, 80, 523-31.

Igwebuike, U. & Tochukwu, A (2013). The morphology of the oropharynx and tongue of the muscovy duck (Cairina moschata). *Veterinarski Arhives*, 83, 685-93.

Jayachitra, S., Balasundaram, K., Iniyah, K., Sivagnanam, S. & Tamilselvan, S. (2015). Morphology of oropharyngeal cavity in guinea fowl (*Numida meleagris*). *International Journal of Advanced Multidisciplinary Research,* 99-102.

King, A. S. & Mclelland J. (1984). Birds. Their Structure and Function, 2nd edn. London: Bailliere Tindall.

Madkour, F.A.M. (2011). Some developmental studies on the roof of the oropharynx of the duck. M.V.Sc. thesis submitted to South Valley University, Egypt.

Mahmoud, F.A., Gadel-Rab, A.G. & Shawki, N.A. (2018). Functional morphological study of the choana in different bird species. *The Journal of Basic and Applied Zoology,* 79, 11. https://doi.org/10.1186/s41936-018-0026-6

McLelland, J. (1979). *Digestive System*; *In Form and Function in Birds*. (Eds.) A.S. King & J. McLelland. Academic Press, London.

McLelland, J. (1990). *A Colour Atlas of Avian Anatomy*. Wolfe Publishing Ltd., Aylesbury, England.

Tadjalli, M., Mansouri, S.H. & Poostpasand, A. (2008). Gross anatomy of the oropharyngeal cavity in the ostrich (*Struthio camelus*). *Iranian Journal Veterinary Research,* 9, 316-23.

Tivane, C., Rodrigues, M. N., Soley, J. T., & Groenwald, H. B. (2011). Gross anatomical features of the oropharyngeal cavity of the ostrich (*Struthio camelus*). *Pesquisa Veterinária Brasileira*, 31(6), 543-50. https://doi.org/10.1590/S0100-736X2011000600015

Short Communication

Preparation of Cost-effective Lung Cast of Goat as Teaching Aid

Roop Kiran, Neelam Bansal*, Anuradha Gupta and Varinder Uppal

Department of Veterinary Anatomy
College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab -141004 (India)
*Corresponding author email: bansal.neelam@rediffmail.com

Received: 23.07.2024 Accepted: 07.08.2024

Abstract

The aim of this study was to prepare three-dimensional specimen of lung cast of goat which is easy to handle, flexible, non-toxic and cost-friendly teaching aid. Fresh lung of goat was used to prepare the silicone cast to study the internal anatomy depicting the branching pattern of bronchial tree. After inflation with air pump, the silicone sealant was injected into trachea and massaged properly till it reached into distal airways. The lung was hanged by an inelastic thread of high tensile strength for 24 hours to allow settling down of the silicone and thereafter immersed in a concentrated solution of the hydrochloric acid for digestion of the lung tissues. After complete digestion, cast was taken out of acid and washed thoroughly in running tap water followed by washing in the soft detergent solution. After drying, the corrosion cast was ready to be used as teaching material, museum specimens and exhibition display.

Keywords: *Goat, Lung, Plastination, Silicon cast,*

The study of bronchial tree of various species have been always a major area of interest for anatomy, physiology, pathology, pulmonary research and surgery students due to its intricate branching and minimal exposure to its three-dimensional anatomy. It has been quite difficult to access the level, number and degree of branching till alveoli, and variations in the caliber of diameter of successive bronchi and bronchioles in formalin fixed, plastinated or even live specimens (Kamath et. al., 2013). Till date, the one and only way to entirely study the bronchial tree was possible with its cast (DeSouza et. al., 2016). Bronchial tree cast can be used by pathologists and surgeons to locate and specify various developmental anomalies and other defects of lungs. The use of endoscopic techniques and locating various associated abscess or other space occupying lesions and teaching of these procedures to the students would be easy using bronchial tree cast. The easy way of making cast by using simple yet very effective technique and small amount of raw material makes it quite favorable as teaching aid and for research purpose. The silicone cast procedure provides the bronchial tree to depict finest branching till alveoli in goat (Ranjan et. al., 2020) and buffalo calf (Singh et. al., 2006). The aim of this study was to prepare three-dimensional specimen of lung cast of goat which is easy to handle, flexible, non-toxic and cost-friendly teaching aid.

Materials and Methods

The low cost procedure of cast preparation is hassle free as standardized by Ramkrishna & Leelavthy (2017) and the same procedure was used in the current study to prepare lung cast of goat.

Material Required

- Fresh lung specimen(s)
- General Purpose Silicon Sealant of 280 ml (WACKER): one bottle is sufficient for one lung.
- Applicator Gun
- Concentrated Hydrochloric Acid (2500 ml): It is reusable at least for 6 times.
- Air pump with valve
- Inelastic thread

Step 1: Procurement and Cleaning of Fresh Lung Specimen

While procurement and transportation of fresh lung specimen immediately after slaughtering (within half an hour), it was highly ensured that the organ was totally free of any type of major or minor cut observed grossly, otherwise silicone will be leaked out from the specimen. The lung was thoroughly washed in running tap water, ensuring removal of all blood clots and debris (Fig.1).

Step 2: Inflation of Lung Specimen

After thorough washing, with the help of a bicycle or motor air pump with valve, the lung was inflated with the valve directed into the trachea. The lung was inflated to its maximal capacity of 3 to 4 times as observed from the actual size of fresh and inflated lungs, ensuring complete dilation of its airways.

Step 3: Injecting Silicon Sealant into the Lung Specimen

Various materials can be used to prepare bronchial tree cast, however each material have its own limitation. So, considering all the materials available in the market, the best material with negligible limitation in making the museum specimen was used i.e. General-Purpose Silicon Sealant. Silicon sealant was readily available in the market, at a very nominal price of Rs 290 per silicon tube of 280 ml. The casting material i.e., silicone sealant was injected into the lungs using applicator gun through trachea, with proper massaging of the lung surface ensuring the sealant entered upto alveoli. Silicon was injected up to the point when the lung specimens become tense i.e. normally one silicone tube was sufficient for one lung of goat, and no more silicone was able to go inside the airways. After complete filling of lung with silicon, trachea was tightly tied with thread preventing silicon from leaking out.

Step 4: Overnight Hanging followed by Digestion of Lung Tissue to Obtain Cast

The silicone injected lung specimens were hanged by a thread of high tensile strength tied to trachea for 24 hours to settle down the silicone, and thereafter dipped in strong acid (concentrated hydrochloric acid) for digestion of lung tissues. After a complete digestion (1 to 2 days of complete immersion of lungs in acid), the casts were washed thoroughly in running tap water, and the remaining fat, debris was removed by mild washing in soft detergent solution.

Further the cast was air dried and the tracheobronchial corrosion cast of goat lung was prepared for using it as a teaching aid (Fig 2). The approximate total cost for the preparation of lung cast was Rs 500.

Precautions: During the procedure following points must be taken care of

- While procurement from slaughterhouse, ensuring the specimen without any cut is of utmost importance to obtain a fine cast with precise anatomical details.
- All the steps of the procedure should be performed with gloved hands.
- Handling of concentrated hydrochloric acid should be done with immense alertness and attention.

Fig. 1. Fresh specimen of goat lung

Fig. 2. Silicone cast of goat lung

Results and Discussion

There have been a lot of materials like LAPOX™ Epoxy resin and techniques proposed for the preparation of bronchial tree cast, having their own merits and demerits. The cast prepared by using LAPOX™ Epoxy resin was hard, non-elastic and brittle as compared to the silicone cast (Narayanan, 2015). The silicon cast of bronchial tree had numerous advantages over other plastination techniques (Bansal et. al., 2022) as it provided detailed internal anatomy and passage of artery, vein, duct, trachea, bronchus and its segmental branches. The cast obtained was flexible and can withstand the pressure

while examining and studying the branching pattern without being broken as in case of epoxy resin casts (Narayanan, 2015). The casts were easy to handle and store without the use of any chemical. The handling of silicon while performing the procedure was quite hassle free as it does not dry easily and was non-toxic. Besides this, the silicon cast technique ensured negligible chemical exposure to students, while handling and studying of bronchial tree cast.

Once the setup has been established, the procedure can be repeated multiple times and just required only single silicon tube for every specimen of goat lung (Kiran, 2024). The procedure is cost-friendly, and all the materials are readily available in the market. Moreover, after the cast prepared it can be washed easily and reused over the years attributed to its indefinitely long shelf life. The bronchial tree cast exhibited precise anatomical detail up to alveoli, as the silicon sealant was able to flow to the alveoli easily when the lung was massaged. The procedure does not require specialized training and the students can also easily make these casts by following all the steps. The bronchial tree cast is useful for researchers, students, teachers and other Veterinary professionals as it is helpful in understanding the pathophysiology of various developmental anomalies and other defects of respiratory tract.

References

Bansal, N., Gupta, A., Uppal, V. & Pathak, D. (2022). Preparation of plastinated specimens by low cost innovative method. *Vet Alumnus*, 44 (2), 82-85

DeSouza, F. M., Natekar, P. E., & Karapurkar, N. M. (2016). A novel and innovative teaching tool using low-cost luminal cast plastination of the trachea-bronchial tree. *Indian Journal of Anatomy, 5*(2), 143-45.

Kamath, V.G., Radhakrishna, K.S., Muhammed, A., & Ramakrishna, A. (2013). A comparative study of tracheobronchial pattern using luminal plastination. *International Journal of Anatomical Research,* 3, 161-64.

Kiran, R. (2024). Gross and histomorphological studies on the lungs of goat. [M.V.Sc. Thesis. Guru Angad Dev Veterinary and Animal Sciences University].

Narayanan, V. R. (2015). Preparation of low cost bronchopulmonary airway cast. *Journal of the Anatomical Society of India,* 64(2), 162-65

Ramkrishna, V., & Leelavthy, N. (2017). Corrosion Cast of Broncho pulmonary Segments. *The Journal of Plastination,* 29 (7), 5-7.

Ranjan, R., Jain, P., & Pandey, M. (2020). Exploratory anatomy of the tracheo-bronchial tree of goat using corrosion cast technique. *Haryana Veterinarian.* 59(1), 80-83.

Singh, A., Singh, G. & Nagpal, S.K. (2006). Broncho-pulmonary tree and segments of the lungs of buffalo calves. *Haryana Veterinarian*, 45, 29-34.

Short Communication

Taxidermy of Quail: Innovative Method of Preservation

Navodita, Santosh Gaikward* and Anuradha Gupta

Department of Veterinary Anatomy,
College of Veterinary Science (Ludhiana),
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
*Department of Veterinary Anatomy, Bombay Veterinary College, Mumbai
*Corresponding author email: anugadvasu@gmail.com

Received: 30.08.2024 Accepted: 22.10.2024

Abstract

Taxidermy is the practice of preserving and displaying animals. The present study was focused on the taxidermy of quail detailing the intricate process of preserving and mounting birds. The procedure started with the meticulous selection of a specimen, which was then followed by careful dissection to protect the quail's skin and borax preservation. After that, the preserved skin was diligently applied to a sculpted identical body, paying close attention to each feather and anatomical detail. The mounted quail's realistic appearance was enhanced by finishing touches like the painting of beaks and feet and the insertion of glass eyes. The finished specimen was displayed in a controlled environment. This case study highlights the combination of scientific technique and artistic skill involved in taxidermy, emphasizing the importance of this technique as a teaching aid and in preserving the beauty of nature for future generations.

Keywords*: Mounting, Preservation, Quail, Skin, Taxidermy*

Climate change is significantly decreasing the number of endangered species. Due to this biodiversity is declining and extinction rates are increasing, especially among bird species. Taxidermy is the practice of preserving and displaying animals. It may play a crucial role in future to understand biodiversity by allowing us to examine species that might otherwise be extinct or endangered in their natural habitats. It will enable us to appreciate and explore the beauty of the natural world, whether it will be showcased in museums or cherished in private collections (Péquignot, 2006).

Through the preservation of quail, the study sheds light on the intricate methods of taxidermy. This technique is a blend of fine arts and science which can be used for educational purposes beyond aesthetics. The process of preserving a quail specimen involves a meticulous set of techniques, requiring a deep understanding of the bird's anatomy and taxidermic preservation. From the initial steps of specimen acquisition to the final stages of mounting and display, each phase must be executed with care and precision to create a lifelike and scientifically accurate representation (Manton, 2022; Winker, 2000). This study on the taxidermy of quails highlights a craft that blends science with artistry. By carefully preserving and mounting quail specimens, taxidermists are

able to honor these remarkable birds and showcase their beauty. This work is important to appreciate and safeguard the natural world so that future generations can continue to enjoy the wonders of quails and other birds in the wild.

Method of Preservation

In this study, the various steps followed for the taxidermy of the quail have been described by Gaikwad (2022).

1. **Preservation:** The preservation process was the most crucial step as it maintained the anatomical integrity of the quail and also added aesthetic value to the specimen (Pequignot, 2006; Winker, 2000). A precise incision was made ventrally, starting from the sternum, and the fascia was separated from the skin with care, avoiding any cuts to the skin. The entire muscular body of the bird with all the viscera was removed through this incision while preserving the skin's vital structures. The skin was peeled back until the legs and wings could be detached. Bones of the wings and legs were left in place while removing all the flesh around them. One important step was done to remove oil glands that if kept inside would lead to faster deterioration of the specimen. The skin was peeled off until the skull and the flesh from the skull including the brain was removed with precision while the eyeballs were taken out without damaging the skin around the eyes. After this, a layer of borax and soap as a preservative was applied thoroughly to every crevice, cavity, and grain part of the skin. Newspaper was placed inside till a duplicate body was made. Newspaper absorbed all the excessive moisture out of the quail's skin halting deterioration, thus extending the specimen's longevity.
2. **Mounting:** The next crucial stage after preserving the skin was mounting. The measurements were made from the removed muscular body that were used to sculpt a duplicate body of the bird with styrofoam and wood wool to resemble a quail's natural anatomy (Maynard, 2022). It provided the framework for carefully preserved skin to be fitted over. The quail was positioned in a natural posture, with every feather and limb arranged precisely. Wires and armature were used to support and pose the bird, allowing for maximum realism and flexibility. The preserved skin was then delicately fitted onto the duplicate body, with meticulous attention. Each feather was individually positioned, and every aspect of quail's natural anatomy was faithfully recreated (Fig. 1).
3. **Finishing:** The finishing touches created a lifelike representation that captured the spirit and vitality of the quail. After mounting, glass eyes were inserted into the eye sockets, providing a lifelike gaze that captured the essence of the bird (Fig. 2). The beak and feet were meticulously painted to match the quail's natural coloring. A duplicate tongue was made from wax and placed inside the beak.

These final touches transformed the biological specimen into a work of art (Fig. 3).

4. **Care and Display:** Upon completion, the mounted quail required proper care and maintenance to ensure its longevity. It was displayed in a controlled environment, away from direct sunlight and moisture. Regular dusting and occasional touch-ups were necessary to preserve its appearance over time.

5. **Interpretation and Challenges:** Anatomical precision was balanced with artistry in carefully positioning the specimen to capture the essence of the quail's natural behavior and grace (Bezan & McHugh, 2019).

Discussion

It took around 3-4 days to taxidermy the quail. Day one was dedicated to skinning the bird and treating it with borax for preservation. It was primarily dehydrating to ensure the long-term preservation of the skin and borax was used for that. By the second day, a styrofoam and wood wool replica body had been separately constructed. Both the materials were available easily in local markets. The support of wire reinforcement by the styrofoam specimen was very solid. But cost-wise, it's pricier than a pile of shredded wood fibers. For more savings newspaper cuttings can be replaced with some wood wool before the reconstruction of the body, indeed that will probably not have as good mechanical properties as wires with styrofoam. Mounting and simpler adjustments like the wings, and legs were also done on second day. The third day concentrated on finer points like placing glass eyes in its sockets to create a vibrant and lifelike gaze from each quail, white petrol was used behind feathers so that these lay naturally. The entire beak and feet were lightly coated with varnish to represent the shiny highlights of a live bird.

The technique is practical. The model is viable for educational purposes and offers a realistic view of an animal. All the materials were readily available though the cost of

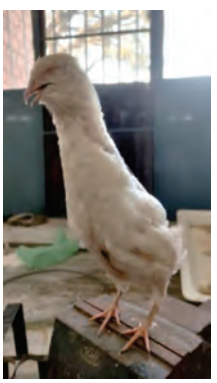


Fig 1. Quail specimen before final touches

Fig 2. Insertion of glass eyes

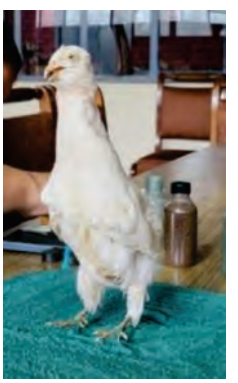


Fig 3. Final specimen: Mimicing natural environment

Styrofoam is slightly more. The technique plays a crucial part in conservation efforts as it enables the preservation of species that might otherwise face extinction due to climate change. One of the most challenging parts of storage is pest control. Therefore, covering specimens with containers of glass or acrylic is crucial to prevent dust accumulation and pest infestation which can deteriorate the quality of the specimen over time. Taxidermy was effective in producing durable specimens. This technique also shows potential for future use in educational settings, museum displays, and conservation initiatives, making it a sustainable and valuable practice.

References

Bezan, S., & McHugh, S. (2019). Introduction: Taxidermic Forms and Fictions. *Configurations,* 27(2), 131-38.

Gaikwad S A. (2022). Taxidermy: The life after death. In Anatomical Techniques: New era tools in Health and Disease, BFC Publications Pvt Limited, Lucknow, pp 47-48.

Manton, W. P. (2022). Taxidermy without a Teacher: Comprising a Complete Manual of Instruction for Preparing and Preserving Birds, Animals and Fishes. DigiCat.

Maynard, C. J. (2022). Manual of taxidermy: a complete guide in collecting and preserving birds and mammals. DigiCat. (https://www.everand.com/book/576733062/Manual-of-Taxidermy-A-Complete-Guide-in-Collecting-and-Preserving-Birds-and-Mammals)

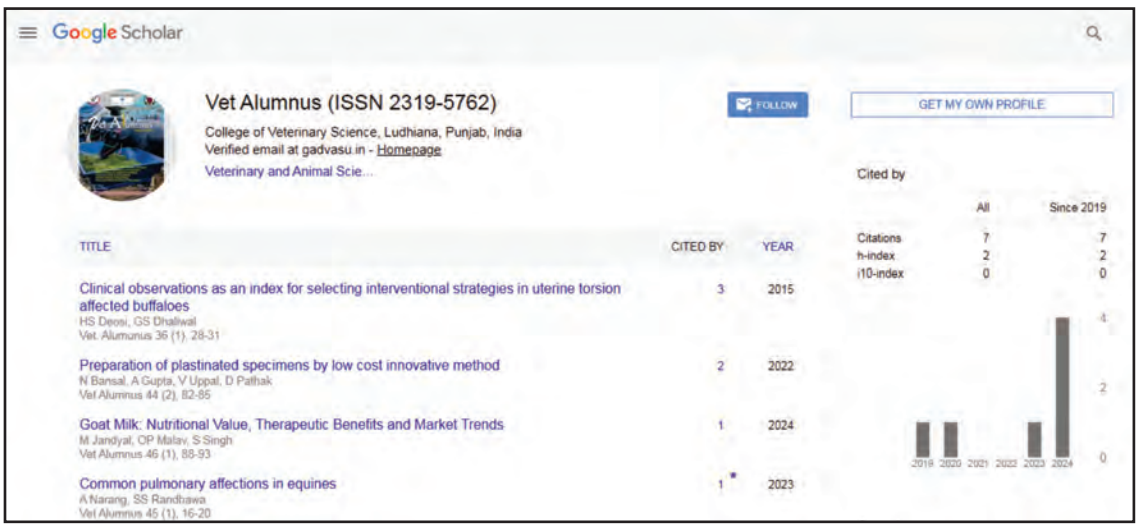
Péquignot, A. (2006). The History of Taxidermy: Clues for Preservation. *Collections*, 2(3), 245-55.

Winker K. (2000). Obtaining, preserving, and preparing bird specimens. *Journal of Field Ornithology*, 71(2), 250-97.

Acknowledgments to Esteemed Reviewers

1. Dr. L.D. Singla
2. Dr. Yashpal Singh
3. Dr. Mandeep Singh Bal
4. Dr. Daljeet Kaur
5. Dr. Vandana Sangwan
6. Dr. Nikita Gupta
7. Dr. Vishal Mahajan
8. Dr. Gurpreet Singh Preet
9. Dr. Sujata Turkar
10. Dr. N. Umeshwori Devi
11. Dr. Jasmeet Singh Khosa
12. Dr. Sunil Kumar B.V.
13. Dr. Jasmine Kaur
14. Dr. M. Honparkhe

Vet Alumnus Articles cited in Google Scholar



GUIDELINES TO AUTHORS

Dear authors, 'Vet Alumnus' - the official organ of the Alumni Association, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India is a registered journal with ISSN 2319-5762 and is published biannually in the months of June and December every year. Recently published issues of the 'Vet Alumnus' are available as OPEN ACCESS on https://www.gadvasu.in/page/vet-alumnus

Types of paper

Original unpublished manuscripts of clinical or practical application interest in the form of a mini-review and research article or short communication, description of emerging diagnostic or therapeutic technique(s), clinical and technical articles will be considered for publication. Each submission will be subjected to the blind peer review process and editorial revisions before the final decision regarding its publication.

Manuscript

- The TITLE should be short, specific, and informative written in the TITLE case.
- The BYLINE should contain the full names of all the authors, affiliation, and the email of the corresponding author, as per example below:

  Example: Varinder Uppal*, Anuradha Gupta, Neelam Bansal and Opinder Singh

  Department of Veterinary Anatomy, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab -141004, India

  *Corresponding author email: v.uppal@yahoo.com
- The maximum number of authors should be restricted to 3. More authors may be included with proper justification given in the cover letter.
- All manuscripts should have an ABSTRACT with 3-5 KEYWORDS, written in alphabetical order. The body of the article must be comprised of a brief INTRODUCTION (without sub-title), METHODOLOGY, RESULTS, and DISCUSSION. The body of other types of manuscripts should be divided into suitable subheads.
- All FIGURES/TABLES must be original (unpublished), well-labeled, titled, and quoted chronologically at appropriate places in the text.
- REFERENCES: List 5 to 7 recent and relevant references in alphabetical order as per the example below:

  Example: Uppal, V., Gupta, A., Bansal, N. & Singh, O. (2015). Anatomical differences in appendicular skeleton of cattle and buffalo. Vet Alumnus, 36(1), 22-23.
- Papers should be TYPEWRITTEN in TIMES NEW ROMAN Font, and DOUBLE SPACED throughout (including references and tables) on A4 size with a 2.5 cm margin at the top, bottom, and left and right-hand sides. Articles (including illustrations) should be submitted in electronic form after a thorough check-up of typographical/grammatical errors and plagiarism check.
- Well-written and structured articles as per the above guidelines can be submitted to the Editor by email: editor.vetalumnus@gmail.com; vetalumnus@gadvasu.in
- Authors shall be solely responsible for the contents of the article.
- There are NO PUBLICATION CHARGES for the authors.

Connect with us

https://www.gadvasu.in/

YouTube

COVS, GADVASU

YouTube Farmer friendly e-extension

Alumni Association
College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana-141004, Punjab, India