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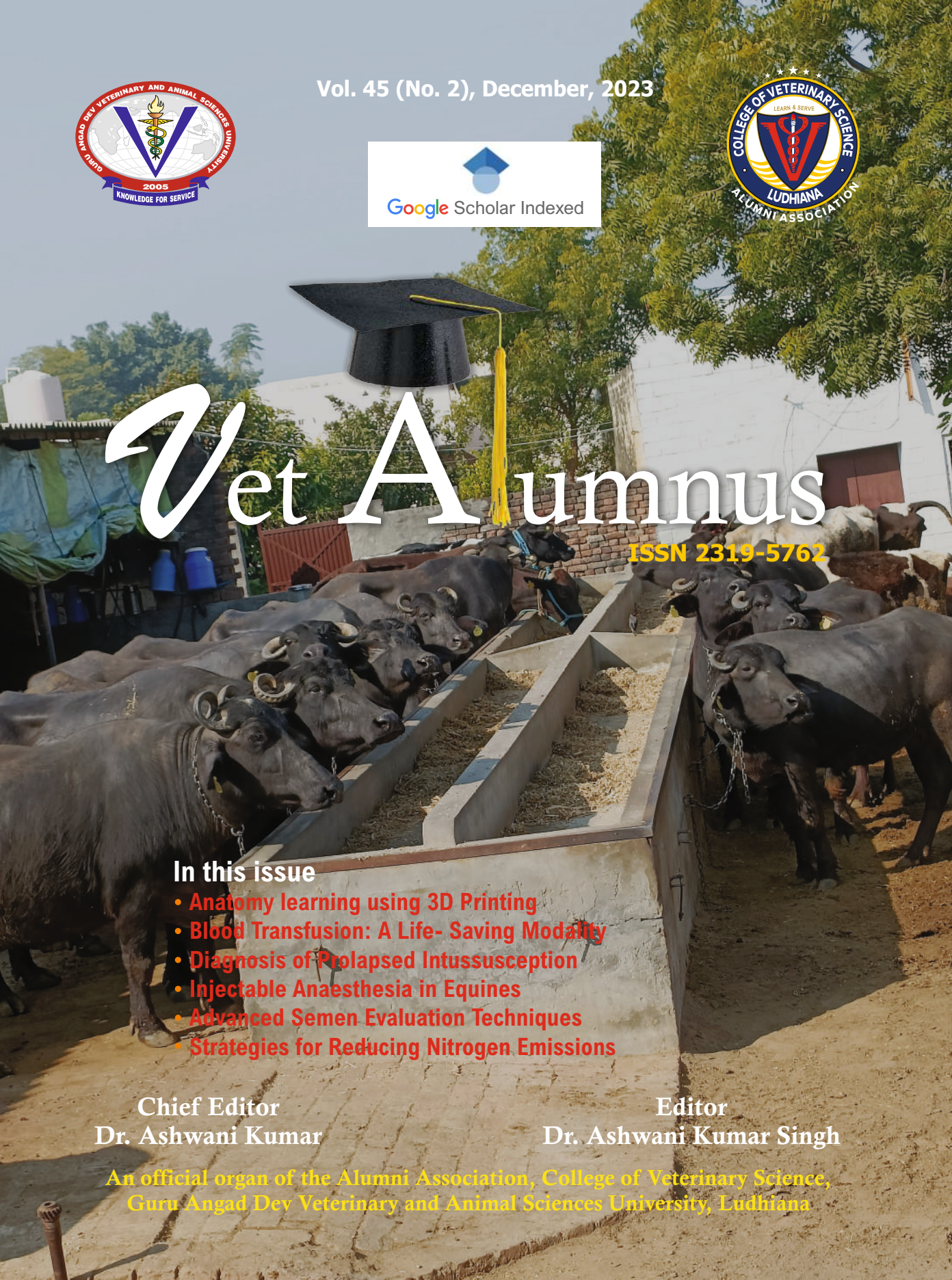

In this issue

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VET ALUMNUS

Vol. 45 No. 2 December 2022

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AUGMENTING LEARNING OF VETERINARY ANATOMY USING 3D PRINTING

Anuradha Gupta, Nirmal Singh[1], Neelam Bansal[*] and Varinder Uppal

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

[1]University Library

*Corresponding author email: bansal.neelam@rediffmail.com

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Abstract

In veterinary education programs, along with theoretical teaching of veterinary anatomy, practical exposure is mandatory to consolidate the theoretical aspects. In this pursuit, accurate anatomic models are indispensable for practical learning of students for honing up their basic understanding of animal structures to sharpen their clinical skills. The models/specimens should be true replica of original object(s) and must be in good condition i.e color, texture, flexibility, and other characteristics, as found in a living animal. The 3D printing technology has opened up new vistas for mimicking the real organs/object(s) at desired scales. Besides ability to generate models with any level of complexity, 3D printing technology have generated products that are cost-effective and easy to use.

Keywords: *3D printing, anatomy models, heart, pelvic cavity, skull*

In veterinary education, theoretical teaching of anatomy is pivotal for developing the basic understanding of animal structures amongst learners and practical exposure is essential to consolidate the theoretical aspects. For practical teaching to be fruitful, the anatomical specimens/models need to be accurate and true replica of original organs/objects bearing all characteristics as found in a living animal. Historically, the teaching of human anatomy in medical and veterinary curricula using cadavers has been a source of significant social controversy, revealing the most contentious medico-legal and ethical debates across other scientific disciplines. Whether dissection of cadavers is still a relevant and appropriate component of a modern medical undergraduate training is one of the major recurring polemics in anatomy education (Parker, 2002). The high cost of preserving cadavers and acquiring equipment needed to maintain them has impelled many laboratories to do away with such practices, ultimately impacting the practical exposure to learners.

The emergence of 3D printing technology and its application to generate osseous models has opened up new avenues to supplement practical learning of veterinary anatomy. The production of high-quality 3D printed replicas of cadaveric material is gaining attention of academicians gradually. This allows highly accurate mimicking of original organs/structures for learning and training of students to hone up their clinical skills. 3D

printing has also emerged as a valuable approach in manufacturing of individualized implants for receiver-specific needs in human and veterinary orthopedics (Memarian et al., 2022). This seems quite helpful to improve animal life and provide them relief from ailments. Flexibility and customization are the key features of 3D printing, enabling generation of models of varying complexity at diverse scales as per ones' requirements. For instance a 3D model printed using this technology allows a surgeon to perform mock surgery before performing the actual surgery on animals (Haleem et al., 2019).

The advantages of 3D printing include design liberty, automation, manufacturing velocity, accuracy, customization, and minimal waste generation. With easy availability and cost-effectiveness, 3D printing has gone from a very expensive specialized process to an everyday affordable tool. The 3D printers come in different sizes, shapes and types. (Wilhite and Wölfel, 2019). This process allows the rapid conversion of information from digital 3D models into physical objects and enables solid products to be obtained by adding two-dimensional micron-level layers and without applying other processes such as cutting and drilling (AbouHashem, et al., 2015). According to Wong (2016), 3D printing process has three components: (i) Image acquisition, (ii) Image processing, and (iii) 3D printing of object.

A Fused Deposition Modelling (FDM) 3D printer procured by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana under the project of Rashtriya Krishi Vikas Yojana (RKVY) was used to generate anatomical models for students. The freely available 3D designs of skull of ox, goat, domestic fowl and parrot, skeleton forming pelvic cavity of dog and bovine heart available in public domain were downloaded from Thingiverse (https://www.thingiverse.com), Cults 3D (https://www.cults3d.com) and Embodi3D-The Biomedical 3D Printing Community (https://www.embodi3d.com) in .stl format. These models were then individually processed in slicing software Ultimaker Cura (https://ultimaker.com), and various parameters of printing including print quality, percentage of infill, size of print, support to print and its percentage, etc., were fixed in consultation with subject expert from the Department of Veterinary Anatomy, College of Veterinary Science, Ludhiana. The output of Ultimaker Cura was then saved as .gcode file. The .gcode file was then transmitted to 3D printer through external storage device supported by 3D printer. The Polylactic Acid was used as input material to generate 3D prints through extrusion. Printing of each model involved varying time depending up on its size, complexity, infill and support percentage involved, which extended from a few minutes to many hours/days. For example, printing of parrot skull took around 55 minutes and printing of bovine heart consumed 60 hours. The models printed were then processed to remove supports as well as to smoothen them for improvising their print quality to make is more realistic and appealing.

The printed models (Fig. 1-3) were then transferred to the Department of

Fig. 1. The 3D printed Skull of ox

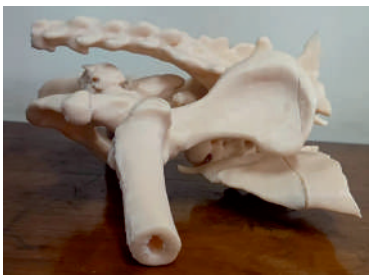

Fig. 2. The 3D printed Pelvic cavity of dog

Fig. 3. 3D printed Heart of ox

Veterinary Anatomy, College of Veterinary Science, Ludhiana, for teaching and extension related activities. The subject expert(s) then labelled the 3D models to enable students' recognition and understanding of different parts of the organs/ structures. These models are being used in teaching of veterinary anatomy and are well accepted and appreciated by faculty as well as students. The easy handling of models without fear of getting damaged boosts the confidence amongst students towards practical learning.

Witnessing the success of 3D printing in supplementing learning of veterinary anatomy, the potential of this technology can also be harvested in other areas of veterinary science such as pathology, surgery, use of prostheses for animal rehabilitation and imaging studies. Besides usage in academic programs, this technology has a vast potential for developing 3D models for education/ awareness of farmers through display at *Pashu Palan Melas*, trainings and other extension activities/events.

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FUNCTIONAL ANATOMY OF DIGESTIVE TRACT OF HORSE

Opinder Singh*, Devendra Pathak, Kritima Kapoor, Varinder Uppal

Department of Veterinary Anatomy, College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004
*Corresponding author email: singhopinder68@gmail.com

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Abstract

Large numbers of livestock farmers in Punjab are involved in horse rearing as an allied farming activity and are earning good income. A basic understanding of the functional anatomy of equine gastrointestinal tract is essential to advise ideal nutrition, management and health care facilities to the animals to get optimal performance. The horses are simple stomach herbivores and hind gut fermenters as large intestine is the main site of fermentation of fibrous feedstuffs which differentiates them from ruminants in which the fermentation takes place in the forestomach. The stomach of horse is comparatively small requiring continuous feeding in low quantity. The large intestine is relatively large for optimal fermentation of fibrous feed that comprise the majority of equine intake. In this article, the basic anatomical features have been correlated with physiological activity to present a fundamental knowledge of the equine gastrointestinal tract.

Keywords*: Functional anatomy, gastrointestinal tract, horse*

Horses are simple stomach herbivores. Horses are called 'hindgut fermenters' because they have a high concentration of microbes in their caecum and large colon that help the horse to digest the tough fibrous portions of the forage in their diet. The horse's digestive tract consists of the mouth cavity, oesophagus, stomach, small intestine and the highly developed large intestine composed of the caecum, colon and rectum. Each part of the digestive tract has a particular anatomy and function (Wong et al., 2011). A basic understanding of the physioanatomy of gastrointestinal tract is important for better management of animals.

Mouth

Mouth cavity of horse is long and cylindrical. Important anatomical structures of the mouth include the lips, cheek, tongue, teeth and salivary glands. Anteriorly mouth is bounded by lips which are highly mobile and thinner than ruminants. The angle of union of lips is about the level of the first cheek tooth. Prehension (grasping) of feed by the horse is done primarily by the lips. Cheeks are less capacious in horse as compared to ruminants and buccal glands are arranged in two rows. Papilla salivalis is present at the level of upper third cheek tooth and the duct of parotid gland opens here. Buccal glands are minor salivary glands arranged in two rows and secrete saliva. Hard palate is

long and narrow and devoid of papillae. In the middle of hard palate, palatine raphe is present. On either side of palatine raphe, 16-18 palatine ridges with concavity directed backwards are present. Sublingual caruncle is present on floor of mouth cavity opposite canine tooth and on it duct of mandibular duct opens. Soft palate is very long in horses. It closes the isthmus faucium along with the epiglottis and hence in horse oral breathing is not possible and in vomiting, the ejected matter escapes through the nose.

Tongue: Tongue is spatula shaped in horse. Gustatory circumvallate (2-3 in number) are present at root of tongue. Foliate papillae are also present in horse. The isthmus faucium is relatively small and not dilatable. Digestion of feeds begins when food enters the mouth. The horse chews reducing feed particle size and increase its surface area and mixing it with saliva to form the bolus and begin the digestive process.

Teeth: All permanent teeth are hypsodont type (High Crown Tooth) specialized tooth, where crown and neck are not easily distinguished and there is only body and root. Dental formula of horse is, 2 (I.3/3 C.I/I P.M. 3 or 4/3, M 3/3) =40 or 42. Entire external surface of tooth (body and root) is enclosed by cementum. Due to differential wear table surface has lamellar pattern. Some horses do not wear their teeth evenly when they chew the feed, so sharp edges on the teeth may develop and eventually impair their ability to chew feed properly. Horses that cannot chew their food properly may suffer problems including a decrease in feed intake, dropping feed from the mouth (quidding), tilting head while chewing, cuts in the oral mucosa, weight loss, and it may predispose the horse to choke.

Salivary Glands: There are three major salivary glands in horse; parotid, mandibular and sublingual salivary glands. Parotid gland is the largest salivary gland in horse. Medial surface of gland is related to guttural pouch and empyema of guttural pouch results in painful swelling of parotid area. Duct of Parotid gland opens opposite third upper cheek tooth. Mandibular gland is smaller than parotid and is medially related to guttural pouch. Sublingual gland has only polystomatic part in horse and extends from incisive part of mandible to 3rd lower cheek tooth. Saliva acts as a lubricant to provide easier passage through the oesophagus and buffers acid in the stomach helping modulate ph. It has low quantity of amylase so the chemical breakdown of carbohydrates is minimum and mostly takes place in the later part of digestive tract.

Pharynx: Pharynx is long and narrow in horse. Nasal septum is not continued into nasopharynx. Diverticulum of auditory tube called guttural pouch is present dorsal to pharynx. The opening of auditory tubes is covered by flap which sometimes become redundant and act as one way valve and trap the air within guttural pouch resulting in guttural pouch tympany. The swallowed food passes through pharynx and its muscular action pushes the food into oesophagus and to stomach. Once swallowed, food or water cannot return to mouth due to long soft palate. If refluxed, these pass through the nostrils.

Oesophagus: It is longer, being about 125 to 150 cm. Most of the oesophagus is present in thorax and it has a small abdominal part. At its origin it is related to the guttural pouches and swelling of guttural pouch due to any pathological condition can result in dysphagia. It moves the food bolus to the stomach via regular, rhythmic muscular contractions called peristalsis. These contractions push bolus in one direction, contributing to the horse's inability to regurgitate. The oesophagus can be site of condition called choke, when it is blocked and swallowed feed can come out of nostrils. Feed enters the stomach from oesophagus through cardiac sphincter, which controls the entry of bolus from oesophagus and prevents already ingested food and gas from entering oesophagus.

Stomach: The equine stomach is small in relation to the size of the animal and to the fodder consumed that makes approximately nine per cent of GI tract volume. It is present in the left half of abdomen. The opening of oesophagus called cardia is fixed at 11th rib. The stomach of horse consists of two limbs. Left limb comprises of fundus and body and right limb comprises of pyloric part. The pronounced fundus called saccus cecus extends under upper part of 15th rib and lowest part of body reaches to ventral part of 9th and 10th ribs when moderately distended.The right limb or pyloric part is much narrower and extends across midline to join duodenum. The cranial surface of stomach is directed against diaphragm above and left lobe of liver ventrally. Caudal surface is in contact with intestine. Cardiac sphincter is well-developed in horse and oesophagus enters obliquely into stomach, so the vomition is difficult in horses. The stomach is divided into glandular and non-glandular parts by margo plicatus (Konig and Liebich, 2014). Microbial digestion is minimum in stomach of horse, however breakdown of proteins takes place due to production of hydrochloric acid and pepsinogen.

Intestine: The intestine occupies the major part of abdominal cavity and is divided into small and large intestine.

A). Small Intestine: The small intestine comprises of duodenum, jejunum and ileum. Duodenum is relatively fixed in position due to short mesentery that makes it difficult to access in usual surgical exposures. The bile and pancreatic duct enter duodenum 12-15 cm distal to pylorus at major duodenal papilla which is inside a pouch called hepatopancreatic ampulla. Accessory pancreatic duct enters opposite these at minor duodenal papilla (Konig & Liebich, 2014). The jejunum is suspended within, free margin of the extensive mesentery called mesojejunum and its gives it greater mobility. Ileum is the terminal part of small intestine and ileocecal fold between ileum and caecum helps in determining the length of ileum. In horse dysfunction of nervous supply to ileum leads to permanent contraction of muscular coat of ileum which can result in impaction and colic

The main function of the small intestine is to break down the digesta into smaller particles and to begin absorption of the nutrients across the small intestinal lining

into the horse's blood supply. The majority of fat, protein, simple sugars, vitamins, and minerals are digested and absorbed in the small intestine. The fibrous components, are digested to a small extent here, and therefore majority pass through to the next section of the digestive tract (cecum). It is important to feed only good quality feed free of mould, toxins, and foreign objects because if present, they may be absorbed by the small intestine and cause sickness in horses. The pancreas and liver assist the small intestine with digestion. The pancreas secretes a mixture containing enzymes into the small intestine, which aids in breaking down protein into amino acids and carbohydrates into simple sugars. The mixture also helps to raise the pH of the digesta for optimal microbial fermentation or digestion of feed by gut microbes. The liver produces bile, which assists with fat absorption by the small intestine. In other animals, bile is stored in the gall bladder and secreted when a meal is eaten. However, in horses the bile is continuously secreted by the liver as it lacks a gall bladder. Despite evolving on low fat/ high forage diets, horses are capable of digesting and absorbing fat from diets that contain as much as 15 percent fat, Fat is an important energy source for horses and it is often increased in diets fed to exercising horses;however feeding too much fat can cause gastrointestinal disturbances in horses. Grain fed to horses often contains large amounts of starches and sugars that are readily digested and absorbed by the small intestine. These compounds are broken down into glucose and other simple sugars and absorbed into the bloodstream within several hours of consumption. Diets containing (more than 2-2.5 Kg of starch and sugar at a t may overload the digestive capacity of the small intestine causing spillage of the grain particles into the cecum so caution should be exercised about the quantity of grains being fed to horse. It takes on average about an hour for the food to reach the cecum (Frape, 2004).

B. Large Intestine

The large intestine is having enormous capacity and characterized by sacculations or haustra form which help to mix and retain plant fibers until digested. Although some microbial fermentation takes place earlier in the digestive tract, the majority takes place in the cecum and later in the great colon. The maintenance of a healthy and effective microbial population in caecum and great colon is very important. In addition, microbes adjust fairly slowly to new diets, so it is best to change the diet of the horse gradually. The caecum is a comma shaped structure occupying right abdominal cavity. It consists of base, body and apex.

i) **Base**: - Bulbous beginning of the caecum in the right paralumbar fossa and partly under cover of the ribs. It has an extensive contact with abdominal roof and sublumbar organs from 15th rib to coxal tuber. Base fuses with root of mesentery medially and right dorsal colon cranially. Cranial part of cecal base forms an overhanging and colon originates from the middle of caudal wall of this overhang.

Gas produced in the cecum causes overhanging part to press upon the origin of the right ventral colon leading to colic

ii) **Body**: - This is continuation of the cecal base cranially along the right wall and floor of the abdominal cavity.

iii) **Apex**: - It is the tapered end of the caecum on the floor of the abdominal cavity, caudal to xiphoid cartilage. The ventral colon wraps around it. (Fig. 1)

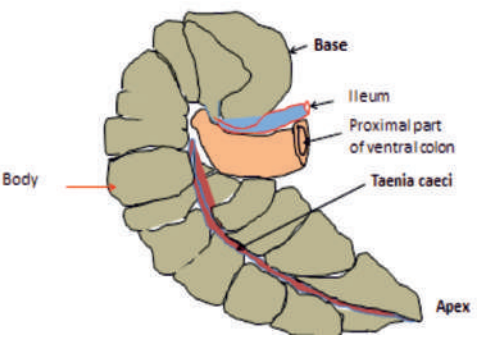

Fig. 1. Caecum of Horse

The ileocaecal and caecocolic openings are present at lesser curvature of base of caecum at a distance of 3-4 inches. The close proximity of the openings, and the shape of the cecum, allows the long fibrous portions of the diet to spend an adequate amount of time there in order to be properly digested. The main function of the cecum is to provide an optimal site for the slow fermentation of fiber by microbes and the absorption of fermented products and nutrients viz; volatile fatty acids and vitamin B and K produces methane, carbon dioxide and water, as well as most of the B-vitamins and some amino acids.. It also provides a site for the absorption of fermentation products and other nutrients. When too much concentrate is fed, it may enter the cecum incompletely digested, in which case the microbes quickly ferment the starch and sugar into lactic acid and gas. The accumulation of gas and lactic acid may lead to colic like condition in horses. In addition, microbes adjust fairly slowly to new diets, so it is best to change the diet of the horse gradually.

Colon: Colon of horse is divided into large / great/ascending colon and descending/small colon. The ascending colon due to its large size is called great colon.Colon in horse is a highly modified structure with great capacity. The different parts of the ascending colon as they receive food can be distinguished on the basis of number of bands. Some of these bands are hidden in mesentery and called as mesocolic bands. Caecum and ventral colon including sternal flexure has four bands (2 free and 2 mesocolic). Pelvic flexure and left dorsal colon has one band which is mesocolic. Right dorsal colon has three bands (1 mesocolic, 2 free). Descending or small colon has two bands (1 mesocloic, 1 free). (Fig. 2)

The most common impaction sites due to change in diameter are- pelvic flexure, caecum and transverse colon. The large colon provides an additional location for fermentation of fibrous digesta and is the site for absorption of the products of fermentation. Another important function is absorption of water from the digesta which plays important role in movement of digesta through the digestive tract. The dehydration in horses predisposes the horse to impaction.

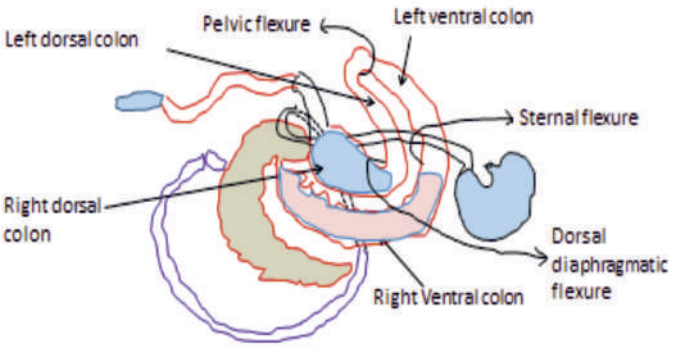

Fig. 2. Large Intestine of Horse

Descending/Small Colon and Rectum

The small colon is approximately 10 feet long. Its main function is to absorb water and form fecal balls. The rectum is approximately 1 foot long. It stores and excretes feces. The equine colic is also caused by presence of intestinal stones called as enteroliths that are most often found in the small or large colon. The formation of stones is related with geographical regions, composition of soil and water, fodder and genetics (House & Warren, 2016). Depending upon shape and size, the presence of such stones may result in onset of colic due to complete obstruction of the intestines, mainly of the small colon and rarely of the large colon. They may also cause periodic colic pain in horses. Certain fodder types such as Lucerne are predisposing factors to intestinal stone formation as they can lead to an increased concentration of minerals and elevation of the pH in the colon which in turn leads to intestinal malfunction and decrease in intestinal motility (House & Warren, 2016). Enteroliths most often consist of magnesium or calcium ions. The enteroliths most often develop in horses aged 5 to 10 years. The highest number of cases is reported in Arabian horses and ponies (Hassel et al., 1999).

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IMPROVING VETERINARY VOCABULARY

Rakesh Kumar Sharma*

Department of Veterinary and Animal Husbandry Extension Education, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004

*Corresponding author email: rkkural@yahoo.com

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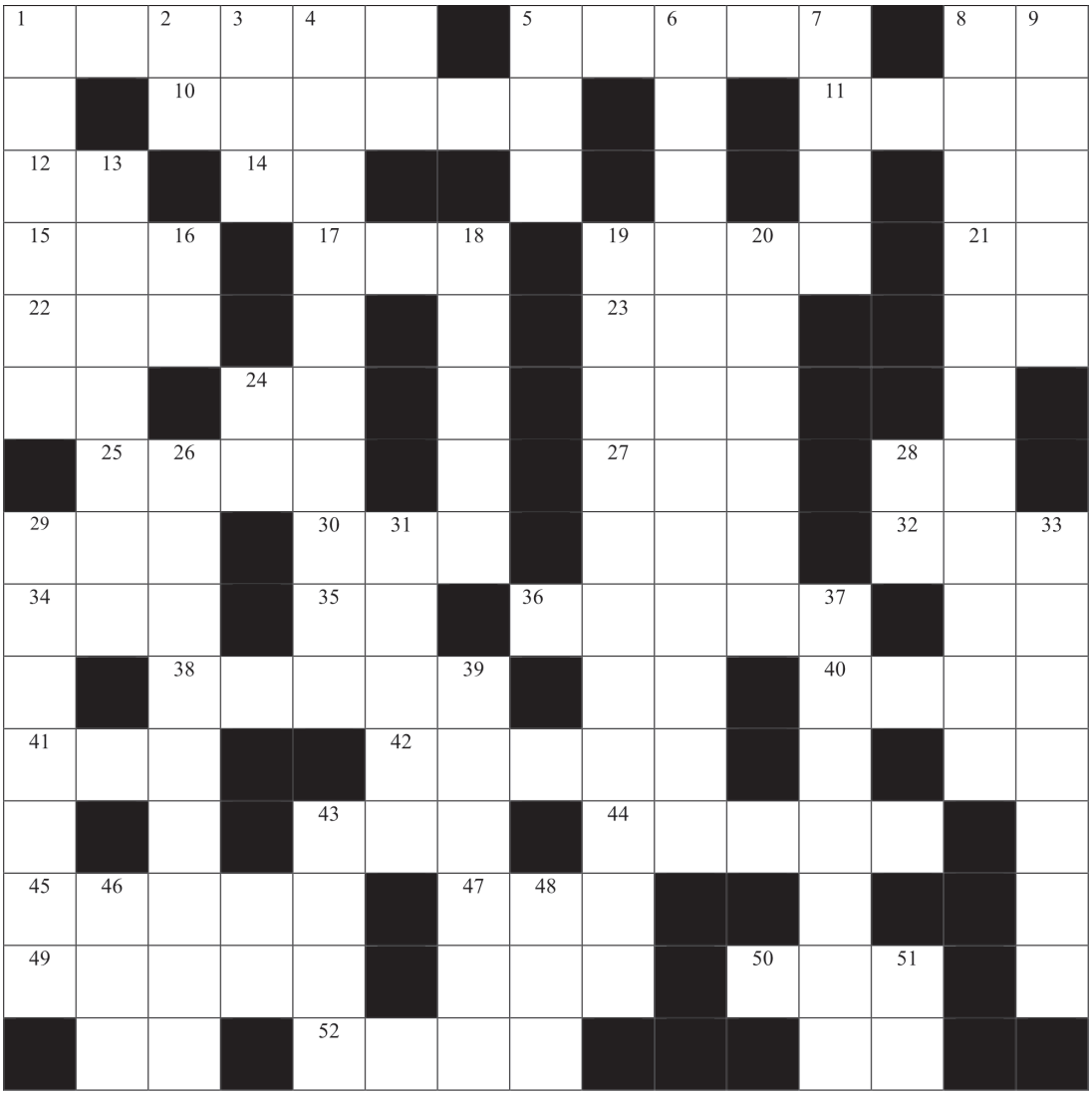
(Note: The spellings for this crossword are according to American English)



ACROSS:

1. A medium sized exotic cattle breed (6)
5. A disease of young chicken, turkeys etc. caused by the worm, *Syngamus trachea* (5)
8. Tuberculosis (Abbreviation) (2)
10. Absence of urine production (6)
11. Egg cell (4)
12. A histocompatibility antigen present in gonads of a bovine freemartin (2)
14. Abbreviation for *foot,* a unit of measure (2)
15. Head- _____, a common clinical sign of equine lameness (3).
17. A male castrated pig (3)
19. Restoration of health of sick animals (4)
21. Abbreviation for *effective dose* (2)
22. Naval-_____ (3)
23. Suffix denoting *resemblance* (Greek word) (3)
24. Chemical symbol of zinc (2)
25. Exudate from a blister (its plural) (4)
27. Abbreviation for *percutaneous coronary intervention* (3)
28. Technical abbreviation for left eye (2)
29. A low-melting, high molecular-weight organic compound similar to fats but lacking glycerides (3)
30. Abbreviation used in medical prescriptions to denote *if necessary* (3)
32. Popular name (Abbreviation) for *M tuberculosis* bacterium attenuated by physician Albert Calmette and veterinarian Camille Guerin (3)
34. Prefix for environment friendly techniques or activities (3)
35. An injection directly given into blood stream (Abbreviation) (2)
36. A foramen (*in Latin*) in the occipital bone through which the spinal cord enters the spinal column (5)
38. Genetically determined characteristic (5)
40. A fine, long appendage of the skin (4)
41. Prefix meaning *new* (3)
42. Prefix meaning *none* (5)
43. A synthetic oestrogenic analogue (Abbreviation) (3)
44. A chronic fever (disease) caused by *Leishmania donovani* (5)
45. Unwanted sound considered unpleasant to hearing (5)
47. Photosensitization occurs when an individual comes under _____ (3)
49. Cannabis (5)

50. In prescriptions, abbreviation for *three times a day* (3)
52. Prefix meaning *nose* (4)

DOWN:

1. A diagnostic agent derived from *M. paratuberculosis* (6)
2. Abbreviation for right atrium (2)
3. Components of milk other than water and fats (3)
4. Painless inducement of a quick death (10)
5. A device to facilitate oral examination of animals (3)
6. False pregnancy common in bitches and goats (12)
7. Bottom of hoof (4)
8. Pin bone (5,6)
9. Brightness modulation in diagnostic ultrasonography (1,4)
13. A structure that develops in the inner cell mass of embryo and expands into a vesicle that later on becomes the primitive gut (4,3)
16. Amino acids that are racemic mixtures of both types of optical isomers (2)
18. Group of species (5)
19. Bulbo-urethral gland (6, 5)
20. The innermost layer of the eye (6)
26. Toxins that are secreted from the body of bacteria during their lifetime (9)
29. Process of separating a piglet from its mother (7)
31. Related to sheep (5)
33. An important hormone in birds and mammals that affects appetite (7)
37. One of the water soluble vitamins (7)
39. Cough (6) (*In Latin language*)
43. Head of a veterinary college (4)
46. A non-leguminous fodder crop of Punjab grown in Rabi season (3)
48. Prefix meaning *urine* (3)
51. A form of current that provides constant voltage over time (Abbreviation) (2)

(Crossword answers on page No. 84)

SHERLOCK – BEGINNING OF A NEW ERA OF CRISPR-BASED DIAGNOSTICS

Shreya Prasher* and Paviter Kaur

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

*Corresponding author email: prashershreya4@gmail.com

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Abstract

Early and accurate diagnosis serves as the cornerstone for effective treatment and formulation of mitigation strategies to combat diseases. Specifically considering the low- and middle- income countries, World Health Organisation has established an ASSURED criteria for an ideal Point-of-Care diagnostic test, emphasising its affordability, sensitivity, specificity, user-friendliness, robustness, rapidity, equipment-free nature, and accessibility to the end-users. Researchers are working round the clock to fulfil the above criteria which has led to the emergence of a new ground breaking diagnostic field i.e. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based diagnosis. This tool integrates isothermal nucleic acid amplification techniques with the collateral activity of CRISPR-Cas enzymes, enabling rapid, precise and on-spot identification of foreign pathogens. This article highlights one such diagnostic test i.e. SHERLOCK - Specific High-sensitivity Enzymatic Reporter unLOCKing system.

Keywords: CRISPR-based diagnostics, SHERLOCK, CRISPR-Cas enzymes, isothermal amplification techniques, PCR, infectious diseases.

Undoubtedly invent of the PCR (Polymerase chain reaction) by Kary Mullis in 1985 has revolutionised the field of diagnostics. This technique has quickly become the gold standard for a countless number of infectious diseases and righteously so owing to its versatility and flexibility.

Nevertheless, even it has its limitations and drawbacks in terms of affordability issues, particularly in the resource limited regions of the world due to high cost of equipment, energy requirement and recurrent cost of reagents. Along with these, two more crucial challenges of PCR based diagnosis are – one is delay in results due to their complex visualisation i.e., performing AGE (Agarose gel electrophoresis) in conventional PCR and use of fluorescent probes in qPCR (real time PCR) and the other being, requirement of trained personnel for performing the test and for result interpretation. These limitations hinder the widespread adoption of this technique as a bed-side test, especially in the remote areas. Although during the COVID-19 pandemic, government and nongovernmental agencies around the world, even in low- and middle-income countries, tried to resolve this but PCR still somehow lacks in fulfilling all the

criteria for an ideal PoC (Point of Care) test by WHO. The criteria being – ASSURED – Affordable, Sensitive, Specific, User-friendly, Robust and Rapid, Equipment free and Deliverable to the end user.

The Invent of CRISPR-Based Diagnostics

Consequently, in order to find the next winner in this race for the gold standard test, a group of scientists, at Broad Institute of MIT and Harvard, USA – Kellner et al. (2019) introduced a new field of CRISPR (clustered regularly interspaced short palindromic repeats)-based diagnostics which includes a combination of nucleic acid pre-amplification techniques with advanced identification methods using CRISPR-Cas enzymes. The technique named as SHERLOCK. To be noted that the name is not based on the popular character of Sir Arthur Conan Doyle's hit detective series but is a shorthand of – Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) system – a CRISPR-based diagnostic platform but yes one can actually say that both are excellent detectives in their respective way. How? Let's see!

Before beginning, a brief overview of CRISPR-Cas system. This is a natural defence system of many bacteria and archaea organisms by which they identify and cleave the foreign nucleic acid molecules and makes them ineffective. CRISPR-associated (Cas) enzymes basically are endonucleases, widely used as gene editing tools in molecular biology. These enzymes are so efficient, precise and cost-effective that their discoverers – Emmanuelle Charpentier and Jennifer Doudna received Nobel Prize of 2020 in chemistry for this. Regarding its functioning, this system is dependent on a guide RNA (gRNA), which guides the Cas enzymes to recognise a particular target sequence and thus, cleave that target. CRISPR-Cas system is broadly divided into 2 classes- class 1 and class 2, which further has many subtypes. Class 2 systems are more commonly used in diagnostics because of their simplicity in designing and property of 'collateral activity'. Once a target molecule is locked by the gRNA, activation of Cas enzyme occurs. Along with cutting the target molecule, this enzyme starts cleaving the nearby non-specific nucleic acid molecules as well which is known as 'collateral activity' of these enzymes. This forms the basis of CRISPR-based diagnostic tests.

In SHERLOCK, CRISPR-Cas13 is used which is an RNA-dependent RNase enzyme responsible for multiple cleavage sites in a ssRNA target molecule (*cis* cleavage) as well as *trans* cleavage of other RNA molecules in near vicinity (collateral activity). The gRNA is designed in a manner that it is complementary to target sequence of our pathogen of interest (infectious agent for which the test is being developed). The bystander non-specific RNA molecules are actually reporter (fluorophore and quencher combination) molecules, which emits fluorescence on cleavage, thus, helping in easy visualisation and facilitating rapid detection of infectious agent once the particular target

sequence is identified.

This technique has a high degree of specificity and sensitivity as it can detect upto a single nucleotide change in the sequence and can detect even a single molecule in 1µl of RNA sample volume. But to detect in a large clinical sample, there is need for nucleic acid pre-amplification strategies to increase the concentration of target sequence in the bulk sample volume. As we want to develop a PoC test, PCR won't be suitable as mentioned above, so the alternative is use of Isothermal amplification techniques.

Coupled with Isothermal Amplification Techniques

Over the past few years, numerous Nucleic Acid Amplification Techniques have been developed which requires a constant temperature unlike PCR. To name a few – NASBA (nucleic acid sequence-based amplification), HDA (helicase-dependent amplification), RCA (rolling circle amplification), RPA (recombinase polymerase amplification), LAMP (loop-mediated amplification), SDA (strand displacement amplification), EXPAR (exponential amplification reaction). RPA can even be performed at ambient temperatures i.e., 37°-42°C although others require higher temperatures like NASBA (40°-55°C), EXPAR (55°C), LAMP (60°-65°C), SDA (60°C) etc. This can be achieved by any heating device such as a water bath which is comparatively easier to operate and maintain than a thermal cycler under field conditions.

Commercial SHERLOCK-based detection kits are already available in market for SARS-CoV-2 detection – comprising of pre-amplification using RT-RPA (reverse transcriptase RPA), followed by T7 transcription and target recognition by Cas13 enzyme. A modified version of this i.e., SHERLOCKv2, which uses RT-LAMP instead of RT-RPA is also available. Other similar assays have also been developed using different types of Cas enzymes – Cas12 and Cas9. CRISPR-Cas12a based tests – DETECTR (DNA endonuclease-targeted CRISPR *trans* reporter) – targeting dsDNA and ssDNA, though these enzymes require a PAM (protospacer adjacent motif) site in the target region of the target DNA molecule unlike Cas13 in SHERLOCK but like Cas13, it also possesses collateral activity and this technique also uses RPA as pre-amplification strategy. CRISPR-Cas9 based tests – NASBACC (NASBA-CRISPR cleavage) – comprises of NASBA as the pre-amplification strategy, a PAM-dependent target detection, Cas9 cleavage of target and a fluorescent sensor (as shown in Fig. 1). Others are CRISDA (CRISPR-Cas9-triggered SDA method), CAS-EXPAR (CRISPR-Cas9-triggered isothermal EXPAR strategy), HOLMES (one-hour low-cost multipurpose highly efficient system) etc.

Applications

These techniques have been developed for numerous infectious agents as mentioned below:

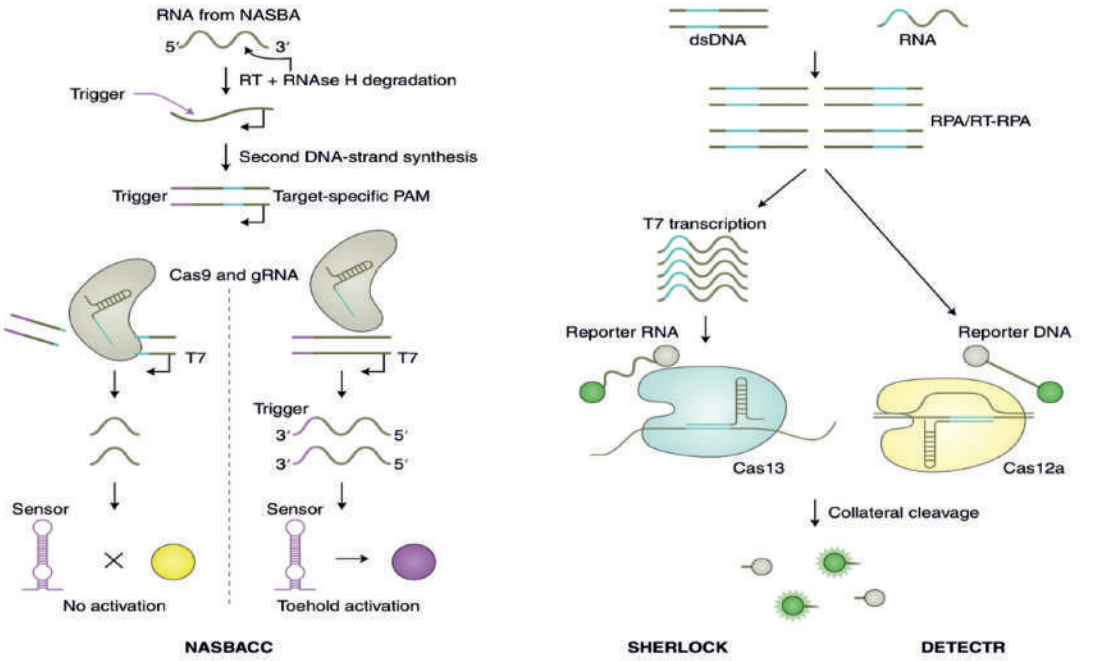

Fig:1. Schematic representation of NASBAC, SHERLOCK and DETECTR (CRISPR- based diagnostic tests) (Kaminski et al., 2021)

Viruses – Parvovirus B19, Dengue virus, Zika virus, Japanese encephalitis virus, Ebola, SARS-

CoV 2, Cytomegalovirus (CMV), Human Papilloma virus, Epstein-Barr virus etc.

Bacteria – *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*.

Parasites – detection of malarial parasite, *Plasmodium malariae, P. vivax, P. falciparum* and *P. ovale*.

Food-borne pathogens such as PCF detection method (PCR-CRISPR-fluorescence based nucleic acid detection) for *Salmonella* spp. with no cross reactions with other microbes in the sample and high sensitivity.

Similar to every other scientific invention, there are some obstacles to this technology as well. The major one is the requirement of amplification strategies as it makes the entire process tedious, time-consuming and complex by increasing the number of steps, thus, ultimately leading to delay in the results. Furthermore, operational characteristics of this technology under field conditions is under scrutiny owing to use

of complex reagents. However, these can be optimized to the field conditions by making low cost, portable, user-friendly, instrumentation free lateral flow assay devices having a one-pot testing reaction which makes the result interpretation convenient for the laymen.

Future Prospects

Nevertheless, despite these limitations, future of CRISPR-based diagnostic tests looks bright. Looking back only a few years ago, CRISPR-based tests were high profile tools used under laboratory conditions, but now actual toolkits are available in the market and many more are under clinical trials, which tells us about the rate with which this platform is growing. Going forward, integration of CRISPR-based sensors onto solid surfaces such as medical devices can be the next big thing as we have seen during COVID-19 pandemic, where nucleic acid sensors are incorporated onto the face masks and immediate detection of pathogen can be achieved. Or incorporating these sensors onto public places such as airports entry terminal, public washrooms (toilet seats) for immediate detection. The possibilities are countless although rigorous testing and trials are prerequisite before advocating for such technologies and installing them in public places.

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MESENCHYMAL STEM CELL UTILITY IN CANINE REGENERATIVE MEDICINE

Samridhi Singh and Ratan Kumar Choudhary*

Animal Stem Cells Laboratory, College of Animal Biotechnology (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: vetdrrkc@gmail.com

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Abstract

Regenerative medicine aims to develop ways to grow, repair, and replace dead or damaged cells, organs, and tissues. In the past few years, it has acquired substantial interest. Regenerative medicine originated from the concept of stem cell therapy. Stem cells found in the body have the potential to give rise to functionally matured cell types. It is found that stem cells can be raised into the mature cell type of interest in vitro, which can be used further to compensate for the body's failure to rebuild damaged tissues and perform metabolic functions. Mesenchymal stem cells appear most favorable because of their availability, easy isolation, and immunomodulatory properties. This article summarises the numerous stem cell types with present status and clinical usage of mesenchymal stem cells in canine research.

Keywords*: Dogs, Mesenchymal stem cells, Regenerative medicine*

Stem cells are a special kind of cells (blank cells), and have the potential to develop into a variety of cells with a specific function in the human/animal body. It can be said that stem cells are unspecialized cells that have the feature of self-renewal, can alter themselves into numerous types of cells, and multiply rapidly.

Stem Cell Types

The classification of stem cells (Fig. 1) can be done in two different aspects, based on their capability of differentiation (totipotent, pluripotent, multipotent, and unipotent stem cells) and others based on their source of origin (Embryonic (ESC), Adult, Mesenchymal, and induced pluripotent stem cells) (Rajabzadeh et al., 2019).

Stem Cells in Canine Regenerative Medicine

Stem cells are a promising tool in canine regenerative medicine, offering a range of applications for improving the health and well-being of dogs. These versatile cells can be harvested from various sources, such as adipose tissue and bone marrow, and then used to treat a variety of conditions (Fig. 1). A mobile app on "Stem Cells - Basic Understanding and Clinical Applications" developed by the College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Choudhary et al., 2023) is free to download. The app provides the fascinating world of

stem cells, tailor-made for veterinary clinicians, researchers, and students. Discover the latest breakthroughs and their practical applications in canine health by understanding the basic knowledge of stem cells and their mechanisms, including information on how to start a stem cell lab.

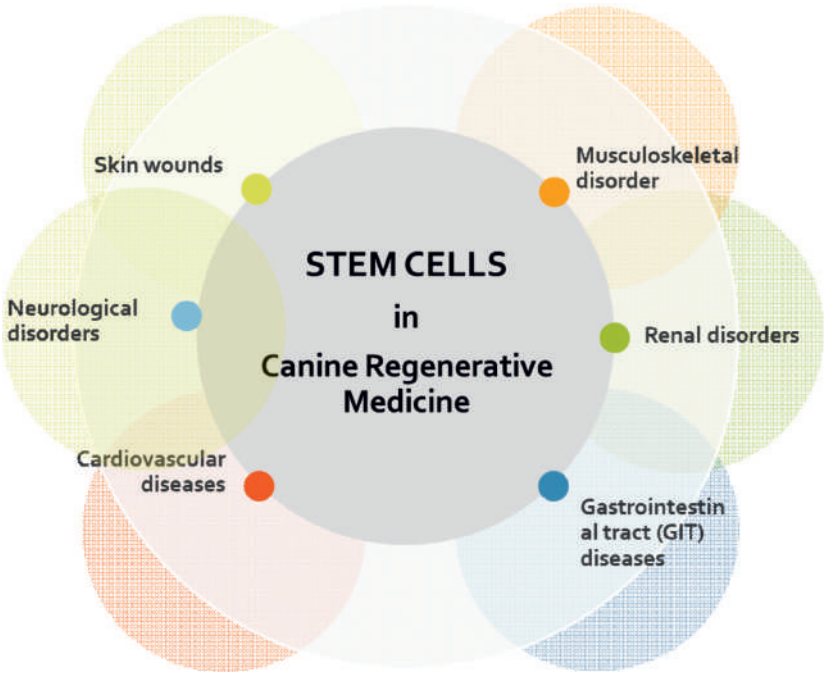

Fig. 1. Various applications of stem cells in canine regenerative medicine, offering promising solutions for improving the health and well-being of dogs.

Musculoskeletal Disorder: Mesenchymal stem cells (MSCs) are obtained from bone marrow (BM). BM-MSCs are easy to procure and have a high capacity to differentiate into different types of skeletal tissue. One pitfall of using BM-MSCs is the long incubation period of about 3 to 6 weeks is required for the growth of MSCs before treatment (Voga et al., 2020). In dogs' bone marrow aspirate is obtained from the proximal humerus, femur, or tuber coxae. MSCs are quite useful for the management of tendon and ligament injuries.

Renal Disorders: Lee et al. (2017) developed an acute kidney injury model of canine by introducing gentamycin and cisplatin and treating it with mesenchymal stem cells procured from the umbilical cord of a dog. They reported low mortality and indicated refined histological results.

Gastrointestinal Tract (GIT) Diseases: Treating gastrointestinal diseases can be complex and composite. It has convoluted morphological and functional characteristics as they are linked to different pathways. IBD is one such disorder that is commonly found in cats and dogs. This autoimmune disorder was earlier treated in dogs using steroids.

However, some animals stop responding to the steroids due to their usage for an extended period and, hence, are not ideal for IBD treatment. Allogenic adipose-derived MSCs were found useful for the management of IBD in dogs and cats. This therapy is promising for animals with IBD because of its anti-inflammatory and immunomodulatory properties (Pérez-Merino et al., 2015).

Cardiovascular Diseases: MSCs are primarily used in cases related to heart attacks because the cure is laborious as the cells that are accountable for provoking contractile force in the heart are infrequently regrown. The protocol used for the treatment depends on varied parameters like the type of cell being transplanted, mode of delivery, the time during which the treatment is carried out, and most importantly its prevalence. Preclinical trials showed that mesenchymal stem cells have the potential to regain cardiac function and regrow the impaired myocytes in a variety of canine models, including acute and chronic diseases (Kang & Park, 2020).

Neurological Disorders: Stem cells are being investigated for their potential to treat neurological conditions in dogs, such as spinal cord injuries, degenerative myelopathy, and other neuronal degenerative diseases (Gouveia et al., 2023).

Skin Wounds: Due to their anti-inflammatory property and regenerative capacity, MSCs may be a promising therapy option for chronic wounds with severe inflammation and hyperplastic response. Several animal investigations have indicated that MSC therapy improves wound healing in various animal species, including dogs (Falcão et al., 2019). At the university, in the College of Animal Biotechnology, work on stromal vascular fractions (SVFs) obtained from canine adipose tissue is underway. The SVFs typically refer to a mixture of cells and cellular components obtained from adipose tissue that are mainly present in the abdominal wall and periovarian area of female dogs. This mixture includes a variety of cells, such as adipocytes, progenitor cells, mesenchymal stem cells, endothelial cells, and immune cells. These fractions are used for the treatment of surgical wounds in dogs, lymphoma and other emerging areas like semen cryopreservation.

Acknowledgements:

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UNVEILING THE FUTURE OF CANINE SKIN HEALTH: CANINE SKIN EQUIVALENT

Shipra Tiwari, Ashwani Kumar[1], and Ratan K. Choudhary*

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

[2]Department of Veterinary Surgery & Radiology,

*Corresponding author email: vetdrrkc@gmail.com

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Abstract

The skin is both a passive and active barrier that shields the body from the surrounding environment, making its well-being vital for fulfilling this crucial function. Over the past several decades, the skin has garnered significant attention across various disciplines, including cell biology, medicine, toxicology, cosmetology, and pharmacology. In this article, we describe the skin equivalent and the methodology to create it in the lab. Next, we describe aspects of tests while using skin equivalent and traditional methods of live skin of the animal use. Finally, we described the utility of skin equivalents and discussed the future of generating canine skin equivalents in our lab. This article underscores the significance of canine skin equivalents as a valuable tool in advancing clinical veterinary science, offering a humane and controlled platform for research, testing, and education in canine dermatology and beyond.

Keywords*: Animal welfare, bioengineered tissue, canine, skin equivalent, veterinary dermatology*

The field of veterinary science has made many advances in recent years, with researchers being busy finding innovative solutions to improve the well-being of our little furry friends. As we know, skin is the largest body organ and thus plays a crucial role in providing a protective barrier against external threats; thus, the development of canine skin equivalent is a major contribution to veterinary research, revolutionizing canine dermatology.

Canine skin equivalents are bioengineered tissue constructs that mimic the structure and function of natural skin. These skin models comprise living cells and biological components, accurately representing the canine epidermal (keratinocytes) and dermal (fibroblasts) layers.

Procedures for Making Skin Equivalent

A canine skin equivalent is made in the laboratory by growing skin cells in three dimensions that mimic the structure and function of real canine skin. In a study, a canine skin model was created and characterized (Serra et al., 2007). Epidermal keratinocytes and dermal fibroblasts were freshly obtained from skin biopsies of healthy dogs. The

fibroblasts were incorporated into a collagen type I matrix protein-based bio-matrix, serving as the foundational structure where the keratinocytes were introduced under air-exposed conditions. After culturing for 3, 7, 15, and 21 days in specialized growth media, the skin equivalents were analyzed using histological, immunohistochemical, and electron microscopy techniques. By the 15th day, the keratinocytes had undergone differentiation, resulting in a multilayered epidermis comprising all histological layers of the skin.

Here are some basic steps for creating artificially grown skin, not specific for dogs but in general.

1) Isolation and Culture of Keratinocytes and Fibroblasts

a. Sample collection: A small skin biopsy is obtained from a healthy skin donor via surgical procedures. The skin tissue sample is brought to the lab in PBS, or media premixed with antibiotics onto the ice for further processing (Fig. 1).

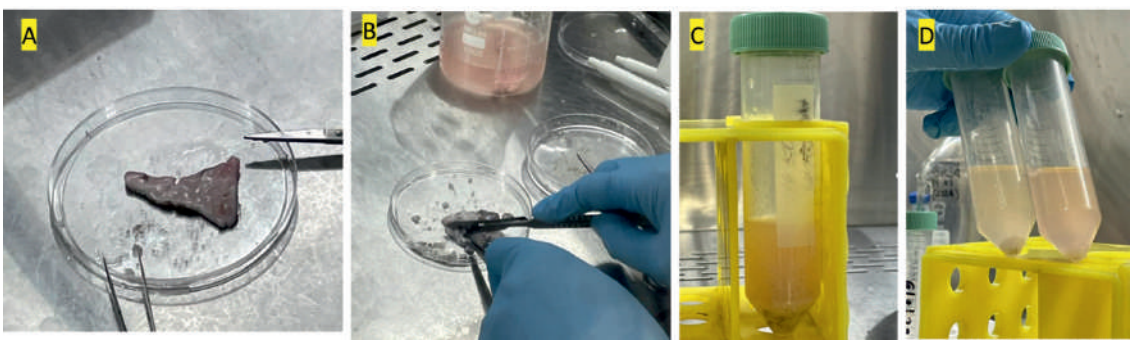

Fig. 1. Preparation of canine skin for harvesting fibroblasts and keratinocytes. Skin tissue is washed with PBS 3-4 times (A) and then chopped into pieces using scissors or a blade (B). Mechanically macerated skin tissue is digested using enzymes (C) followed by centrifugation (D) to harvest various skin cell types.

b. Keratinocytes isolation: Digest the skin sample with an enzyme mixture (dispase, trypsin) to dissociate the epidermis and collect Collect keratinocytes from the epidermal layer using centrifugation.

c. Fibroblast isolation: After keratinocyte isolation, the remaining dermal tissue containing fibroblasts is digested using an enzyme mixture (collagenase/trypsin).

2) Scaffold-based or scaffold-free techniques for 3D culture

- 3D matrices like collagen or animal-derived extracellular matrix (ECM) or spheroid formation in scaffold-free culture are used to cast the collagen gel in culture dishes to create the bio-matrix. In scaffold-free technique, non-adherent

cell aggregates called spheroid are forms that mimic solid tissue and secrete their own ECM.

3) Obtaining skin equivalent

- Cell seeding: Seed keratinocytes onto the surface of the collagen gel bio-matrix and keratinocytes are grown. Co-culture keratinocytes with fibroblasts within the collagen gel to stimulate the dermal layer.

4) Tests for the characterization of skin equivalents

a. Histological Analysis: To visualize tissue structure, fill and section the skin equivalent for histological staining (Hematoxylin and Eosin, Masson's Trichrome).

b. Immunohistochemistry: Using immunohistochemical staining, assess the expression of specific skin markers (e.g., cytokeratin for keratinocytes, collagen for fibroblasts). Readers are referred to the article for characterization of the organization of canines by IHC and qPCR (Wiener et al., 2021).

c. Barrier Function Assessment: Measure the permeability and barrier function of the skin equivalent by assessing trans-epidermal water loss and dye penetration.

d. Viability and Proliferation: Evaluate cell viability and proliferation rates within the skin equivalent through calorimetric assays like reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals (called MTT assay).

e. Monolayer Scratch Assay: This assay tests the fibroblast and endothelial cell migration response to drug/compound on wound healing and the proliferation potential of skin cells, especially if someone wants to know.

Table 1. Differences between the use of canine skin equivalent and skin graft

Aspect	Canine skin equivalent	Traditional methods (live animal testing)
Ethical considerations	Humane & cruelty-free	This may raise ethical concerns
Animal welfare	No harm to live animal	Potential harm & suffering in animals
Control over variables	Precise control	Variables influenced by animal's physiology, stress & health
Cost-effectiveness	Long term	Higher costs associated with animal care

Reproducibility	Consistent & and standardized models	Variability due to individual animal differences
Speed of examination	Faster due to controlled conditions	Many require longer experimental timelines
Human safety	Reduces risk of animal-related injuries	Potential zoonotic risk to researchers
Applicability to in-vitro tests	Suitable for in-vitro testing of drugs, cosmetics and therapies	Limited to in-vivo testing
Regulatory approval	Follows the cruelty-free research standards	Subject to strict regulatory & and ethical conditions

Skin equivalent applications

1. **Advancing Veterinary Research -** Skin equivalents provide an ethical and controlled platform for researching various skin conditions that affect dogs. Skin diseases like dermatitis, allergies, and infections can be tested using skin equivalents to provide a better and more effective way of skin diseases in dogs.
2. **Product Testing for Dermal Toxicity –** Pet care products that could cause allergies, drugs, or dressing materials can utilize skin equivalents for testing (do Nascimento Pedrosa et al., 2021).
3. **Clinical Use in Burns and Chronic Wounds -** The placement of skin equivalence within a chronic wound may share structural attributes with a skin graft and accelerate wound closure. Skin cell culture and growing appendages like hair and sweat glands called "skin organoids" have the advantages of a tissue system full of complexity (do Nascimento Pedrosa et al., 2021; Lee & Koehler, 2021).
4. **Education and Training –** Veterinary students and faculty can use canine skin equivalents as education tools. It provides hands-on experience, allowing them to learn about canine health and various skin problems.
5. **Viable Substitute as Skin Graft** – Severe loss of skin due to degloving injuries or following resection of larger cutaneous masses creating larger wounds that pose difficultly in suturing. Even advanced skin flaps fail to cover such larger wounds effectively (Kaur et al., 2023). Such skin equivalents would be highly useful in such clinical situations requiring wide surgical resection.

Skin equivalents contain the majority of the *in vivo* state response. It is a good model to study cell-to-cell response that is linked to mesenchymal to epithelial cell signaling pathways (Stabell et al., 2023).

Future Directions

Guru Angad Dev Veterinary and Animal Sciences University has started working on developing a canine skin equivalent under a research project supported by the Department of Biotechnology (DBT, New Delhi). The research aims to develop mesenchymal stem cell-based regenerative therapy for enhanced closure of surgical wounds in dogs. The *in vitro* study will utilize canine skin equivalent to test the safety, efficacy, and insights of regenerative therapy in canines. Canine skin equivalent holds great promise for canine dermatology and other veterinary research, offering ethical and scientific advantages that significantly benefit pet animals. Culturing skin cells along with appendages, mainly sweat glands and hairs, called "skin organoids," will have advantages in reconstructing damaged skin.

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BLOOD TRANSFUSION IN CANINE PATIENTS: A LIFE SAVING MODALITY

Sujata Turkar and Silpa S. N.

Department of Veterinary Medicine, College of Veterinary Sciences (Ludhiana),
Guru Angad Dev Veterinary and Animal Sciences University,
Punjab-141004, India
*Corresponding author email: singhopinder68@gmail.com

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Abstract

Blood transfusion is a critical and life-saving modality in Veterinary Medicine, allowing veterinarians to address a wide range of medical conditions and emergencies in dogs. It helps to restore blood volume, improve oxygen delivery, and stabilize patients in critical conditions, ultimately improving the chances of recovery and survival of canine companions. This article will give a brief knowledge about indications, blood group, cross matching, selection of donor, collection of blood from donor and post transfusion complications.

Keywords*: Anaemia, Blood transfusion, cross matching, dogs, transfusion reactions*

Blood transfusion is one of the crucial life-saving procedures in small animal practice. Transfusion is performed to replace blood or its components from an individual of same species. Blood is a biological drug they do not provide a cure and is not a definitive treatment but it provides support until disease is diagnosed or treatment is initiated.

The history of blood transfusion dates back centuries. Richard Lower is credited with performing first animal-to-animal transfusion in dogs in 1665. Since then, transfusion medicine has made significant advancements, moving from administration of whole blood to targeted component therapy which meets specific demand and requirement of the patient (Yagi & Holowaychuk, 2016). As with any medical procedure, blood transfusion requires careful consideration, proper matching of blood types, screening of potential infections and vigilant monitoring during and after transfusion.

Indications

a) Anaemia

Anaemia is the main indication for blood transfusion. Anaemia can occur as a result of blood loss due to haemorrhage or red blood cell destruction or reduced red blood cell production. The most common causes of anaemia in dogs are haemoprotozoan infections (*Babesia gibsoni, Ehrlichia canis* and *Hepatozoon canis*) and chronic liver and kidney diseases (Khan, 2021). Usually, transfusion is indicated when PCV falls below

20% and haemoglobin falls below 5 g/dl (Choudhary *et al.,* 2017). Animals with non-regenerative and haemolytic anaemia are usually normovolemic. Transfusion trigger for normovolemic anaemia is much less than hypovolemic anaemia, as it is well tolerated. Transfusion is recommended when the animals begin show clinical signs of anemia like tachycardia, tachypnoea, and weakness. In such cases packed RBCs (pRBCs) are the component of choice and should be administered slowly to avoid volume overload. Hypovolemic anaemia is due blood loss in which whole blood transfusion is necessary along with volume replacement by crystalloids or colloids if the haemorrhage is severe (Rozanski & Laforcade, 2004).

b) Haemostatic disorders

- Acquired or congenital coagulopathies

  Plasma transfusion is indicated in coagulopathies. Haemophilia A or B and von Willebrand's disease are the main inherited coagulopathies that is benefitted from plasma transfusion. Aetiology for acquired coagulopathy includes anticoagulant rodenticide toxicities, sepsis with and without disseminated intravascular coagulation (DIC), neoplasia, liver failure, and over-heparinization.

- Thrombocytopenia

  Platelet rich plasma or platelet concentrate can be used but severe thrombocytopenia cases are benefitted from fresh blood transfusion (Helm and Knottenbelt, 2010a).

c) Trauma with subsequent haemorrhage to restore blood volume and oxygen carrying capacity (Rozanski & Laforcade, 2004).

Blood Groups

Blood groups are identified based on antigen (glycoprotein or glycolipid) present in the red cell membrane, known as Dog Erythrocyte Antigen (DEA).Over 13 blood types are present in dogs but only six dog erythrocyte antigens have been identified as per international standard which are DEA 1.1, DEA 1.2. DEA 3, DEA 4, DEA 5 and DEA 7. DEA 6 and DEA 8 were also recognized but antisera of these are not available (Hale, 1995). DEA 1.1 and DEA 1.2 are the major blood group found in about 60% of the population. A dog donor with blood type negative for DEA 1.1, 1.2 and 7 is considered as true universal donor as they are highly antigenic. Apart from DEA, Dal is a common blood type identified in Dalmatian (Blais et al., 2007).

Donor Selection (Helm & Knottenbelt, 2010b)

One of the most important steps in ensuring that the blood collected is safe and useful for transfusions is choosing blood donors. Canine blood donors must fulfil specific requirements in order to be eligible for donation, much as human donors. The following are some considerations that are frequently made when selecting canine blood donors:

1. Healthy and friendly
2. Normal on physical examination
3. Properly vaccinated and dewormed
4. The ideal age for the donor falls between 1 to 8 years
5. Recommended body weight of the donor is 22.7 kg (25-30 kg)
6. Packed cell volume of ≥35%
7. No previous pregnancies and female donor should be neutered.
8. No previous history of transfusion
9. Free from infectious diseases like Babesiosis, Brucellosis, Ehrlichiosis, Anaplasmosis, Trypanosomiasis etc.
10. DEA 1.1 and 1.2 negative

Cross Matching and Blood Typing

Cross matching is a serological test that is necessary before transfusion especially in pre-transfused dogs to prevent immune related post transfusion reactions except in emergency conditions. It can be used along with blood typing or when blood typing is not available. However, cross matching does not ensure that the animal is safe from transfusion complications. Cross matching does not provide any guarantee over delayed transfusion reaction, reaction to donor plasma protein, white blood cells or platelet compatibility (Lanevschi & Wardrop, 2001)

Cross matching test is divided into major and minor cross matching. In major cross matching, compatibility between donor RBCs and the recipient plasma is determined to find the presence of alloantibody against donor red blood cell antigen in recipient body. In minor cross matching compatibility between donor plasma and recipient RBCs is determined to find the presence of alloantibody in donor's blood. However, if the donor has not received any transfusion before, the chance of haemolysis or agglutination to occur in minor cross matching is very low. If agglutination or haemolysis is absent, the donor blood is can be used for transfusion. Minor cross-matching is insignificant since the volume of plasma provided is little and is diluted in the recipient. When there is autoagglutination or no suitable units are available, transfusing the least incompatible unit may be necessary.

The following are steps of crossmatching technique (Lanevschi & Wardrop, 2001):

1. Collect blood samples in both an ethylenediamine tetra acetic acid (EDTA) tube (purple top) and a non-additive tube (red top) from both the donor and the recipient.
2. Centrifuge the samples to separate plasma and serum from RBCs. Transfer the extracted serum in separate tube, discarding the plasma

3. Rinse the RBCs from the EDTA tube using saline. Centrifuge the tube for 1 minute, pour off the saline and repeat the process for three times, discarding the supernatant on the final round
4. Re-suspend the RBCs in saline solution to make a solution of 2 to 4%
5. For major cross match mix 2 drops of patient serum with 1 drop of donor RBC suspension and 1 drop of patient RBC suspension with 2 drops of donor serum for minor cross match in a tube and label accordingly. For control mix 1 drop of patient RBC suspension with 1 drop of patient serum
6. Incubate the mixture at 37°C for 15 to 30 minutes
7. Centrifuge the mixture for 15 minutes
8. Interpretation: If there is visible macroscopic haemolysis or hemagglutination, or if microscopic agglutination is present, then the donor blood is not a suitable match (fig1)

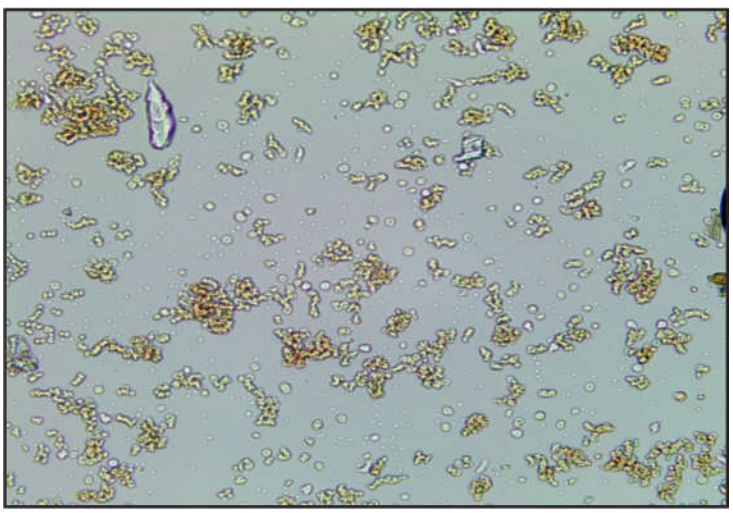

Fig. 1. Incompatible cross matching

Now blood typing kits are also commercially available for DEA 1.1 and 1.2. These kits are readily available for typing in practice and easy to use and accurate (Fig 2).

Blood Collection and Administration

Dogs can donate 10 per cent of their total blood volume without any adverse effect (blood volume = 90 ml/kg in dogs), ie 10 ml of blood per kg of body weight can be collected from a donor dog. Collection of up to 20 per cent of blood volume should not result in clinically significant anaemia provided the donor has a normal PCV at the time of collection, although it can produce transient hypovolaemia in the short term. Collection of >20 per cent of blood volume can produce significant hypovolaemia and

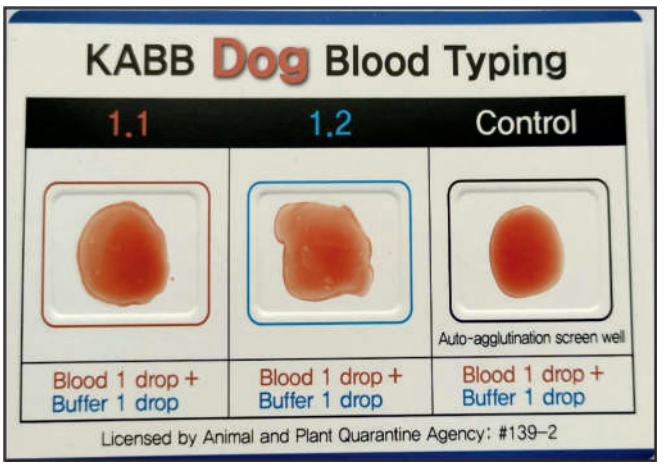

Fig 2. Dog blood typing kit (DEA 1.1 and 1.2 negative)

compromise the health of the donor and is not recommended.

Canine blood is often collected directly into a human blood collection bag which contains acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD) anticoagulant for approximately 350 ml of blood. The use of ACD or CPD as anticoagulant allows the blood to be stored for up to three to four weeks without significant loss of red blood cell viability, provided it is kept at 4 to 5°C. Citrate phosphate dextrose adenine (CPDA) is used at the ratio of 1:7 (1ml anticoagulant for 7 ml blood). If a donor is very agitated, aggressive or difficult to handle, opioid such as butorphanol (0·1 to 0·3 mg/ kg intramuscularly or intravenously) alone or in combination with acepromazine (0·01 to 0·03 mg/kg intravenously) can be used. But, due to the risk of hypotension, intravenous fluids should be given for up to an hour following donation (Davidow, 2013; Yagi and Holowaychuk 2016). Donor's blood is usually collected from jugular vein aseptically into blood collection bag (fig 3).

Fig 3. Collection of blood from donor in CPDA bag

The amount of blood to be transfused can be determined by the following formula (Choudhary et al., 2017).

$$\text{Amount of blood required} = \text{Body wt. (kg)} \times 90 \times \frac{(\text{Desired PCV} - \text{PCV of recipient})}{\text{PCV of donor}}$$

A basic guideline for smaller animals suggests that infusing 20 mL/kg of whole blood can raise the PCV by approximately 10%, assuming the donor's PCV is around 40% (Kumar, 2017).

For example, a recipient dog's body weight =10 kg, recipient's PCV = 10% and desired PCV=25%. Donor's PCV = 45%.

Now, the amount of blood required for transfusion = 10x90x(25-10)/45= 300 ml.

Blood should be infused into the cephalic or jugular vein of recipient via an intravenous catheter. In severely hypotensive or paediatric patients, blood can also be administered by intraosseous route (trochanteric fossa of the proximal femur). Marrow transfusions are nearly as successful as direct intravenous infusions because the absorption of blood from the marrow cavity into the circulation is very efficient. It is not advised to provide blood intraperitoneally since it is ineffective and only absorbs 40% of the blood administered.

For the first 15 minutes, the rate of transfusion should be 0.5-1 ml/kg/hour, to detect immediate transfusion reaction. All vital signs should be monitored every 15 minutes in the first hour, followed by every 30 to 60 minutes afterwards. Rapid transfusions can result in vomiting, muscle fasciculations or vomiting. However, rate of transfusion can be assessed individually depending on patient status (hydration status and severity of anaemia). The entire procedure should be completed within 4 hours to minimize bacterial contamination of the collected blood unit (Davidow, 2013).

Blood should not be administered concurrently with intravenous fluids containing calcium or glucose, or with lactated Ringer's solution. No medications or solutions other than 0·9 per cent sodium chloride should be added or infused through the same tubing as blood products.

Post Transfusion Complications

Even though transfusion is a lifesaving technique, potential risks are unavoidable. Transfusion reactions are classified as immunologic and non-immunologic reactions (Table 1).

Handling Suspected Haemolytic Transfusion Reactions

- Stop the transfusion immediately.
- Maintain the intravenous line and administer crystalloid solutions.
- Monitor the animal for evidence of shock or disseminated intravascular coagulation, and check the patient's temperature. In addition, test serum and urine for evidence of haemoglobinaemia and haemoglobinuria, respectively.
- Administer supportive therapy (eg, oxygen supplementation, antihistamines, adrenaline).

Table 1. Common Clinical Signs of Transfusion Reactions (Nusbaum, 2021)

Immunological Reaction			
Haemolytic	**Hypersensitivity**	**Febrile**	**Delayed**
Fever Tachycardia or bradycardia Hypotension Dyspnea Cyanosis Salivation Lacrimation Urination Defecation Emesis Collapse Opisthotonos Cardiac arrest Hemoglobinemia Hemoglobinuria	Hives Edema Redness Vomiting Diarrhea Tachypnea Tachycardia Hypotension	Rise in temperature immediately or within 2 hours of the start of a transfusion	Anemia Fever Icterus
Non-immunological Reaction			
Sepsis	Circulatory overload	Hemolysis	Citrate intoxication
Fever Tachycardia Tachypnea Hypotension Hypoglycemia Hemoglobinemia Hemoglobinuria	Tachypnea Orthopnea Dyspnea Cyanosis Coughing Abnormal lung sounds Peripheral edema Jugular distention	Hemoglobinuria Hemoglobinemia	Face rubbing Vomiting Tremors Tetany Cardiac arrhythmia

- Check the blood bag for evidence of lysis by capillary tube centrifugation and collect a sample of blood for culture and sensitivity testing.
- Check blood typing or cross-matching.

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CLINICAL DIFFERENTIATION OF PROLAPSED INTUSSUSCEPTION AND RECTAL PROLAPSE

Ameya Jadhav* and Nikita Gupta

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: ameya0612@gmail.com

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Abstract

Rectal prolapse is principally associated with endoparasitism or enteritis in young animals, and tumors or perineal hernias in middle-aged and older animals. Intestinal intussusceptions most often occur in young dogs. Intussusceptions may protrude from the rectum and can be mistaken for a rectal prolapse. To diagnose intussusception, ultrasound is the only reliable technique. But, the prolapsed intussusception and rectal prolapse can be differentiated clinically without ultrasound or radiography. This article describes the technique which will allow veterinarians to identify the intussusception prolapse from the rectal prolapse in the absence of ultrasound facility and would aid in appropriate choice of surgical correction of the condition.

Keywords: *Dogs, intussusception, rectal prolapse, surgical, young*

Prolapse of mass from rectum is very common affection observed in routine canine practice. The animal is presented with certain mass protruding out of its anal opening and the dog is in weak dehydrated state. Such dogs usually have history of diarrhoea or constipation leading to straining. These cases may be misdiagnosed as rectal prolapse and are manually repositioned along with purse string suturing. The prolapsed mass cannot be identified to be rectal prolapse or intussusception prolapse by appearance itself. Untreated intussusception may lead to severe necrotic changes of the affected intestinal segment and further reducing the prognosis of the condition or even death.

What is intussusception?

- It is a medical condition in which a part of the intestine prolapses or invaginates into the lumen of another part of bowel (Fig. 1).
- Intussusceptum is the invaginated segment of the alimentary tract, whereas, the intussuscepiens is the enveloping segment.

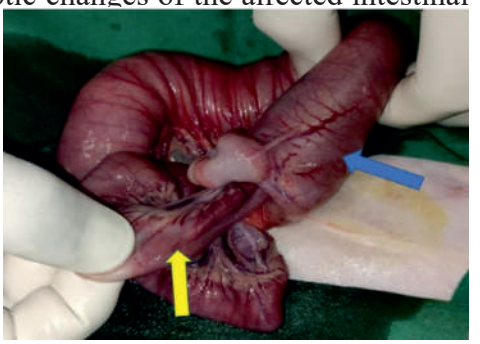

Fig. 1. Invagination of Intussusceptum (yellow arrow) into Intussuscipien (blue arrow).

- Diagnosis of the intussusception can be made by abdominal palpation, radiography and ultrasonography (Patsikas et al., 2019).
- The affected bowel may be palpable as a sausage-shaped intra-abdominal mass.

Clinical Signs of intussusception:

- Iintermittent vomiting, progressive loss of appetite, mucoid bloody diarrhoea and a palpable cylinder- shaped mass in the cranial abdomen, depression and anorexia.
- Ultrasonography (USG) is considered an accurate diagnostic method to diagnose intestinal intussusception in Dogs (Fossum et al., 2013).

Rectal Prolapse

- Rectal prolapse is protrusion of a portion of the rectum or rectal mucosa through the anus, usually caused by an underlying disorder. Rectal prolapse grossly appears similar to intussusception prolapse.
- Straining or recent perineal surgery is common, but is most commonly seen in young brachycephalic breeds with endoparasitism or kept in poor hygiene or more dry/constipated diet.

Differential Diagnosis

- A prolapsed intussusception can be differentiated from true rectal prolapse, as a finger or small blunt probe (like thermometer) can be passed between a prolapsed intussusceptum and the rectal wall (Kumar et al., 2007) more than 5-6cm (Fig. 2),

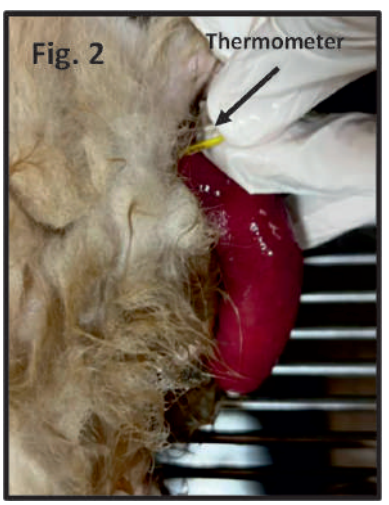

Fig. 2. Photograph showing a thermometer (Black arrow) is inserted more than 5-6cm inside lateral to prolapsed tissue.

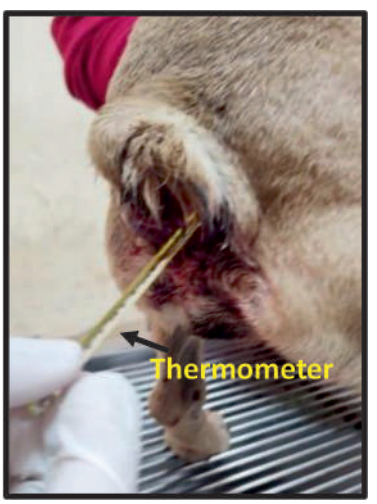

Fig. 3. Photograph showing a thermometer (Black arrow) is not inserted more than 1cm inside lateral to prolapsed tissue.

whereas with a true rectal prolapse (Fig. 3) nothing can be inserted lateral to the prolapse more than 1-2cm (Fossum et al., 2013; Moores et al., 2021).

Treatment

- **For Rectal prolapse,**
  - **Purse string sutures:** In early presented cases.
  - **Colopexy:** If no improvement with purse string, chronic condition but the rectal mucosa is still clean without erosions.
  - **Rectal resection/Amputation:** Chronic condition with no improvement with purse string and mucosa is injured.
- **For Prolapsed Intussusception:** Midline **e**xploratory laparotomy is required.

The present report describes the differential diagnosis of rectal prolapse and prolapsed intussusception, clinically. This will help in decision making and plan for early exploratory laparotomy for the correction of intussusception prolapse when identified.

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TECHNIQUE OF UMBILICAL HERNIA REPAIR IN CALVES

Abhishek Verma, Kartik Sharma 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: sangwanvandana1@gmail.com

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Abstract

Umbilical hernia is a common condition in cattle calves and mostly need surgical repair. The repair in small calves can be done under local anesthesia and injectable general anesthesia/ sedation under field conditions. The article describes the technique of umbilical hernia repair in young calves.

Keywords*: Calf, congenital, cow, umbilical hernia*

Umbilical hernia is primarily a developmental and hereditary in origin. The umbilicus in newborn calves consists of urachus and the remnants of umbilical vessels that transport blood from fetus to its mother. Umbilical hernia develops due to faulty closure of umbilical opening at birth or hypoplasia of the abdomen muscles (Fig. 1) (Tyagi & Singh, 2010). Umbilical hernia when contaminated with abscess, bear poor prognosis. Such cases may be drained first for abscess and later repaired for hernia when infection heals. Besides, delayed cases may lead to strangulation and rupture / fistulation of the herniated organ which is abomasum in most cases (Sangwan et al., 2011).

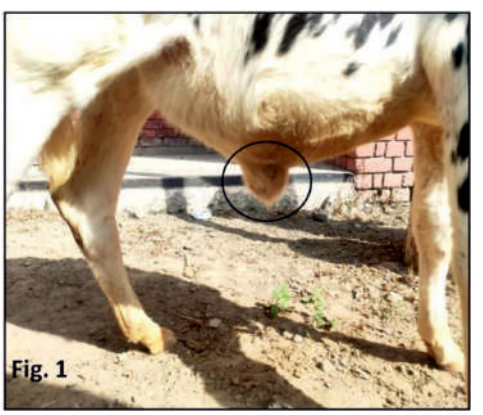


Fig. 1. Reducible umbilical hernia in a cow calf

The repair of umbilical hernia should be preferred in dorsal recumbency to allow least pressure on the suture line and to avoid interference of the abdominal organs during herniorrhaphy procedure. Maintenance of dorsal recumbency with minimal movement and to maintain asepsis, the calves may need general anesthesia. The young calves can be maintained on injectable general anesthesia and local anesthetic. While the older calves/ heifer/cows need inhalant general anesthesia for dorsal recumbency. Break in asepsis and placement of loose sutures, are the two prominent reasons for re-occurrence of hernia following repair. The technique of umbilical hernia repair described in this article is mainly for small calves (1-3 months) which can be easily managed with injectable anesthetics.

Materials Required

Sterile surgical pack, Sterile gloves, Syringes, Local anesthetic, Antibiotics, Analgesics, Sterile silk thread no. 2, Inj. Diazepam and Inj. Ketamine or inj. xylazine

Pre-surgical preparation

- The young calves are kept off-milk for 3 hrs if only on milk. The 2-3 month or older calves taking some fodder need off feed and off water for 12 hrs.
- Place the calf in lateral recumbency on a table/trolley and restraint.
- Place a 20 gauze IV cannula in the ear vein and start NSS fluid.
- Load 2ml inj. diazepam and 2ml inj. ketamine in a single syringe, if available. Otherwise, the young calves can be managed on local anesthetics alone and the older ones may be given 0.1ml xylazine for a 100 kg body weight, IV before incision. Xylazine should be avoided in less than 80Kg calves.

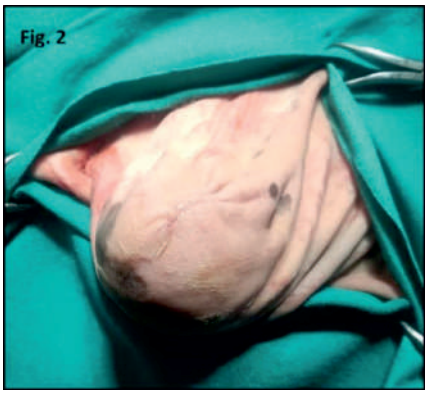


Fig. 2. Aseptically prepared and draped site of surgery.

- Shave the site of surgery. In-filter local anesthetic subcutaneously and into muscle margins at the site of incision. Load local anesthetic in 5ml syringe, especially, in very young calves to avoid over-dose. For calves upto 50kg, 5 ml local anesthetic is sufficient and safe.
- Scrub the site of surgery with savlon (5-6 minutes) (Fig. 2) and surgeon should also scrub hands.

Technique

- Initially the surgery may be started in lateral recumbency which can be converted to dorsal while applying sutures. In young calves, the calf limbs (both fore and both hind) may be hand held in dorsal recumbency.
- Laterally, separate the subcutaneous tissue underneath the incision with a scissors.
- Blunt dissection of the tissue is done deeper but always laterally so that the center stump is not incised blindly. The center stump sometimes may contain adhered abdominal organs/intestines which may be incised accidently if approached blindly (Fig. 4).
- When reaching the center stump/peritoneum, give a small stab incision and carefully cut it laterally all around.

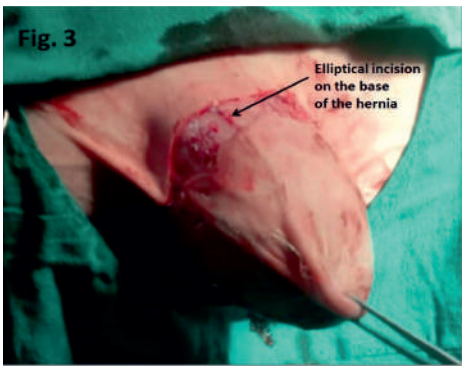


Fig. 3. An elliptical incision is made at the base of the hernia.

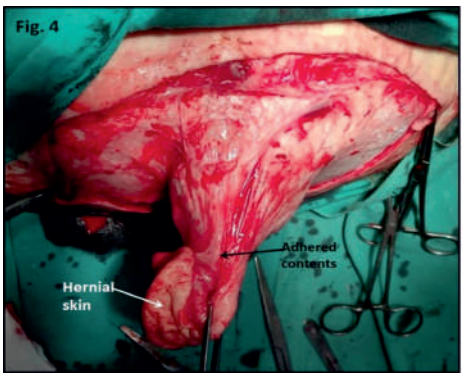


Fig. 4. A part of abdominal organs or omentum adhered to the inside the tip of hernia,

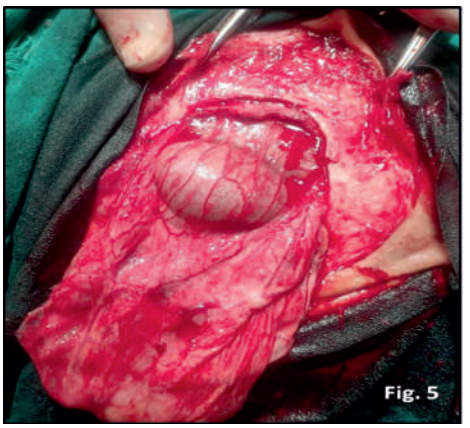


Fig. 5. Visible clear hernia ring with omentum and contents protruding out

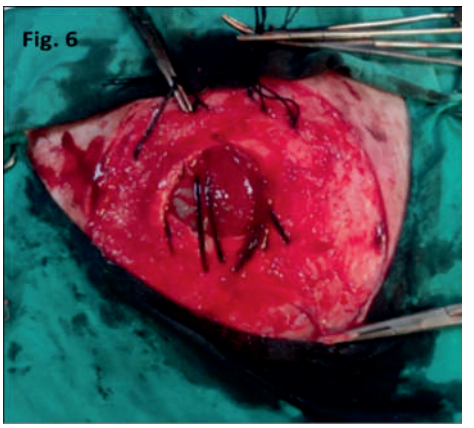


Fig. 6. Vest over pant sutures applied with silk no. 2

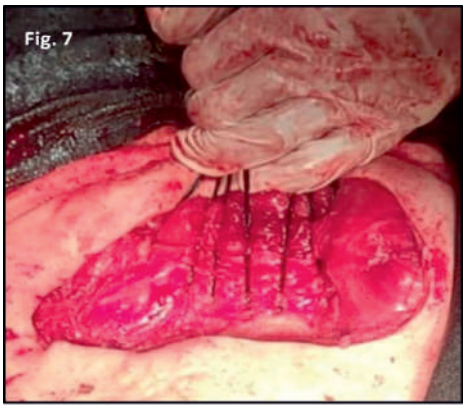


Fig. 7. Pulling of vest over pant sutures and applying the knot.

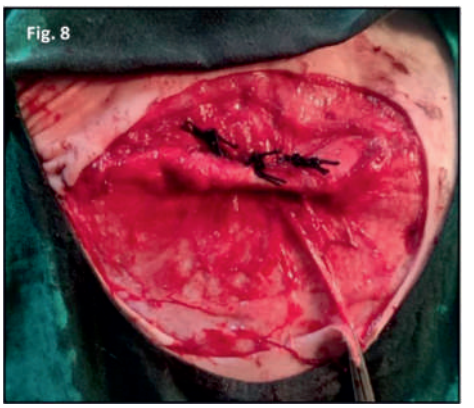


Fig. 8. Applied vest over pant sutures.

- Now the contents are free and can be reposed back into the abdomen (Fig. 5). At this stage the calf must be made in dorsal recumbency.
- The hernial contents are manually replaced back into the abdomen.
- Close the hernia ring with vest-over pant sutures using sterile silk thread (no. 2, double) (Fig. 6, 7, 8).
- One more layer of single thread continuous suture may be applied over the vest over pant embedding the muscle edges with abdominal muscles.
- Skin is closed with cross mattress suture pattern using sterile silk thread (no. 2) (Fig.9a).
- The Suture line may be covered with a sterile gauze piece and tied with stay sutures (Fig. 9b) or with a Dynaplast tape (Fig. 9c).
- The Suture line need to be bandaged with a cotton cloth (in small calves) or with cotton *Nivar* (in sub-adult and adult) calves till sutures are removed (approximately at 14 days) (Fig. 10).

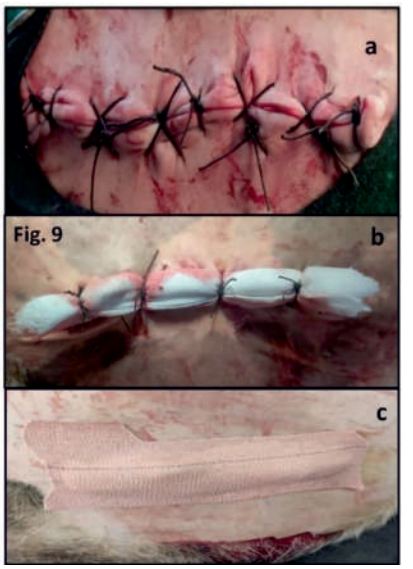
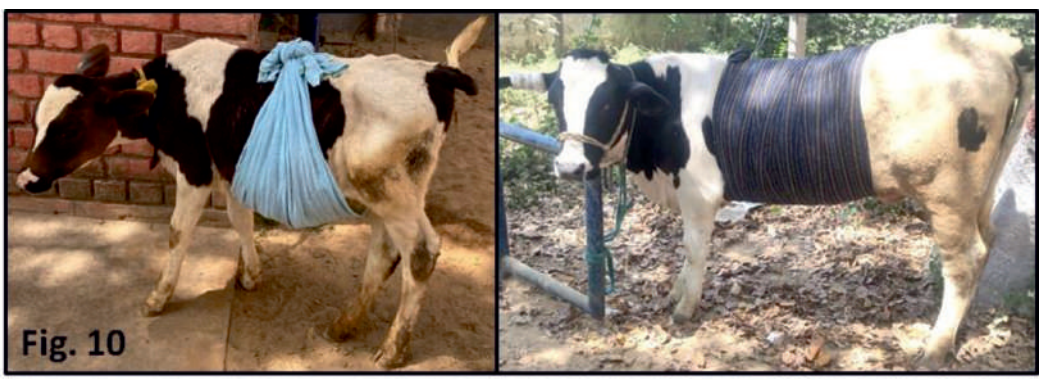


Fig. 9. Photograph showing cross mattress skin sutures (a), sterile gauze cover with stay sutures (b) or wound covered with dynaplast tape.



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BRACHYCEPHALIC OCULAR SYNDROME: COMMON OPHTHALMIC CONSIDERATIONS

Ajaybir Singh Dhaliwal* and Ashwani Kumar

Department of Veterinary Surgery & Radiology, College of Veterinary Science (Ludhiana)
Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141004, India
*Corresponding author email: dhaliwalajaybir31@gmail.com

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Abstract

Small brachycephalic breeds, which include the Pug, Shih Tzu and French bulldog, are currently in tremendous demand. These breeds' conformation is part of their attractiveness to owners; however, they are susceptible to ocular surface illness such as corneal ulcers and pigmentation. The write up describes the various considerations relating to eyes of brachycephalic breeds.

Keywords: *Brachycephalic, canine, canthoplasty, ophthalmic surgery*

Brachycephalic ocular syndrome refers to the ophthalmic difficulties linked with certain breeds. The tear film, eyelids, and cornea are frequently in close contact with dolicocephalic and mesocephalic dogs. This does not appear to be the case with breeds with brachycephalic ocular syndrome, which is characterized by inadequate cranium & eyelid conformation, corneal sensibility, and tear films, as well as ocular issues that include corneal ulceration and pigmentation, in addition to a proclivity for globe proptosis. The report describes the various conditions of eyes in brachycephalic breeds long with their suggested precautions and treatment options.

Corneal Disorders in Brachycephalic Breeds

Inability to efficiently blink predisposes the eyes to prolonged exposure, which can result in a broad variety of corneal disorders, including, but not restricted to:

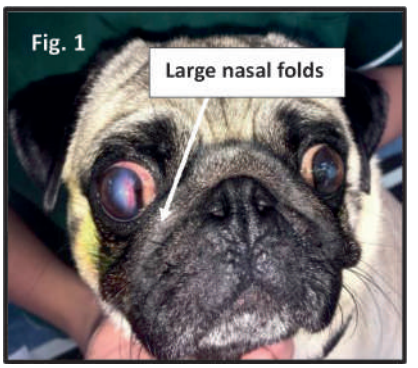
- Corneal ulcerations and erosions
- Vascular keratitis
- Pigmentary keratitis
- Corneal fibrosis.

All of these issues may further lead to keratoconjunctivitis sicca, unclear cornea and can cause pain and poor vision.

Features of Brachycephalic Ocular Syndrome

1. **Macroblepharon**: Also known as Euryblepheron (Fig. 1). It is a condition that involves abnormally long eyelids and large eyelid fissures. It can cause the cornea

and the white of the eye to be overexposed contributing to Keratoconjunctivitis Sicca (KCS). It can be corrected by Medial and/or lateral Canthoplasty.



2. **Medial Trichiasis due to Entropion:** It is a condition where an inversion or inward turning of eyelid margin is reported which further results in pointing of eyelashes towards the globe, ultimately leading to corneal abrasion and corneal ulceration. This can be corrected by Lateral Canthotomy.

3. **Ectropion:** It is an abnormality of the eyelids in which the eyelid (mostly Lower) is rolled outward or is everted (Fig. 2).

   It exposes the delicate conjunctival tissues, lining the inner surface of eyelids and cover the eyeball, causing drying of the tissue. It can be corrected by any of the following procedures: Lateral Eyelid Wedge excision, V to Y plasty (Wharton-Jones Procedure).

4. **Large Nasal Fold:** Brachycephalic breeds have large nasal folds, whose hair may contact the medial nictitating membrane, conjunctiva and cornea resulting in epiphora, conjunctival hyperemia leading to vascularization, pigmentation and even ulceration. It can be corrected surgically, by removing of the upper one-half or entire nasal fold (Fig. 1).

5. **Pigmentary Keratitis:** It is condition in which black or brown pigment is deposited on the cornea of a dog's eye. Chronic topical medical therapy using corticosteroid ointment and/or a tear stimulator (cyclosporine or tacrolimus) are most effective in promoting regression of granulation and pigmentation. Desired results will be visible after 4 to 6 months.

6. **Tear Deficiency:** Eyes with Keratoconjunctivitis Sicca (KCS) develop a thick (mucous) discharge, increased redness, and keratitis, that when uncontrolled or untreated results in vision loss. Topical cyclosporine is very effective in stimulating tear production in dogs. Tacrolimus is also an excellent alternative. Liberal use of tear substitutes is highly beneficial.

7. **Shallow Eye Orbits:** Provides significantly less protection to the globe than their mesocephalic or dolichocephalic counterparts' deeper orbits.

General considerations, a brachycephalic pet owner can pay attention to for avoiding eye conditions in these breeds by

- Daily cleaning in between the eye folds and eyes with a wipe/ cloth/towel.
- Use a moist, warm cloth to remove debris at the corner of the eyes or on the skin around the eyes.
- Avoid walking/playing in long grass or for short breeds your clothes also might injure the eyes.
- Regular hair trimming of facial hair in Shih Tzu, Lhasa Apso breed.
- Early treatment for even a small injury.
- Systemic precautionary medication like deworming etc., liver health should be taken care.
- E-collar may be applied, when taking out for a walk.

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COMPLICATIONS OF BURDIZZO CASTRATION IN CALVES

Nikita Gupta* 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: guptanikita086@gmail.com

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ABSTRACT

Burdizzo castration is the most common and simplest method of castration in male cow calves. The procedure of castration done using Burdizzo may be associated with certain complications if not rightly done. This article describes the complications of Burdizzo method of castration, namely scrotal skin necrosis, urethral rupture and failure of procedure, along with the cause, treatment and precautions for each complication, so that this technique could be effectively used by the veterinary practitioners.

Keywords: *Burdizzo, Castration, Complication, Scrotum*

Burdizzo method of castration is the most common method of castration for farm animals and is being widely used at field level due to the simplicity of the procedure. It is a non-surgical, bloodless method of castrating young farm animals, which only requires an instrument called as Burdizzo castrator (Capucille et al., 2002; Tyagi & Singh, 2012). Even though the procedure is easy to perform, if not executed correctly, it may lead to certain life-threatening complications, which warrants surgical treatment. These complications are explained as follows:-

Scrotal skin necrosis/sloughing of scrotal skin/Scirrhous cord

Scrotal skin necrosis is the most common complication of Burdizzo castration (Fig. 1a).

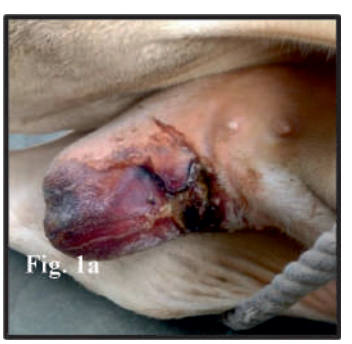
Fig. 1a

Presentation: The skin of the scrotum, below the point of application of the castrator gets necrosed and slowly gets sloughed off, exposing the testicles.

Cause- It happens due to the stoppage of blood supply to the scrotal skin along with the testicles.

Precautions during castration to avoid the condition:

- Do not apply the castrator to the entire length of scrotum and should only be applied to the isolated spermatic cord to the lateral aspect of scrotal wall.
- Place a thin gauge piece over the isolated spermatic cord so that the jaws of the castrator do not directly press over the scrotum (this can be for very young animals with soft skin and the castrator is big and strong)
- Most important, once the castration is complete with burdizzo, move the testicles

up and down through site of castration underneath the skin. This will separate the skin from the testicles and cord and its blood supply will be restored.

This condition if not treated at time, might further lead to sloughing of the ventral scrotal skin and development of scirrhous cord (Fig. 1b) as the spermatic cord might get infected on exposure to the environment.

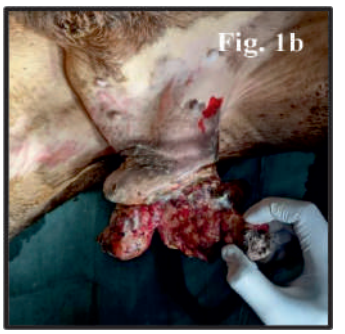
Fig. 1b

Treatment – Scrotal ablation along with the removal of testicles and if required removal of scirrhous cord need to be done.

2. Urethral rupture

Urethral rupture is a possible complication of Burdizzo castration.

Cause – Carelessness during placement of Burdizzo castrator over spermatic cord might include urethra as well, as it runs along the ventral midline, just at the base of the scrotum. It leads to crushing of urethra along with the spermatic cord and hence urine leakage from there.

Precautions – at start of castration, do not hold the spermatic cord to pull the testis downwards. Instead hold the testis and pull it downwards. By doing this you will not mistakenly hold the urethra (genitalia) along with the spermatid cord. Before the final application of the Burdizzo double check that the urethra is running straight at the base of the abdomen and is not in your hands.

Treatment – if urine starts accumulating in the subcutaneous space in the prepucial region after 1-2 days of castration, it suggests that the urethra has been ruptured. It may require a tube cystostomy, urethrostomy or may be multiple stab incisions on the swelling to let the urine drain out.

3. Castration Failure

The size of the testicle reduces within a few weeks after the procedure. However, in some cases it might not be true, indicating the failure of the procedure. Also the behavior of such animal does not change.

Cause – using inadequate size of the Budizzo, i.e., a small castrator for a large animal. This will lead to improper crushing of the spermatic cord leading to failure of the procedure. Another reason can be that the calf is overgrown for Burdizzo castration as its spermatic cord is thick which do not get crushed with Burdizzo.

Precautions – use appropriate sized Burdizzo castrator and at right age.

Treatment – Surgical castration is required.

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SURGICAL MANAGEMENT OF INTUSSUSCEPTION IN A GERMAN SHEPARD MALE DOG

Nima Wangdi*, Pema Tshewang, Karma Phuntshok, Karma Tshomo, J. Mohindroo[1]

National Veterinary Hospital, Motithang, Thimphu-11001, Bhutan
[1]Department of Surgery and Radiology, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab, 141001, India
*Corresponding author email: nimavet2012@gmail.com

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Abstract

A 1-year-old male German Shepard dog, weighing 29 kg presented with a history of weight loss, chronic anorexia, bloody diarrhoea and vomiting which was unresponsive to medicinal treatment at District Veterinary hospital. Blood biochemistry showed high values of glucose and low values total protein and albumin. Radiograph of the abdomen revealed distended intestines with gas and fluid suggestive of obstruction. Exploratory laparotomy diagnosed the condition as an intussusception cranial to the caecum with fibrosis and lack of blood supply to the affected portion of the intestine. The diseased portion of the duodenum was resected and end-end anastomosis was performed. The dog recovered uneventfully and follow up for six months show no recurrence.

Key words: *Dog; laparotomy; intussusception; anastomosis.*

Intussusception is a condition in which a part of the bowel is prolapsed or invaginates into the lumen of an immediately adjoining part (Valiei & Beheshti, 2011). Commonly the Intussusceptum, a proximal segment of bowel which slides into a distal segment and this pattern follows the normal direction of peristalsis. The incidence of intussusception is more in German shepherd dogs and occurs when excessive peristaltic motility forces one segment of the intestine into another slightly larger part of the intestine (Rahman et al., 2020). It can occurs at any location in the gastrointestinal tract from the stomach to the large intestine; however, previous studies have indicated that the majority of intussusceptions in small animals are enterocolic (Atray et al., 2012). The predisposing factors for the intussusception can be multiple, like intestinal parasitism, bacterial and viral enteritis, intestinal foreign bodies, prior abdominal surgery, intestinal neoplasia, and extra/intra-luminal mass lesions that cause intestinal motility disturbances (Rahman et al., 2020).

Diagnosis of intussusception can be done by palpation, radiography, ultrasonography, and computed tomography (Patsikas et al., 2019). However, Ultrasonography is a non-invasive, cost effective, non-hazardous diagnostic imaging modality which can confirm

intussusception. Target Sign or Bull's eye appearance in ultrasonogram is characteristics of intussusception (Rahman et.al, 2020). Manual reduction of intussusception can be performed otherwise resection of the affected segment of intestine is indicated followed by anastomosis if irreducible or the involved segments are unhealthy.

History and Clinical Findings: A 1 year old German Shepard male dog weighing 29 kg was presented to the national veterinary Hospital with a history of lethargy, weight loss, anorexia, chronic bloody diarrhoea, severe dehydration and vomiting. Clinical examination revealed hypothermia (99° F), Tachycardia and bounding pulse.

Diagnosis: Rapid antigen test was done to rule out canine distemper and faecal examination revealed *Toxocara canis* ova. Radiographic examination presented radiolucent, severely distended colon cranial to the caecum. Blood biochemistry showed increased level of glucose, reduced total protein and albumin pack system (Table 1).

The right lateral radiographs revealed radiolucent, severely distended colon pushing the liver ventrally and the stomach cranially. The stomach couldn't be visualized as it was superimposed by the distended fluid and gas filled colon (Fig. 1).

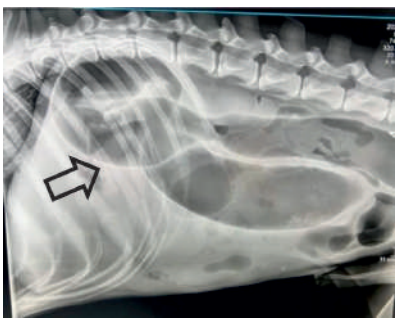

Fig. 1. Right lateral radiograph showing gas and fluid distended colon

Table 1. Blood Biochemistry Results

Test	Result	Normal range
Magnesium	4.39 mg/dl	1.80-2.60 mg/dl
Uric Acid	4.6 mg/dl	3.6-7.0 mg/dl
SGPT	30.2 U/L	21.0-102.0 U/L
SGOT	13.1 U/L	16.0-91.0 U/L
Cholesterol	104 mg/dl	135-270 mg/dl
Glucose	158.6 mg/dl	65.0-118.0 mg/dl
Total Bilirubin	0.15 mg/dl	0.00-0.20 mg/dl
Urea	9.9 mg/dl	21.4-59.9mg/dl
Total Protein	4.22 g/dl	5.40-8.00 g/dl
Triglyceride	54.9 mg/dl	40.0-160.0 mg/dl
Albumin	1.58 g/dl	2.30-4.10 g/dl
Creatinine	0.28 mg/dl	0.50-1.50 mg/dl
Alkaline Phosphatase	93 U/L	0-140 U/L

The clinical, laboratory finding and radiographic findings suggested intestinal obstruction either mechanical or functional.

Anaesthesia and Treatment: The animal was stabilized with intravenous fluids, prophylactic antibiotic, gentamicin @ 10 mg/kg body weight for 7 days, antiemetic, ondansetron @ 0.2 mg/kg and anti-diarrhoeal, Metronidazole @ 20mg/kg body weight.

The dog was premedicated with xylazine @ 1mg/kg body weight (b.wt) and atropine sulphate @ 0.04 mg/kg b.wt, intramuscularly. Following intravenous cannulation, general anaesthesia was induced with combination of ketamine + diazepam @ 5 mg/kg and 0.5 mg/kg b.wt. Pre-emptive analgesia was achieved with meloxicam @ 0.4 mg/kg b.wt through intravenous route. Anaesthesia was further maintained with the same drug combination through bolus administration.

The dog was positioned in dorsal recumbency for ventral midline celiotomy and surgical site was prepared was prepared aseptically with povidone-iodine (0.5% w/v) surgical scrub. The peritoneal cavity was opened after incising the skin, subcutaneous tissue, at linea alba and the peritoneum. On exploration, reducible intussusception was found cranial to the caeco-colic junction involving the caecum and colon (Fig 2).

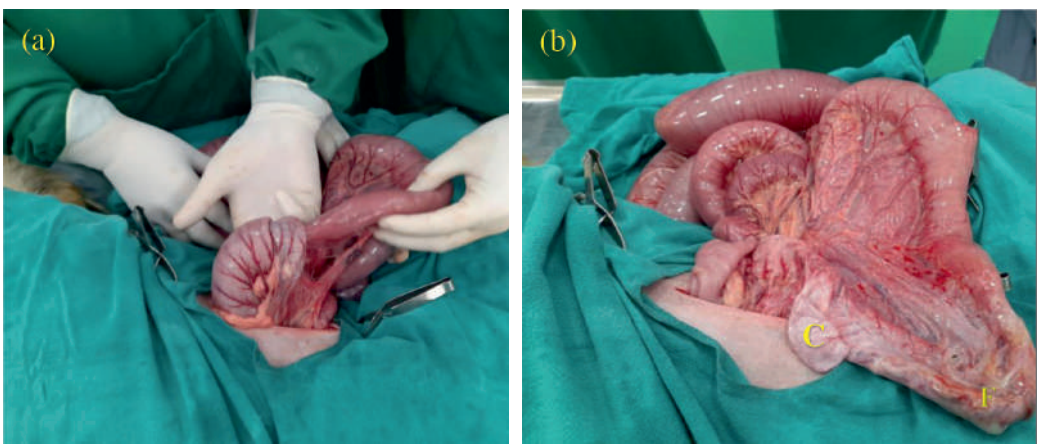

Fig 2. (a) Caeco-colic intussusception (b) Post reduction of intussusception showing caecum and fibrosed portion of intestine ,C- Caecum, F- Fibrosed portion of colon

Intestinal clamps were applied on proximal and distal ends of the colon before resection. The fibrosed loop was resected after ligating the mesenteric blood vessels. End to end anastomosis as performed using 5/0 polydioxanone suture in simple continuous pattern followed by Cushing (Fig 3b). The abdominal cavity was lavaged with normal saline. Laparotomy wound was closed in standard procedure.

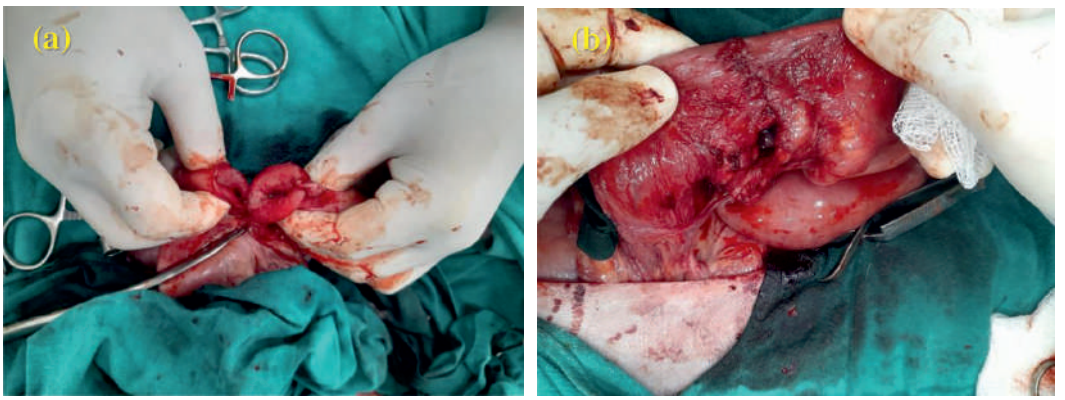

Fig. 3. (a) Proximal and distal of the colon after resection (b) End to end anastomosis

Post-operative follow up: Post operatively, the dog was treated with fluids (Dextrose normal saline and Ringer's lactate), antibiotics (cefotaxime @ 50mg/kg b.wt twice daily for 5 days), analgesic meloxicam (0.4 mg/kg b.wt once daily for three days) and injection omeprazole (1.5 mg/kg b.wt once daily for three days) intravenously. The owner was advised to give easily digestible liquid diet in small quantities and gradually shift to normal food. Post-operative follow up for 6 months showed an eventful recovery (Fig. 4)

Fig. 4. Six months follow up

Discussion

Intussusception needs to be differentiated from gastrointestinal foreign body, intraluminal lesions, Gastric dilatation and volvulus (GDV), Enteric form of canine distemper (CD) and bacterial gastro-enteritis. Canine distemper was ruled out using a CD rapid antigen test and radiographically, could diagnosed the case as intestinal obstruction either mechanical or functional. Exploratory laparotomy confirmed the condition as caeco-colic intussusception. Anorexia, vomiting and bloody diarrhoea were the predominant clinical signs of intussusception reported in this dog and in other studies (Rallis et al., 2000). Atray et al., 2012 also reported similar uniform gas-filled intestinal loops occupying the entire abdomen in radiograph. Exploratory laparotomy confirmed the condition as caeco-colic intussusception with no adhesion between the intussusceptum and intussuscipiens resulting in successful manual reduction of intussusception but anastomosis has to be performed after resection of the unhealthy, fibrosed portion of intestine. Further severe adhesion was found between the intussusceptum and intussuscipiens whereas, no such adhesions were appreciated in this case. Most of the intussusception in dogs are reported to occur as a sequela to a number of conditions such as linear foreign bodies, non-specific

gastroenteritis, viral-induced enteritis (parvovirus, distemper), leptospirosis, intraluminal masses and prior abdominal surgery (Rallis et al., 2000), whereas the aetiology in this study was suspected to be intestinal nematode as the faecal sample was positive to *Toxocara canis* eggs.

Intussusception is the invagination or telescoping of one part of the bowel into an adjacent part which causes bowel obstruction, and compromises blood flow to the affected portion and can be successfully treated by exploratory laparotomy with intestinal resection and anastomosis (Munif & Alam, 2021) which agreed to this study. Recurrence rate ranging from 3-25% that are surgically treated for an intussusception without entero-plication have been reported (Smeak, 2020). However, there was no recurrence although entero-plication was not done.

This study reports the successful surgical management of ileocaeco-colic intussusception with manual reduction, resection of fibrosed ileum and end to end anastomosis.

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INJECTABLE ANESTHESIA IN EQUINES FOR SHORT DURATION SURGERIES

Hussain Khan and Vandana Sangwan*

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

*Corresponding author email: sangwanvandana1@gmail.com

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Abstract

The write up highlights the injectable anesthetic protocols suitable for short duration surgeries in equines at filed level. Injectable anesthesia offers rapid onset, cost effectiveness, and avoidance of risks associated with inhalation anesthesia. But, in the same time it is not suitable for long surgeries as it delays recovery and recovery is violent. Overall, injectable anesthesia proves valuable for short surgeries in equine.

Keywords*: castration, dissociative anesthesia, equine, eye worm injectable anesthesia, horse.*

Equine patients are pre-disposed to injuries, which require early suturing and care under general anesthesia (Garcia et al., 2002; Staffieri & Driessen, 2007). In general, equine anesthesia presents a number of difficulties. Their temperament and sizes can make them more likely to harm both humans and the animal itself (Taylor & Clarke, 2006). The surgical procedure necessitates multiple personnel to safely handle the horse during the induction process and recovery because of the size of an equine patient. This write up describes the various anesthetic protocols that can be used in equine patients for short duration surgeries.

Indications

1. Castration of colts and stallions
2. Repair of umbilical hernia in colts and fillies
3. Small tumours of skin, vulva, eye lids etc.
4. Suturing of lacerations (if equine do not allow in standing)
5. Eyeworm removal
6. Plaster application

Even if the planned surgery is minor and only takes a short while to complete the surgical procedure, the horse must be properly monitored during this time. It includes respiration and palpation of the pulse.

Advantages of Injectable anesthesia for short duration surgeries

1. Shortened recovery times
2. Increased safety

3. Better post- operative results as can be done in field at initial hours.

Common injectable anesthetic protocols in adult horses

Always take care that the induction in horses is only successful if the sedation is sufficient and to full effect, and it may take 5-10 minutes for the pre-anesthetic to act.

Clinical Signs of full sedation with pre-anesthetic are:

- Head lowering to the level of knee joints
- Mild swaying with bending of knee but not falling

Topping of anesthesia may be required just after lying down or in between the surgery. So, always keep a cocktail of **5ml Xylazine (20mg/ml) and 5ml Ketamine (50mg/ml)** ready in a single syringe. Short duration surgery means the one that last for 15-20 minutes.

Various protocols of injectable anesthesia for short duration surgeries

1. Xylazine and Ketamine: (Xylazine –1.1 mg/kg IV, ketamine- 2.2 mg/kg IV)

Ketamine is administered 3-5 minutes after xyalazien of when the equine head is lowered down upto knee level.

Preparations available:

Xylazine= Xylaxin (23.32 mg/ml of xylazine hydrochloride)

Ketamine= Aneket (50 mg/ml of ketamine hydrochloride)

Following the injection of ketamine, the horse goes into a recumbent position within 90 to 120 seconds, and the horse stays anaesthetized for 15-20 minutes depending on its age and the intensity of the surgical stimulus.

If ketamine is required for topping, recovery is anxious and may need some sedative or xylazine (half dose, IV) during recovery.

Recovery: smooth.

2. Detomidine + Ketamine: (detomidine – 0.02 mg/kg IV, ketamine- 2.2 mg/kg IV)

Detomidine is 80-100 times more potent and twice as long – lasting than xylazine.

Detomidine is more cardiovascular depressant than xylazine.

Recovery: pronounced ataxia, which can be challenging to control at times.

3. (Xylazine + Butorphanol) + Ketamine: (Xylazine -1.1 mg/kg IV + butorphanol- 0.02 mg/kg IV, ketamine- 2.2 mg/kg IV)

Recovery: satisfactory.

Anaesthesia lasts 10 to 30 minutes and good analgesia is ensured due to the addition of butorphanol.

4. **Xylazine-diazepam and ketamine:** (xylazine- 1.0 mg/kg IV + diazepam- 0.02 mg/kg IV, ketamine- 1.5-2.0 mg/kg IV)

   Recovery: smooth without excitation and ataxia periods

   Anaesthetic duration: 15-20 minutes

5. **Xylazine and tiletamine/zolezepam:** {Xylazine @ 1.1 mg/kg IV, tiletamine-zolezepam mixture (*comes under the trade name Zoletil 50 contains- tiletamine as hydrochloride – 125 mg and zolezepam as hydrochloride – 125 mg in the form of freeze dried powder*) given at 1.1- 2.2 mg/kg}

   Full analgesia for 10 minutes at 1.1mg/Kg dose rate

   Full analgesia for 20 minutes at 1.6-2.2 mg/Kg dose rate

   The anaesthesia lasts for 30 to 45 minutes.

   More respiratory depression.

   Recovery: straightforward & rough, with noticeable ataxia and occasionally hyper-responsiveness. One fourth dose of xylazine may be repeated at recovery.

6. **Guaiphenesin and Ketamine:** Preparation of Guaiphenesin need autoclaving of the solution, so may not be possible in field.

7. **Barbiturates based induction for short surgeries:** It is not preferred as the recovery is violent and severely ataxic.

8. **Propofol based induction:** The induction quality is inconsistent with propofol (alone) induction ranging from good to poor quality. When guaifenesin the induction quality may be good to excellent, but with xylazine or detomidine, no improvement in the quality is seen.

This report describes the various protocols of injectable anesthesia in equines for short duration surgeries which can be practiced in field. The above mentioned protocols offers convenience and suitability for brief procedures, but careful consideration of the horse's health, proper dosing, and monitoring during the procedure are crucial to ensure safety and successful outcomes. It is essential to select the most appropriate anesthesia protocol as per the individual horse and the specific surgery required.

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INJECTABLE ANESTHETIC PROTOCOLS IN FOALS FOR SHORT DURATION SURGERIES

Kokkilagadda Vivek Vardhan* and Ashwani Kumar

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: vivekvardhan1358@gmail.com

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Abstract

Foals are more sensitive to the effects of the drugs than adults. There are certain short duration procedures which may be accomplished under injectable anesthesia and mild restraint in field level. This article deals with the unique physiology of foals which enables the practitioner to perform sedation and general anesthesia with few adverse outcomes.

Key Words: *Anesthesia induction, juvenile foal, neonatal physiology*

Foals exhibit advanced developmental maturity at birth compared to other domestic species, achieving juvenile status within 6 to 8 weeks. Equine anesthesiologists categorize foals as neonates up to 1 month, pediatric from 1 to 3 months, and juvenile from 3 to 4 months. The first four weeks pose the highest mortality risk.

Neonatal Physiology

- The cardiac index of foals is double that of adults but with a smaller stroke volume, and so a higher heart rate. So, they have a poor compliance with Xylazine.
- Foals have oxygen demands, and so increased respiratory minute ventilation but limited neuromuscular control of ventilation and underdeveloped lungs contribute to the foal's vulnerability to hypoxemia and hypercarbia.
- The immature central nervous system and permeable blood-brain barrier lead to distinct pharmacokinetic behaviors, affecting responses to sedatives, analgesics, and anesthetics.
- The incomplete functional maturity of the liver impacts drug metabolism, potentially causing drug accumulation and prolonged effects in neonates.
- Foals have greater total body water, blood plasma, and extracellular fluid volumes compared to mature horses, influencing drug distribution.
- Limited glycogen reserves in the liver and muscles make foals susceptible to hypoglycemia, especially if not nursing shortly after birth.
- They have a higher body surface area to weight ratio, thin skin, and limited subcutaneous fat, making them prone to heat loss. And anesthetic drugs can interfere with thermoregulation, leading to extended periods of hypothermia.

Indications for Short Duration Anesthesia

1. Contracted tendon
2. Fractures
3. Umbilical hernias
4. Pervious Urachus

Preanesthetic Evaluation and Preparation

1. Complete blood count
2. Blood glucose
3. Total Protein
4. Nursing foals up to 2 months of age prior to anesthesia have free access to their mother milk. Older foals with more solid food intake withheld feed for 3 to 6 hours prior to anesthesia.

General Injectable Short Duration Anesthetic Protocols

Always explain the risk of anesthesia to the owner and the limited facility available with a risk note signed.

***Anesthetic Management of the Neonate*: -**

1. Benzodiazepine derivatives are the favored choice.
2. Eg. Midazolam (MEZOLAM) (0.05–0.1 mg/kg IV) may be a superior option in upto 2-3 weeks foals compared to Diazepam (LORI) (0.1–0.25 mg/kg IV) which can lead to metabolic acidosis, nephrotoxicity, hyperosmolarity, tissue irritation, and hemolysis.
3. In >2–3 weeks foals, benzodiazepines + opioids like butorphanol (BUTODOL) (0.05–0.1 mg/kg IV), or a low dose of xylazine (XYLAXIN) (0.05–0.1 mg/kg IV) or dexmedetomidine (DEXTOMID) (0.001–0.03 mg/kg IV) to enhance sedation and provide analgesia.
4. Induction with
    a. Ketamine @ 2.0–2.5 mg/kg IV
    b. Propofol @ 2.0–2.5 mg/kg IV, Slow to prevent respiratory depression and apnea.
    c. Total intravenous anesthesia techniques involving constant rate infusion (CRI) of injectable anesthetics, such as Propofol 0.2–0.4 mg/kg/min can be used for maintenance of anesthesia.

Anesthetic Management of the Pediatric/Juvenile Foal

1. Lower doses of xylazine @ 0.2–0.3 mg/kg IV. Kicking can take place after xylazine.

2. Induction with
    a. Ketamine @ 2.0–2.5 mg/kg IV.
    b. Ketamine @ 2.0–2.5 mg/kg IV + Diazepam @ 0.1–0.25 mg/kg IV)
    Duration of anesthesia: 7 min to 20 min.
    Recovery: Smooth.
    c. Ketamine @ 1.5 mg/kg IV + propofol @ 0.5 mg/kg IV) can be combined for induction of anesthesia.
    d. Propofol @ 1.0–3.0 mg/kg IV alone can be used for induction.

Propofol (0.1–0.3 mg/kg/min) as CRI can be a viable option for anesthesia maintenance.

Monitoring During Anesthesia:

Fluids should always be given even in smaller surgeries.

Various parameters, including heart rate, Pulse, and respiratory rate should always be monitored.

The temperature of environment where surgery is conducted should be comfortable, around 24°C.

Recovery

Try to not let the foal stand for 10 minutes of last topping given. Or for smaller foals make him stand with support. As foals trying to stand up too quickly and yet with poor muscle coordination may predispose to fracturing limbs or other types of injury.

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TRAUMATIC RETICULOPERITONITIS IN CATTLE: INSIGHTS INTO PATHOGENESIS AND DIAGNOSIS

Omer Khalil Baba*, Sonam Sarita Bal, L. Geeta Devi, 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: dromerbaba@gmail.com

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Abstract

Traumatic reticuloperitonitis in cattle develops when they consume nails, wire, and other metallic objects that damage the reticular wall. Sharp objects can penetrate the reticulum, letting ingesta and germs into the peritoneal cavity causing peritonitis and abdominal adhesions along with rumenoreticular atony, stomach pain, decreased milk yield, arched back, guarded abdomen. Increase in total and differential leukocyte counts, total protein and total fibrinogen concentration are seen in laboratory examination. Gross examination of dead animal shows reticular perforation by metallic wire, fibrinous pericarditis (bread and butter appearance), diffuse peritonitis, adhesions, gelatinization of body fat. Treatment can be done by the oral administration of specially designed magnets or by surgical rumenotomy.

Keywords*: Adhesions, fibrinous pericarditis, peritonitis*

Traumatic reticuloperitonitis/ traumatic reticulopericarditis is a localized inflammation in the reticulum of cattle, often caused by ingestion of sharp metallic objects. The small distance between the reticulum and the pericardium facilitates the perforation of the contaminated foreign body resulting in traumatic pericarditis (Athar et al., 2012). These are common foreign body diseases in ruminant species, with cattle being more susceptible due to their lack of prehension and tendency to consume chopped feed. It results in decreased feed intake, reduced rumen motility, mild fever, and pain. This disease is less common in other ruminants like goats and sheep, which are discriminating eaters.

Etiology and Pathogenesis of Traumatic Reticuloperitonitis in Ruminants

Ingested wires can come from cut tires, metallic fragments from mixer wagons, and wire fencing in production systems. Pregnancy, ruminal tympany, alimentary tract obstruction by phytobezoars can increase intra-abdominal pressure, potentially allowing foreign bodies to enter from reticulum to other organs. Contractions of the reticulum promote penetration by the foreign object, which leads to further complications such as peritonitis or damage to other organs, including pericarditis, cardiac tamponade, pneumonia, pleurisy, hepatic, splenic, pulmonary or diaphragmatic abscesses. In suppurative pericarditis, the pericardial surface is thickened by white, rough, and shaggy

fibrous connective tissue. In chronic inflammatory lesions, viscera and pericardium have fibrous adhesions.

Diagnosis of Traumatic Reticuloperitonitis in Ruminants

Diagnosis of Traumatic Reticuloperitonitis is done by various methods like testing for the presence of a reticular foreign body (e.g, back grip, pain percussion, or pole test). Radiography is also an important tool to visualize metallic foreign bodies in the reticulm, whereas, ultrasonographic evaluation of the ventral abdomen is important for identifying localized peritonitis adjacent to the reticulm.

Laboratory testing for traumatic reticulopericarditis, in cattle plays a vital role in diagnosing and managing this condition. Following are the commonly used laboratory methods.

i) Blood Smear Examination

Laboratory tests are helpful to diagnose traumatic reticuloperitonitis with differential leucocyte counts being more accurate than total leucocyte counts. Localised acute peritonitis causes neutrophilia with a regenerative left shift in the first 3 days, while diffuse peritonitis causes leukopenia with a degenerative left shift (Constable et al., 2017).

In hematological evaluation, anemia occurs because of different degrees of dehydration, blood loss during penetration of the object or as a result of the chronic inflammatory process. Hematologic tests such as a leukogram, fibrinogen concentration and plasma protein concentration can be performed.

In acute TRP, a neutrophilia (>4000 cells/uL) is the most common response seen, sometimes with a regenerative left shift (>200 immature neutrophils/uL). This usually last for 3-5 days in uncomplicated cases of TRP, at which point the counts begin to return to normal. In chronic, TRP the counts may return to normal after several days or weeks, although sometimes these animals maintain a moderate neutrophilia and monocytosis for chronic periods (Harvey, 2008). Severe cases that involve the pleura or pericardium tend to generate higher total leukocyte counts (14,000-20,000 cells/uL), and cases that progress to diffuse peritonitis and show leukopenia (total cell count <4000 /uL) with a degenerative left shift (greater absolute number of immature neutrophils than mature neutrophils). Overall, leukogram changes that occur with TRP can be variable and must be interpreted with other findings to arrive at a diagnosis.

ii) Serum Biochemical Analysis

Hyperfibrinogenemia and hyperproteinemia are more reliable in specifying the traumatic reticuloperitonitis. Increased fibrinogen concentration indicating the condition as early as 2-3 days after onset. In chronic cases, an increase in gamma-globulin concentration leads to an increase in total protein concentration. The glutaraldehyde

coagulation test can determine these concentrations quickly, positively correlated with gel formation time.

Elevations of plasma fibrinogen (>1000 mg/dL) and total plasma protein levels (>10 g/dL) tend to occur fairly consistently with TRP (Reddy et al., 2014). These parameters begin to rise just a few days after the onset of acute TRP and usually maintain consistent levels in chronic cases. Caution must be used to account for dehydration in the animal, thus it is recommended that a ratio of plasma protein to fibrinogen of less than 10: 1 be used as finding consistent with TRP.

Hyperproteinemia, hyperglobulinemia and hypoalbuminemia are described due to the characteristic inflammatory response. Albumin reduction occurs by prioritizing the synthesis of acute phase proteins by the liver, in order to prevent inflammation and contribute to healing. The increase in the glutamyl transferase, aspartate aminotransferase, serum bilirubin, dehydrogenae enzyme and creatine kinase also occurs. Elevations in serum concentrations of liver enzymes generally indicate chronic lesions associated with right-sided heart failure with secondary hepatic congestion.

iii) Peritoneal Fluid Analysis

Peritoneal fluid analysis can help to detect TRP. Ultrasonography-guided abdominocentesis can yield samples for assessment of amount, color, transparency, odour, and consistency. The specific gravity and total solid concentration can be evaluated using refractometry. Exudates indicating traumatic reticuloperitonitis are cloudy, watery to viscous fluids with a foul odour and may clot quickly. Flecks of fibrin are common and the specific gravity is >1.015. Also measurement of D-glucose isomer concentrations in peritoneal fluid and serum can assist in diagnosis, particularly peritoneal fluid D-dimer concentration is the best criterion due to its high sensitivity and specificity. A lower glucose concentration in peritoneal fluid suggests bacteria metabolizing glucose (dos Santos et al., 2021). Because of the marked fibrinous response and localization of peritoneal lesions in cattle, failure to obtain fluid on abdominocentesis does not preclude the presence of peritonitis. If fluid is obtained, visual examination for color and turbidity should be performed. A total nucleated cell count of greater than 6000/uL, total protein concentration greater than 3.0 g/dL, and a differential cell count with greater than 40% neutrophils and less than 10 % eosinophils is consistent with peritonitis in cattle. Also, bacterial culture and sensitivity can aid in both diagnosing and formulating a treatment plan for TRP (Harvey, 2008).

iv) Necropsy and Histopathological Examination

Gross examination shows the penetration of an electrical wire/ metallic wire from the reticular wall into the pericardial sac (Fig.1) In acute cases, distension of the pericardial sac along with the presence of amber flocculent fluid and extensive fibrin deposit (Bread

and butter appearance) is observed (fibrinous pericarditis/shaggy heart) (Fig. 2) along with myocarditis and cardiac tamponade. In chronic cases due to excessive fibrinous inflammation the pericardial sac is adhered to the pericardium by fibrinous junctions and thickened (Athar et al., 2012). In addition to that extensive adhesion between heart, lungs can be also seen. (Fig. 3). Abdominal cavity shows large quantities of turbid, foul-smelling peritoneal fluid with fibrinous clots indicating chronic diffuse peritonitis (Fig. 4). Adhesions and abscesses of the reticulum, hepatomegaly and enlarged gall bladder are also seen. In the lungs severe pneumonic changes, consolidation, and abscess are also observed. Rumen and reticulum often contains undigested feed due to ruminal atony. Body fat often shows gelatinisation due to chronic anorexia.

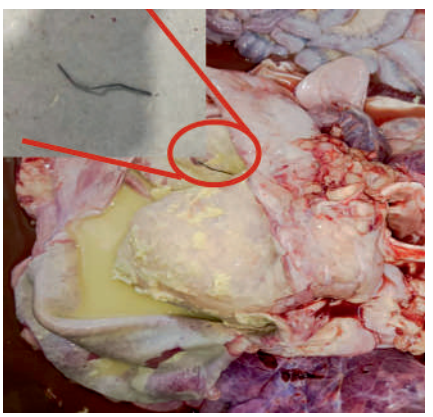

Fig. 1: Traumatic reticulopericarditis: Metallic wire piercing the pericardium (Inset: Metallic wire)

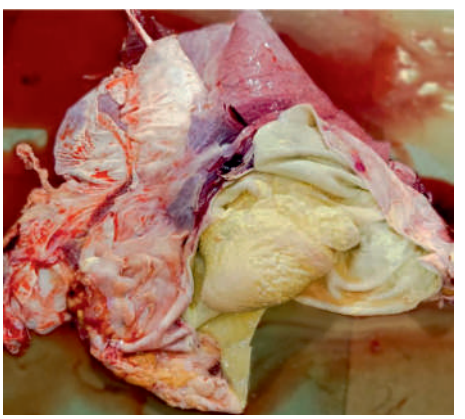

Fig 2: Fibrinopurulent pericarditis due to the traumatic reticulo pericarditis. Extensive fibrin deposit (Bread and butter appearance) in the pericardial sac, lungs- pneumonia.

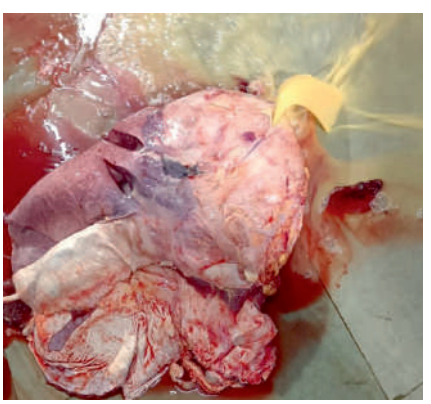

Fig 3: Extensive adhesion between heart and lungs; large quantities of turbid, foul-smelling fluid containing fibrin clots

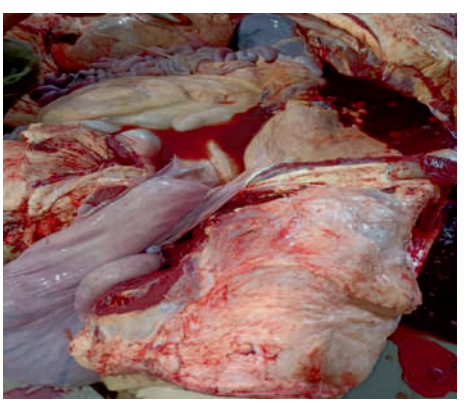

Fig 4: Diffuse Peritonitis; showing large quantities of turbid, foul-smelling peritoneal fluid

Histopathology shows the presence of nodular hyperplasia of the stratified squamous epithelium in the reticulum (high infiltration of inflammatory cells in the pericardium and myocardium, mainly of neutrophils and mononuclear cells, in addition to myocardial hyalinosis.

Prevention of Traumatic Reticuloperitonitis

To prevent traumatic reticuloperitonitis, avoid baling wire, pass feed over magnets, keep cattle away from construction sites, and remove old buildings and fences. Magnets can be administered orally to all cattle at about one year of age that can minimize the incidence of TRP.

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ADVANCED TECHNIQUES FOR SEMEN EVALUATION

A K Singh* and Dipti Nain

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab-141 004, India

*Corresponding author email: assengar2001@yahoo.co.in

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Abstract

Semen quality evaluation is the most important part in predicting fertility of domestic animals. However, due to the complexity of the fertilization process, it is not possible to predict the fertility of semen using a single semen evaluation test. Therefore, a combination of semen tests must be selected with high relevance for important sperm traits and low redundancy of assay results. This article updates various advanced techniques for the semen evaluation in animals

Keywords*: Semen handling, semen evaluation, sperm motility*

Successful fertilization needs healthy and functionally competent sperm. Information about the functional integrity of spermatozoa cannot be obtained from a single test. Furthermore, information about the functionality and fertilizing ability of spermatozoa is not sufficiently provided by conventional approaches. Due to inconsistent and lower relationship of physical characteristics with the sperm fertilizing potential, there is a need to identify suitable tests which can assess the functional integrity rather than physical integrity. Following are some latest techniques used for semen evaluation:

1. Computer Assisted Semen Analysis (CASA) Based Motility: Sperm motility is responsible for sperm transport within the female reproductive tract primarily for penetration of the zona pellucida. Traditionally, the assessment of sperm motility was done subjectively using a microscopic analysis of both mass and individual motility. However, as a result of the observer's skill, training, subjectivity, and bias, the subjective method's results showed a great deal of variation. As a result, emphasize has been put on developing an objective method for semen evaluation. Through CASA, all of the semen traits can be objectively evaluated overall. Using digital pictures of each sperm's track, the CASA machine can process and provide information about the tracks of spermatozoa. Compared to other methods, it provides more precise information on the kinematics of spermatozoa. It offers consistent estimations of various sperm motility characteristics, viability, and morphology viz. total motility (TM, %); progressive motility (PM, %); rapid motility (RM, %); slow motility (SM, %); velocity average path (VAP, μm/s); velocity straight line (VSL, μm/s); velocity curvilinear (VCL, μm/s); amplitude of lateral head displacement (ALH, μm); beat cross frequency (BCF, Hz); distance curve line (DCL, μm); distance average path (DAP, μm); distance straight line (DSL, μm); straightness

(STR, %); linearity (LIN, %); sperm size (SS, μ) and sperm nucleus (SN, μ). Motility, LIN, curvilinear velocity, and average path velocity can be used to predict sperm fertility, but not VSL. Among all the CASA parameters, some parameters like VCL, VAP, PM, LIN may be much more related to fertility, and these parameters may be higher in the bulls having high fertility based on conception rate.

2. Membrane integrity: It includes the following:

a. **Hypo-Osmotic Swelling Test (HOST):** The functional integrity of sperm plasma membrane is of fundamental importance for fertilization. The capacitation, acrosome reaction and binding of spermatozoa to oocyte require biochemically active plasma membrane. The HOST evaluates the functional integrity of sperm plasma membrane. Hypo-osmotic swelling test is based on the principal that a functional sperm plasma membrane allow the entry of fluid inside to maintain the equilibrium in its surrounding environment. The volume of spermatozoa increases when placed in hypo-osmotic solution. The increase in volume is associated with the spherical expansion of sperm plasma membrane and as the thickness and elasticity of membrane covering the tail is not uniform; the flagellum is forced to coil inside the membrane. Hence, the tail curling in sperm occurs as an effect of hypo-osmotic medium. Biochemically active sperm exposed to hypo-osmotic solution, swells until equilibrium is established between fluid compartment and the external environment. The optimal hypo-osmotic medium should exert enough osmotic stress to cause an observable changes in the sperm structure but not enough to induce lysis of plasma membrane due to influx of excess amount of water.

b. **Acrosome Integrity:** It is considered one of the important semen quality test to predict fertility. Acrosome encapsulates a variety of enzymes essentially required to penetrate ovum for fertilization. Spermatozoa have on their head a discrete deeply staining area mostly antero-laterally which appears as a refractile area. Acrosomal abnormalities may be hereditary (knobbed acrosome) or acquired (ruffled acrosome) and may lead to sterility or infertility. The different stains employed to evaluate acrosomes include Giemsa stain, Wells-Awa stain, Aslseth and Saacke's stain, chondroitin sulfate or India-ink. Giemsa stain has been mostly employed for acrosomal assessment. Giemsa is specific for the phosphate groups of acrosome and attaches itself to regions with high amounts of adenine-thymine bonding. It is also a differential stain with a mixture of methylene blue, Eosin and azure B.

3. DNA Integrity: Damage to DNA has been studied as a cause of male subfertility. The testis is characterized by very high rates of cell proliferation and excess germ cells are eliminated by apoptosis. One characteristic of apoptosis is endonuclease

cutting of DNA into discrete sizes demonstrating the presence of fragmented DNA in mature sperm. During the freeze-thaw process, the primary damage to DNA is caused by excessive accumulation of hydrogen peroxide (H_2O_2) which is the end product of superoxide radical. Acridine orange (AO) is the most commonly used staining method to evaluate sperm DNA integrity. AO intercalates into native DNA and fluoresces green (double-stranded) when exposed to blue light and fluoresces orange, yellow or red when associated with single stranded DNA because of the metachromatic properties of AO. Red and green sperm obtained from sub-fertile bulls could be seen under the fluorescent light microscope and roughly corresponded to the proportion of green and red sperm as measured by flow cytometry. Therefore, AO fluorescence staining of semen samples has been suggested as a practical and clinically significant procedure to determine sperm quality during infertility investigations.

4. Detection of Antisperm Antibodies

a. Immunoperoxidase Assay: This technique is used for determination of antisperm antibodies in serum and seminal plasma and is based upon the principle of enzymatic antigen detection in which the antibodies are visualized through peroxidase-catalyzed reaction. It is also used in pathology to demonstrate hormones, tissue-specific antigens, structural proteins, tissue enzymes, onco-fetal antigens, microorganisms and viruses.

b. Sperm-Mar Test: The Sperm-Mar IgA test is used for detecting antisperm antibodies of the IgA class in semen which otherwise can interfere with sperm function and zona binding and the acrosome reaction.

5. Sperm Zona Pellucida Binding Assay: Zona pellucida binding assays (ZBAs) have proven useful in determining the fertilizing ability of spermatozoa in several species. This test analyses the sperm capability to bind and penetrate the zona pellucida. Briefly, each zonae pellucidae of oocytes are inseminated with 10^5 to 10^6 motile spermatozoa/ml, prepared by a direct swim-up method. Following 4 h of incubation at 37^0C in humidified air, the zonae pellucidae are 'washed' by vigorous pipetting to remove any loosely attached spermatozoa. The zonae are then placed individually in microwells and dissolved by exposure to acidified (pH < 2.0) medium to form a fluid monolayer. The slides are sealed and the number of spermatozoa in the monolayer are counted within 24 h.

6. Hemizona Assay: The hemizona assay (HZA) has been developed as a diagnostic test for the tight binding of spermatozoa to the zona pellucida to predict fertilization potential. In this homologous bioassay, the two matching hemizona halves are functionally equal surfaces allowing controlled comparison of binding from a fertile control versus a test sample, with reproducible measurements of sperm binding obtained from a single oocyte. Oocytes from different sources are salt-stored and used after micro-bisection. Extensive

clinical data have demonstrated excellent predictive power of the HZA for the outcomes of intrauterine insemination and IVF, and therefore the assay has relevance in the clinical diagnostic setting in infertility.

7. Cervical-Mucus Penetration Test (CMPT): The *in vitro* CMPT is used to diagnose bovine infertility by measuring the distance travelled in millimeters by the most progressive or vanguard spermatozoa in cervical mucus of homologous species after 60 minutes of incubation. The structural arrangements of glycoproteins are responsible for the permissive or inhibitory effect of mucus on sperm migration. Cervical mucus acts as a natural barrier for the spermatozoa and as the first medium into which the sperm penetrates on their way into the reproductive tract. The cervical mucus penetration test can be used to distinguish between good and poor progressive motility of bovine sperm but it is merely useful to define the fertility level of semen sample. Grading of the semen samples is carried out as mentioned below:

Excellent: Distance travelled more than 30 mm.
Good: Distance travelled between 20 mm to 30 mm.
Medium: Distance travelled between 12 mm to 20 mm.
Poor: Distance travelled between 8 mm to 12 mm.

8. *In Vitro* Capacitation / Acrosome Reaction: The capacitation can be accomplished *in vitro* in numerous species by incubating ejaculated or post-thaw sperm under a variety of conditions in defined media that mimic the electrolyte composition of oviductal fluid. Ejaculated mammalian sperm need a period of incubation in the female reproductive tract in order to acquire the capacity to fertilize an egg. This period of attaining functional competence, referred to as capacitation, is required for undergoing the acrosome reaction induced by physiological stimuli such as zona pellucida. The most popular *in vivo* method of capacitation is by using heparin. In addition, *in vitro* treatment with calcium ionophores (Hoescht A23187) can also be used to induce acrosome reaction.

9. Oviductal Epithelial Cell Explant Test: Prior to fertilization, mammalian sperm attach to the oviductal epithelial cells (OEC) where they are stored for varying periods of time. The attached sperm are maintained at this site and then sub-populations of sperm are released over time. The sperm oviductal binding, which appears to be mediated through lectins, help the sperm maintain motility for prolonged periods of time while maintaining membrane integrity. In addition sperm calcium influx, a necessary prerequisite for acrosome reaction, is delayed while sperm are attached to OEC. *In vitro* studies using sperm and oviductal cell culture have been used as a bioassay to evaluate different bull spermatozoal treatments. The number of spermatozoa bound to the epithelial cells indicates its viability.

10. Heterospermic Insemination: It is also called as competitive fertilization. It is a powerful test for male fertility with mixtures of sperm from different males and involves direct assessment of fertilizing ability of spermatozoa. This procedure controls variation in environmental factors, technicians, and sperm numbers and is not affected by male-female interactions. Thus, when males differ slightly in fertility using homospermic insemination, heterospermic insemination can be used to exaggerate the differences. Heterospermic insemination has been estimated to be up to 170 times more efficient in assessing the fertility of bulls compared with homospermic procedures.

11. Zona free Hamster Oocyte Penetration Test: The hamster zona-free ovum test (HZFO test), or hamster test is a method for diagnosing male infertility due to the inability of the sperm to penetrate the ova. The method for the zona-free hamster egg penetration assay was originally described by Yanagimachi *et al.* (1976). In this test, sperm are incubated with several hamster eggs. Mature ova are collected from female golden hamsters (*Mesocricetus auratus*). After 24 h incubation, the number of sperm penetration per egg is measured. Results are expressed as the percentage fertilization rate of the oocytes and the sperm penetration rate per oocyte. This test enables direct assessment of the ability of spermatozoa to undergo capacitation, the acrosome reaction, fusion with the oolemma, penetration of the oocyte and to undergo decondensation in the cytoplasm of the oocyte. This is not a test of 'true fertilization' rather a test of sperm-oocyte fusion (Rashid *et al.,* 1998).

12. Sephadex Filtration: A good deal of heterogenesity in spermatozoa morphology is encountered in mammalian semen and significant reduction in the percentages of dead and abnormal spermatozoa has been reported following sephadex filtration in semen. Sephadex can be used to reduce the ejaculate rejection rate by filtering the dead spermatozoa from a poor quality ejaculate. The separation of spermatozoa is based on complex and interacting properties of sperm plasma membrane and the medium suspending sperm and sephadex particles. Different types of sephadex G-25, G -50, G-100, G-200 and G50-200 are used which have a significant effect on sperm motility. Samples filtered through both sephadex G-75 and G 50-200 reveal higher semen quality than those filtered through sephadex 25, 50, 100 and 200. Sephadex ion exchangers are also being used now a days and it gave better results in comparison to sephadex filtration method (Bhakat *et al.,* 2014). Mechanism of action of sephadex ion exchanger is considered that positively charged dead sperms interact with the negatively charged CM-cellulose and are trapped (Anzar and Graham, 1993) and live sperm containing negative charge pass easily through the ion exchanger.

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DO YOU KNOW GOATS CAN HAVE CANCER?

Hitesh Rana and Ratan K. Choudhary*

Animal Stem Cells Lab, College of Animal Biotechnology (Ludhiana), Guru Angad Dev Veterinary and Animal Sciences University, Punjab - 141004, India

*Corresponding author email- vetdrrkc@gmail.com

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Abstract

Studies on cancer in small ruminants are increasing in veterinary sciences, as its incidences increase day by day. New spontaneously occurring tumors arise due to rapidly changing environmental factors (ultraviolet rays, carcinogens), which favor the incidences. Although the frequency of cancer is increasing, reports are still very low in India, especially in small ruminants like goats. This is mainly due to misdiagnoses of cancer with other diseases due to a wide gap in the literature as there are very few data available. Based on different types of goat cancers, symptoms, and their etiology along with preventive measures, prevalent types of goat cancer can be Adenocarcinoma (cancer of glands) and Squamous cell carcinoma (skin cancer), followed by Sarcoma (cancer of muscles and bones) and Lymphoma (cancer of lymphatic system). Good management practices and early diagnoses and treatment can reduce the loss of an animal. Understanding cancer in goats is not only crucial for the well-being of these animals but also for advancing our knowledge of cancer in general.

Keywords*: Cancer, diagnosis, goat, research model, treatment*

In India, goats are very important livestock species as they contribute greatly to the country's agricultural economy. They are especially useful in areas where other livestock animals cannot be kept, and the land is not suitable for cultivation (Rajasthan and Gujrat). They provide livelihood and a constant source of income to small and marginal farmers of our country. The productivity of goats in India is not low, but rather it's on a higher scale than other livestock animals. Factors such as the environmental conditions in which they are reared, type of feed they get, housing space requirement, feed conversion percentage, quality of their milk and meat, and labor requirements that are more efficient than other livestock animals.

When we think of cancer, we often associate it with humans or our beloved pets like dogs and cats. However, goats can also suffer from cancer. Indeed, goats can fall victim to this relentless disease, shedding light on the intricate world of cancer in the animal kingdom. In this article, we will explore incidences of cancer in goats, its types, case reports and what we can learn from these studies. Here the various types of goat cancer are described in detail and have been published mainly as case reports.

Lymphomas

Lymph nodes are small swellings present in the body where white blood cells are formed, which provide immunity to the animal. When cancerous growth starts from lymph nodes, the cancer is termed Lymphoma. Lymph nodes play a vital role in the body by filtering lymph to detect foreign particles and infections. It is the most common type of cancer reported in goats (14-18%), but still, its occurrence is irregular (Schlemmer *et al.* 2019). It has also been reported in ruminants other than a goat. It can occur in goats of any age and gender. The clinical signs are different in every case because they depend on the severity and the area of the body where the affected lymph node is present. Still, it mostly affects the area around the liver, spleen, and lungs. The first sign that the animal may show is swelling, which is due to a build-up of pus around the external lymph nodes present around the neck and abdomen. When this pus invades nearby tissue, it causes difficulty ruminating, eating, and drinking. If it reaches the lungs, the animal may find it difficult to breathe and show signs of respiratory stress (Valentine et al. 2011). Other symptoms include fever, loss of appetite, and weight loss. The cause of this cancer is not confirmed yet but may be due to viral and environmental agents. In the case of viral agents, it may enter from the eyes, nose, and mouth. Animals may get infected from contaminated water and feed. Goats suffering from Lymphoma should be milked last, and all the equipment should be cleaned and sanitized after use to avoid the potential risk of transmission. It is also very important to separate the infected animals from the herd.

Cancers of Muscle and Bones (Sarcomas)

It includes a wide range of tumors that arise from muscle and bones. Scarcoma occurs when the cells of these tissues divide abnormally and, as a result, form a lump of cells. As muscles are present all over the body, Sarcoma can develop anywhere in the body. Therefore, sarcomas are classified according to the location where they are found in the body. Depending on their location, they may or may not invade other body organs, but may develop in the reproductive tract and intestine (Scaglione *et al.* 2015; Chen *et al.* 2017). If the tumor develops in the muscle of the hind limbs, the animal may show lethargic behavior and pain in walking. Swelling in the affected area can be noticed. Tumors that arise in the stomach or intestine may cause the animal to show symptoms of diarrhea, loss in appetite, abdominal pain, and weight loss. Tumors that arise in the mouth may cause bad breath, difficulty eating, and bleeding from the mouth.

Skin Cancer (Squamous Cell Carcinoma)

Skin cancer starts as a small neoplastic lesion in the skin, which looks like a thickened reddened area. Later on, it makes the skin around the lesion flaky, and an ulcer may also develop, which finally leads to bleeding. In poor management and hygiene, flies worsen the condition, and the animal quickly loses its body condition (Baipoledi

2001). The major cause of skin cancer is direct exposure to ultraviolet rays present in the sunlight. Most cases of skin cancer are reported in the region with high sunlight exposure around the year. Goat species with light pink skin and light coat color are at higher risk of this cancer, like the Jamunapari goat breed. It is also reported in different species of cattle and sheep. As it is caused by exposure to sunlight, body parts that are not covered by hair-like, face, perianal region, vulvar region, udder, eyelids, and area near the tail, are at risk of developing this cancer (Ramadan 1975). Viruses can also cause cancer, but it is rare. Animals graze together and get an almost equal amount of sunlight exposure and live together, making it possible for the flies to transmit the virus from diseased animals to healthy animals.

Cancer of Glands (Adenocarcinoma)

When the tumor develops in different body glands, it is called adenocarcinoma. It is one of the most common cancers found in goats worldwide. When we talk about the tumor in glands, it means that the size of the gland increases due to the uncontrolled growth of cells, and its secretions also increase. Cancer may invade other body organs if it releases its secretions inside the body. If the affected gland releases its secretions outside the body from the nose or genital tract, it may also reach and affect other herd animals. Carcinogens and viruses usually cause this cancer. Carcinogens are substances that cause cancer when ingested by animals in high amounts. The most common carcinogen that can affect goats is aflatoxin produced by a fungus (Aspergillus flavus). This fungus usually grows on the feed when conditions are suboptimal, like high temperature and humidity, due to poor management. When an animal eats the contaminated feed, the carcinogens start depositing in the body, and with time the concentration becomes high enough to cause cancer. Therefore, the prevalence of adenocarcinoma is reported more in older goats (5 years and older). It can also be caused by viral agents (Enzootic nasal adenocarcinoma virus or ENAV). From where the virus reaches the animal, is still unknown, but it could probably be from contaminated feed and water. However, once the animal is infected with ENAV, mucus is constantly secreted from the nose (Fig. 1). When it reaches other herd animals through common water sources, then cancermay spread to the other animals.

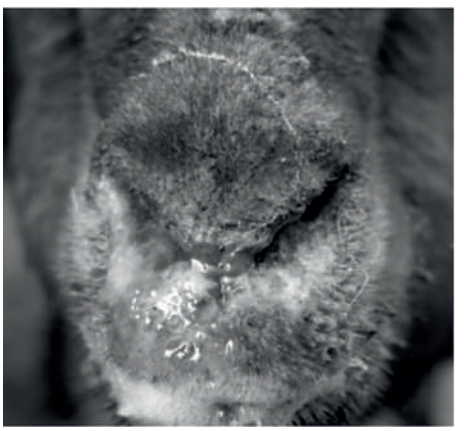

Fig. 1: Mucus secretion from the nose in goat affected by enzootic nasal adenocarcinoma (Yi et al. 2010)

The uterine body and uterine horns can also be susceptible to developing cancer. In a case report published recently reports, metastatic endometrial adenocarcinoma in seven yar old female goats (Consalter et al., 2022). The animal was that was

brought for progressive ataxia and sternal recumbency. When examined and treated, the animal was unresponsive to the treatment and was finally euthanized. At necropsy, 57 cm^3 volume of tumor mass was found in the uterine body and uterine horn.

Mucin 1 (MUC1) is a membrane-bound protein that provides a protecting barrier and cell-signaling platform to the glandular epithelium MUC1 is heavily glycosylated and expressed by luminal epithelial cells. Heterogeneity of MUC1 expression has been seen in many breast cancer cell lines (Baldus *et al.* 2005), and hence can be a useful marker to evaluate the health of the caprine mammary gland. We have examined MUC1 protein expression in apparently normal lactating goats using immunohistochemistry and found aberrant expression of MUC1 and increased collagen density in goat mammary cancer (Choudhary et al., 2017). In normal conditions, MUC1 localization is seen on the luminal side of the mammary epithelium (Fig. 2A), while in abnormal conditions, nuclear localization can also be seen. Aggregation of cells and nuclear localization of MUC1 is shown in a lactating goat (Fig. 2B), and could be utilized to detect early developing caprine mammary cancer. Detection of this cancer in the early stages, separating the affected animal, and proper treatment are crucial for good productivity of the livestock farm.

Other Types of Cancer

Mesothelioma is a cancer of thin layer of tissue that covers the majority of your internal organs (mesothelium). Pleural mesothelioma in an eight-year-old Saanen goat (Fry et al., 2023). Mesothelioma has rarely been reported in the goat. The differential diagnosis of mesothelioma should be made from thoracic masses in small ruminants like parasitic nodular lesions.

Diagnosis of Cancer

Identification of tumors in goats, in general, is difficult. Due to the rarity of cancer in ruminants, goats generally do not develop a tumor. An outgrowth of tissue mass usually occurs in the advanced stages of cancer. A pathologist does a core tissue biopsy of tumor mass to look for abnormal cellular growth, lack of tissue architecture, abnormal cell size, mitotic cells, and other parameters of cancer cells. Immunohistochemical examination of tissue biopsy of nodular lesion shows highly polymorphic neoplastic cells exhibiting positive immunoreactivity for cytokeratin (epithelial cell markers) and vimentin (mesenchymal cell marker).

Fine needle aspiration cytology (FNAC) is a reliable and non-invasive method doctors prefer to accurately identify small breast lesions and is a reliable diagnostic tool (de Cursi et al., 2020). Likewise, FNAC is being used to diagnose canine mammary tumors (Ghisleni et al., 2006). FNAC can be used as a diagnostic tool to identify tumors

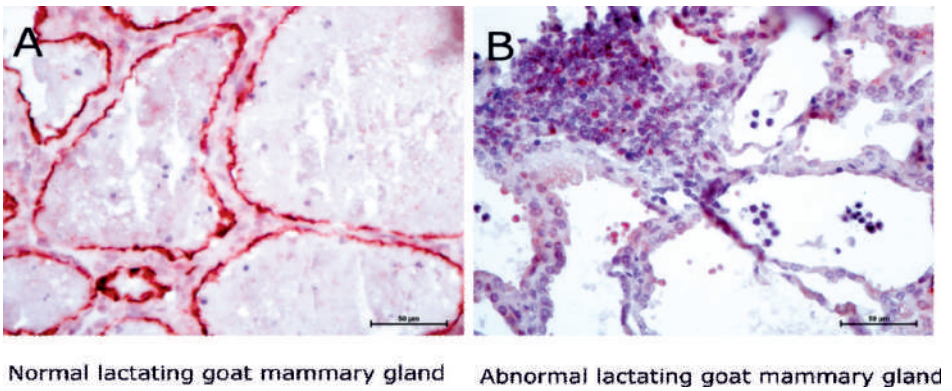

Fig. 2: Differential localization of MUC1 (a cell differentiation marker) in mammary tissue of normal and abnormal lactating goat. A: MUC1 protein is generally expressed in the cytoplasm of the luminal layer of mammary epithelial cells shown in healthy goat mammary tissue. B: Aberrant and nuclear (not cytoplasmic) localization of MUC1 was seen in the cancerous mammary gland of a goat. However, no tumor-like outgrowth of the goat mammary gland was observed in those animals.

in the small lesion in a goat suspected of cancer similar to the technique applied to identify canine mammary tumor (Luhache et al., 2022).

Treatment and Presentation

Early detection of cancer is the key for improving the prognosis of goats with cancer. Regular monitoring of goats for any signs of illness, including unusual lumps, changes in behavior, or a drop in milk production can aid in early diagnosis. Regular veterinary check-ups and preventive measures can also help to reduce the risk of cancer. In the advanced stage of cancer and unresponsive to treatment, animals can be euthanized to relieve chronic pain and suffering.

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STRATEGIES FOR REDUCING NITROGEN EMISSIONS IN DAIRY PRODUCTION THROUGH RUMEN DIETARY PROTEIN MANIPULATION

Shasta Kalra, Digvijay Singh, Jujhar Singh Sidhu 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

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Abstract

Mitigating nitrogen emissions in dairy production is crucial for environmental sustainability, animal health, and economic viability. Manipulating rumen dietary proteins by optimizing the balance of Rumen Degradable Nitrogen (RDN) and Undegraded Dietary Nitrogen (UDN) presents a promising approach to reduce nitrogen emissions, especially in high-yielding cows. NRC (2001) has given recommendation on RDP (9.5-10.5%) and RUP (6.5-8.0%) level for high yielder animal as per their satge of lactation and milk yield. Supplementation of rumen protected or analogs of Amino acids (AA); primarily Methionine and Lysine are the recent stratregies which can be used to limit the nitrogen emission through reducing protein level in the diet of animals.

Keywords*: Dietary protein, Enviornment, Nitrogen emission, Ruminant*

The dairy production industry faces a dual challenge: meeting the increasing demand for dairy products while minimizing adverse effects on the environment, animal health, and human nutrition. One of the primary environmental concerns associated with dairy production is the excessive nitrogen emissions, primarily in the form of ammonia (NH_3), which contributes to air and water pollution, health issues, and climate change. Dairy cow milk grossly contains around 30% of the protein and energy that dairy cows consume. An estimated 30% of energy is lost in faeces, 3% in urine, 5% in gases, and 25% is lost as heat. Each third of the feed protein taken is lost through faeces and urine. Therefore, reducing the losses experienced during digestion and metabolism can boost output and its effectiveness. An excessive amount of ammonia, a major environmental pollutant, builds up in the rumen and is then expelled, which is the primary source of nitrogen-containing emissions (NCEs), according to NRC 2001.

The Impact of Nitrogen Emissions

Excessive nitrogen emissions pose a significant environmental and health risk. Ammonia, a major component of these emissions, can have various detrimental effects. In its gaseous form, NH_3 can damage foliage and, when converted to ammonium (NH_4+), can pollute surface waters. Furthermore, NH_3 can reduce air quality by catalyzing the

formation of fine particles (PM 2.5), contributing to global climate change, and causing visibility degradation. NH_3 is estimated to cause two million premature deaths annually and is associated with health issues such as decreased lung function, aggravated asthma, and respiratory symptoms (USEPA 2004). Typical ammonia levels in well-ventilated, environmentally regulated buildings are 10 to 20 ppm with liquid manure systems and 50 ppm where manure and urine are deposited on solid floors. Levels can exceed 50 ppm with lower winter ventilation rates and reach 100 to 200 ppm in poorly ventilated buildings.

Sources of Nitrogen Emissions in Dairy Production

Dairy cattle are a significant contributor to nitrogen emissions, accounting for 13% of total NH_3 emissions. The nitrogen excreted by dairy cows is mainly in the form of urea in their urine and is a major source of NH_3 emissions. The quantities of dietary protein and non-protein compounds degraded by ruminal microorganisms into peptides, amino acids (AA) and NH_3, and excessive urinary N at about 60 to 80% is excreted in the environment (Vander Pol et al. 2009). Therefore, strategies aimed at mitigating nitrogen emissions must focus on reducing urinary nitrogen output.

Manipulating Dietary Protein for Nitrogen Mitigation

Reducing dietary protein in the animal ration has been identified as a promising approach to decrease NH_3 volatilization from manure. This strategy takes into account the degradability of the protein during digestion and categorizes it into Rumen Degradable Nitrogen (RDN) and Undegraded Dietary Nitrogen (UDN). It is a better system to calculate requirement levels, especially for high-yielding cows which have been shown to benefit from protein that escapes microbial degradation in the rumen and is absorbed as amino-acids in the small intestine.

Inorganic nitrogen sources from plants as well as other non-protein nitrogen, such as urea, are completely degraded by microbes in the rumen. Hence, the RDN is broken down by rumen microbes and used for their protein synthesis by the microbes. Later in the digestion process the microbes are themselves digested and the microbial protein becomes available to the animal. Nevertheless this microbial synthesis is only optimal when the animal receives sufficient energy supplements. Therefore, if sufficient RDP is not available, the rate of digestion of fibrous as well as concentrate-rich diets will be reduced. This leads to a reduction in intake, lower energy supply and reduced milk production.

On the other hand, some protein nitrogen can resist microbial breakdown in the rumen and can pass directly to the cow's intestine. This feed protein fraction is called by-pass protein which is especially profitable for high-yielding cows. At a low level of productivity a cow can meet her protein requirements entirely from microbial protein

and the diet only needs to contain degradable protein. This explains why such a cow can be fed with urea or chicken manure instead of high quality protein can meet the protein requirements. It is therefore important to have the optimum balance of UDN and RDP in the diet.

Partition of Dietary Nitrogen

Faeces: Nitrogen excretion in faeces typically represents a constant proportion of total nitrogen intake, around 7.5 g/kg dry matter ingested or 0.6% of the dietary DM intake (Cannas et al 2004.). Faeces are composed of undigested feed N, undigested microbial N and endogenous N (Tamminga, 1992), but reduction in faecal N excretion did not appear to be a promising way to achieve any substantial reduction in N loss from the animal (Tamminga, 1992). This is due to the true digestibility of feed protein in most dairy cow rations being high, whilst digestibility of microbial protein is also high, so suggesting little improvement in digestibility is possible (Tamminga, 1992).

Urine: Urinary N excretion on the other hand, appears to be a more promising means by which N output in dairy cattle can be managed. About 57-78% of urinary N is excreated in the form of urea and rapidly converted to NH_3 during manure collection and storage as compared to faecal N. Various routes contribute to urinary N output including ruminal and metabolic losses (Tamminga, 1992). Moreover, increase in dietary protein or N intake generally lead to substantial increase in urinary loss with almost all N ingested in excess of animal requirement excreted in urine.

Milk: Ruminants are only 30% efficient in converting intake N to milk or tissue N (Tamminga et al 1992) and the remaining N is lost into the surroundings. Nitrogen efficiency could be as high as 80% in pigs, assuming similar potential, increasing N efficiency in lactating cows will decrease N excretion from dairy farms. Great improvements in N efficiency could be achieved by reductions in feed N if milk production could be maintained (Ipharraguerre and Clark, 2005).

Balancing Protein Levels and Amino Acid (AA) Supplementation

Maintaining the right balance of rumen degradable protein (RDP) and bypass protein in the diet is essential for both economic and environmental reasons. Feeding RDP to meet microbial needs while minimizing excess can minimize nitrogen excretion. Feeding inadequate amounts of RDP can compromise dry matter intake, microbial protein production, and overall energy and protein supply to the cow.

The NRC (2001) recommends 9.5 to 10.5% dietary RDP and 6.5-8.0% RUP for lactating dairy cows and these recommendations are generally followed. However, it may be concluded that decreasing dietary RDP than recommended will have positive economic and environmental impacts if diets can be constructed to maintain milk

and milk protein production in dairy cows. Milk production may be maintained when essential AA requirements are met by maximizing ruminal microbial protein outflow. Reducing dietary CP concentration has been recognized as an efficient strategy to reduce N emission through rumen protected and analong amino acid (AA) supplementation in diet. Various studies (Patton, 2010; Lee et al., 2014) has investigated benefits of first limiting AAs; methionine (Met) ad lysine (Lys) supplementation with or without protein deficite diet of lactating cattle.

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BOOSTING MILK PRODUCTION WITH BETAINE: THE SCIENCE BEHIND THIS GAME-CHANGING NUTRIENT

Jujhar Singh Sidhu*, Neeti Lakhani, JS Hundal and Udeybir Singh

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

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Abstract

Betaine, also known as trimethyl glycine, plays a pivotal role as a feed additive in dairy animal physiology and nutrition, exerting its influence on various aspects of growth, productivity, and reproductive performance. Betaine, as an osmolyte, helps balance the osmotic pressure between the cell's interior and its external environment, thus protecting cell membrane integrity. Betaine helps stabilize proteins and enzymes, preventing their misfolding or inactivation under extreme osmotic conditions. Betaine provides methyl groups that are required for the synthesis of numerous substances, such as creatine, phosphatidylcholine, carnitine, adrenaline, methyl purines, and methylated amino acids. The role of betaine as a methyl donor helps maintain the rumen pH, enhances the nutrient digestibility of animals, and in turn increases milk yield. The mammary growth-promoting activity of betaine reflects higher milk production by maintaining cellular osmolarity and promoting cell proliferation. Betaine, after microbial fermentation in the rumen, gets converted to methylamine and acetate. The higher concentration of acetate in rumen volatile fatty acids has a positive relationship with milk fatty acid synthesis, which leads to higher milk production. Betaine has been shown to improve the digestibility of feed in cows by increasing the activity of digestive enzymes in the small intestine. Betaine's impact on rumen fermentation, metabolic diseases, digestibility and milk production makes it a valuable feed additive for dairy farmers.

Keywords*: Digestibility, Methyl Donor, Milk production, Osmolyte.*

Betaine, naturally present in wheat and sugar beets, is an oxidative product of choline and a trimethylated derivative of glycine. Commercially available betaine is a coproduct of the sugar beet industry and is extracted from molasses by water-based chromatographic separation and crystallization. Betaine functions as an osmolyte to maintain cell function and volume, a methyl donor to increase methionine and decrease homocysteine concentrations, and is fermented to acetate in the rumen (Mitchell et al., 1979). Due to both its methyl donor and its amino acid function, betaine is involved in protein and energy metabolism.

Betaine also help fight heat stress. Heat stress affects the production performance of dairy cows in the summer season. Through environmental modification methods, the production performance of dairy cows can be increased, but the cost of these techniques

is high, and poor farmers cannot use such techniques because of the high price. The use of feed additive may be a cost-effective and easily accessible way to decrease heat stress in animals, and it can increase the production performance of dairy cows (Shah et al., 2020). Betaine's impact on rumen fermentation, metabolic diseases, and digestibility makes it a valuable feed additive for dairy farmers. Betaine functions and mechanism of action has been discussed in details further below.

Dietary Sources of Betaine

In plants and bacteria, betaine is often produced and accumulated in order to cope with salt and temperature stress. In Table 1, the betaine content of selected feed ingredients is summarized. Sugar beets contain exceptionally high levels of betaine, which accumulate in condensed molasses solubles, a by-product of sugar beet processing. In addition, considerable amounts of betaine have been found in wheat bran and wheat.

Table 1. Betaine content of selected feed ingredients (mg/kg)

Feed Ingredients	Betaine content (mg/kg)
Condensed molasses soluble	116000
Wheat	1400
Peas	160
Groundnut meal	2520
Wheat Bran	2675
Lucerne Meal	3150-3850
Fish Meal	400-1180
Oats	590
Barley	730

Betaine as an Osmolyte

1. Osmotic Balance: Betaine can accumulate inside cells and tissues to help balance the osmotic pressure between the cell's interior and its external environment. When cells experience hyper tonic conditions, betaine helps maintain cell volume by counteracting the water loss that would otherwise occur through osmosis.
2. Protecting Proteins and Enzymes: High osmotic stress can denature proteins and disrupt cellular processes. Betaine helps stabilize proteins and enzymes, preventing their misfolding or inactivation under extreme osmotic conditions.
3. DNA and RNA Stability: Betaine can protect the structural integrity of nucleic acids like DNA and RNA under osmotic stress which is vital for the replication and functioning of cells.

4. Cellular Integrity: Betaine contributes to the overall integrity and health of cells by preventing cell shrinkage or bursting, which can occur under severe osmotic stress. It helps cells maintain their shape and function even when exposed to changing osmotic conditions.

Betaine as Methyl Donor

1. Homocysteine Methylation: One of the primary functions of betaine as a methyl donor is in the methylation of homocysteine to form methionine. The reaction is catalyzed by enzyme betaine-homocysteine methyltransferase (BHMT). In this reaction, betaine donates a methyl group to homocysteine, converting it into methionine. Methionine is an essential amino acid and a precursor for various important molecules, including proteins and S-adenosylmethionine (SAM).
2. SAM Production: S-adenosylmethionine (SAM) is a critical methyl donor in numerous methylation reactions throughout the cell. Betaine indirectly contributes to SAM production by providing a pathway to regenerate methionine from homocysteine and is involved in DNA, RNA and protein methylation, and synthesis of various bioactive compounds (Coleman et al., 2019).
3. Epigenetic Regulation: DNA methylation is an epigenetic modification that involves the addition of a methyl group to cytosine residues in DNA. This process can regulate gene expression by modulating the accessibility of genes to transcription factors and RNA polymerases. Betaine's role in providing methyl groups indirectly influences these epigenetic mechanisms by contributing to the production of SAM, which is a primary methyl donor in DNA methylation reactions.
4. Neurotransmitter Synthesis: Betaine can also be involved in formation of certain neurotransmitters such as dopamine and norepinephrine, through its role in the methylation of related compounds.

How Betaine Can Boost Milk Production

Betaine serves as a methyl donor in the animals which increases methionine concentration (Monteiro et al., 2017) and affects many functions in the animal body such as hepatic function, growth and lactation. The role of betaine as a methyl donor help maintain the rumen pH, enhancing nutrient digestibility of animals and in turn increase milk yield (Shah et al., 2020). The mammary growth promoting activity of betaine reflects higher milk production by maintaining cellular osmolarity and promoting cell proliferation (Monteiro et al., 2017). The increased milk yield is also related to the functions of betaine as an organic osmolyte that maintains the cell function by stabilizing cellular proteins and promoting proper protein folding. The same role might have been played by betaine in ruminants which gets escaped to the intestine (Monteiro et al., 2017).

Betaine, naturally found in plants and an oxidative product of choline, which after microbial fermentation in the rumen gets converted to methylamine and acetate. The higher concentration of acetate in rumen volatile fatty acids has a positive relationship with milk fatty acids synthesis which leads to higher milk production (Peterson et al., 2012). The acetate produced is also used for milk fat synthesis. Another reported mechanism is that betaine being itself a methyl donor reduces the requirement of same from choline and thus has sparing effect for choline which becomes available for lipid synthesis (Loest et al., 2002). Milk contains a large number of methylated compounds and choline is a limiting amino acid for lactating animals supporting high milk production in lactating animals.

The Science behind Betaine's Effects on Rumen Fermentation

Rumen fermentation is a complex process that plays a vital role in the digestion of feed in cows. Betaine could alter ruminal fermentation by serving as a source of either ruminally available nitrogen or methyl groups (Loest et al. 2002). VFA, acetate, and propionate concentrations increased with the supplementation of betaine (Shah et al., 2020) in dairy animals. Moreover, betaine is metabolized by ruminal microbes to produce acetate and trimethylamine (Mitchell et al., 1979). Supplementation with betaine decreases ruminal ammonia N content due to an enhanced growth of ruminal microbial populations that would increase ammonia N utilization, especially by the fibre-degrading populations. Also, betaine supplementation has an Osmo-protective effect, and it promotes the growth of favourable microbiota in rumen under environmental stress conditions (Wdowiak-Wróbel et al., 2013).

Digestibility is a critical factor in cow health and milk production. Betaine has been shown to improve the digestibility of feed in cows by increasing the activity of digestive enzymes in the small intestine (Wang et al., 2010). Similarly, dairy cows fed a diet supplemented with betaine had increased concentrations of pancreatic enzymes in their small intestine, essential for the digestion of carbohydrates, proteins and lipids. Betaine has also been shown to increase the activity of brush-border enzymes, which are responsible for the final stages of digestion in the small intestine. Betaine provides methyl groups that are required for the synthesis of carnitine, which has a major role in the transfer of long-chain fatty acids to mitochondria for subsequent β-oxidation. Moreover, the osmoregulatory property of betaine also improves digestion and absorption of nutrients in the gut. Increased nutrient availability is the main reason behind the increase in milk production and milk fat synthesis.

Supplementation in Diet

Betaine is available in powder form (Biochem: **Betaine HEPATRON® 85%)** and can be added to a cow's feed at a rate of 10–20 grams per day. The most popular

forms of feed-grade betaine are anhydrous betaine, betaine monophosphate, and betaine hydrochloride.

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ANSWER TO THE CROSSWORD

(Quiz on page No.10-12)

[1]J	E	[2]R	[3]S	[4]E	Y	■	[5]G	A	[6]P	E	[7]S	■	[8]T	[9]B
O	■	[10]A	N	U	R	I	A	■	S	■	[11]O	V	U	M
[12]H	[13]Y	■	[14]F	T	■	■	G	■	E	■	L	■	B	O
[15]N	O	[16]D	■	[17]H	O	[18]G	■	[19]C	U	[20]R	E	■	[21]E	D
[22]I	L	L	■	A	■	E	■	[23]O	D	E	■	■	R	E
N	K	■	[24]Z	N	■	N	■	W	O	T	■	■	I	■
■	[25]S	[26]E	R	A	■	U	■	[27]P	C	I	■	[28]O	S	■
[29]W	A	X	■	[30]S	[31]O	S	■	E	Y	N	■	[32]B	C	[33]G
[34]E	C	O	■	[35]I	V	■	[36]G	R	E	A	[37]T	■	H	H
A	■	[38]T	R	A	I	[39]T	■	G	S	■	[40]H	A	I	R
[41]N	E	O	■	■	[42]N	U	L	L	I	■	I	■	I	E
I	■	X	■	[43]D	E	S	■	[44]A	S	S	A	M	■	L
[45]N	[46]O	I	S	E	■	[47]S	[48]U	N	■	■	M	■	■	I
[49]G	A	N	J	A	■	I	R	D	■	[50]T	I	[51]D	■	N
■	T	S	■	[52]N	A	S	O	■	■	■	N	C	■	■

DEVELOPMENT OF ACARICIDE RESISTANCE IN TICKS AND STRATEGIES FOR MITIGATION

Lalnunpari Vangchhia, Jyoti*, Harkirat Singh and Nirbhay Kumar 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

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Abstract

Tick and tick-borne diseases affect a large population of the animals and cause massive economic losses to the livestock industry. Aggressive and indiscriminate use of chemical acaricides has led to the development of drug resistance in ticks which has become a major problem faced by the livestock sector. Integrated control measures must be implemented to delay the development of resistance. Alternative control strategies like effective managemental strategies, biological control agents (fungi, nematodes), anti-tick vaccines, herbal acaricides, essential oils, nanopesticides and semiochemicals/pheromones have been suggested.

Keywords: *Acaricide resistance, control strategies, ticks.*

Ticks affect 80% of the world's cattle population and cause significant losses due to their blood sucking, toxins and transmission of several pathogens. Almost all the economically important livestock species are suffering from ticks and tick-borne diseases. In India, the annual economic losses due to tick and tick-borne diseases is estimated to be 6107.65 Cr INR (Singh et. al., 2022). Global tick parasitism has serious negative effects on human, veterinary, and public health. The most popular tick-control strategy used by cattle owners worldwide including India is use of synthetic acaricides. These medicines are readily available over-the-counter and infected animals are treated with them on a regular basis. However, indiscriminate use of these drugs has led to the emergence of resistance against these compounds. Resistance to acaricides in tick has occurred in every region of the world where chemical control has been used to control ticks on cattle (FAO, 2004). As per WHO acaricide resistance is defined as "An inherited characteristic that imparts an increased tolerance to a pesticide, or group of pesticides, such that the resistant individuals survive a concentration of the compound(s) that would normally be lethal to the species. There are several types of acaricide resistance:

- Acquired resistance: Decreased sensitivity to the drug with time.
- Cross resistance (CR): Resistance develops against chemically related drugs with similar mechanism of action.
- Multiple resistance (MR): Resistance against several classes of chemically unrelated drugs with different mechanism of action.

The main problem faced by entomologists is the resistance to chemicals, due to the presence of CR and MR between ticks, progressive decrease in effective acaricides and high cost of developing new acaricides. There is an exponential growth pattern between the discovery of new acaricides and the development of strains resistant to these new products.

Development of Acaricide Resistance: The development of resistance is an evolutionary process that occurs by genetic selection. The speed with which resistance develops in a population depends mainly on the initial frequency of resistance genes, selection intensity, the degree of dominance of the gene and the relative ability of the genotype. In general, the frequency of resistance genes is very low in populations that have been under selection pressure. The development of resistance is divided into following three phases:

1. Establishment phase: It arises when the resistant allele appears in a population, usually by natural mutations and independently to selection pressure.
2. Development phase: Is the phase of increasing number of resistant individuals and occurs by the preferential survival rate of resistant ones after the use of chemicals.
3. Resistant phase: Occurs by a high rate of selection pressure in a short phase and the resistant allele is common enough in the population to demonstrate a reduction in the effectiveness of the acaricide.

Mechanisms of Resistance: According to the type of response to acaricides, resistance has been grouped into four categories:

1. Resistance behaviour: It adjusts behaviour to avoid contact with the acaricide.
2. Penetration resistance: It is a modification of the exoskeleton to inhibit or retard the penetration of the chemical, and generally has to do with the concentration of lipids that facilitate or retard the penetration of pesticides through this structure
3. Metabolic resistance: It is the detoxification of the acaricide by enzymatic processes by the modification of metabolic pathways of the tick. The most important metabolic resistance involves multifunctional oxidases, glutathione S-transferase and esterase.
4. Target site insensitivity: Is modifying the site of action of acaricide to decrease the sensitivity of the chemical. When this is the cause of resistance, regular resistance levels are very high (1000x) as compared to the detoxification (50x).

Control or Mitigation Strategies: Acaricide resistance has usually been managed after emergence, by the use of new products. However, there are various non-chemical alternate control strategies for tick control which are more environment friendly. These approaches are generally advocated to be used as Integrated Tick Control strategies.

1. Managemental Strategies: The primary strategies that can be employed to delay the development of acaricide resistance include:

- Regular monitoring of resistance so as to make appropriate choices for treatment of tick infestation.
- Reduction in frequency of treatments to reduce selection pressure.
- Synergists: The main synergists that have been used for tick control are Piperonyl butoxide, triphenylphosphate, diethyl maleate.
- Combination of drugs: Products that contain two or more drugs with different mechanism of action may be used *viz*. Deltamethrin, thymol and essential oil, Neonecotinoids and pyrethroids.
- Rotation of drugs: The alternate use of two or more active substances with different mechanisms of action and no chance of cross-resistance is referred to as rotation.
- Dosing and method of administration: Exposure of ticks to sub lethal doses or excessive doses of drugs favours the development of resistance. Tick resistance is closely associated with the manner in which acaricides are applied. Plunge dips or spray races were considered superior as hand spray method may cause under dosage.
- Refugia: Targeted selective treatment which leaves a proportion of the tick population unexposed is crucial in delaying development of resistance as the unexposed ticks will dilute the resistant allele in the next generation.
- Manual removal: Feasible in small farms with lower infestation rates.
- Grazing management: Integrated tick control can involve pasture management when grazing patterns are used to disrupt the life cycle of ticks.

2. Biological Control: This technique requires the use of other living organisms or natural enemies of ticks to reduce tick population. However, these organisms should have host specificity, high fecundity, good host searching ability and adaptability. Some examples of biological control agents include predators (Birds, ants), pathogens (bacteria, nematodes, fungi) and parasitoids.

Entomopathogenic Fungi: Entomopathogenic fungi has shown to be one of the most promising options as it shows effective acaricidal activity against various tick developmental stages (egg, larva, nymph and adult) both in laboratory and the field. The most widely studied strains are *Metarhizium anisopliae* and *Beauveria bassiana*. Several strains of *M. anisopliae* induced a mortality of 90–100% in populations of *R. microplus* multi-resistant to acaricides (OP, SP, Am) and ivermectin. These fungi have numerous advantages in that they do not produce any harmful effects to non-target hosts, they affect all life stages of the ticks and leave no chemical residues.

Entomopathogenic Nematodes: *Steinernematidae* and *Heterorhabditida* have been successfully used worldwide to control different insect pests (Singh et. al., 2018). Bacterial symbionts carried by the Infectious Juveniles (IJ) of these nematodes cause tick death within 24 to 48 hours.

Parasitoid Wasps particularly *Ixodiphagus* wasps have been reported to infect numerous tick species. Female wasps oviposit on unfed nymphs then develop into larvae which then feeds on the ticks' tissues and eventually results in death.

3. **Herbal Acaricides**: A wide range of plant species have been examined and analyzed by numerous research group and have demonstrated considerable potential as tick repellent and control. Some of these plant species are *Piper longum* (Indian long pepper), *Atropa belladonna* (Deadly nightshade), *Dalbergia sissoo* (North Indian rosewood or shisham), *Nicotiana tabacum* (Common tobacco), *Azadirachta indica* (Neem). The acaricidal effects of plant extracts can be attributed to secondary metabolites (terpenoids, steroids, alcohols, fatty acid derived substances, coumarins, sulfurated compounds and aldehydes). Since, multiple active compounds are present in these plants, ticks are less likely to develop resistance.

4. **Essential Oils**: When used against *Rhipicephalus* ticks on cattle, EOs and its active ingredients have demonstrated high acaricidal action. This is achieved by 3 main mechanisms: Neurotoxic effect which is due to inhibition of acetylcholine esterase and antagonism of GABA and octapamine receptors; mechanical effect is due to their hydrophobic nature which produces disruption of the cuticular waxes and obstruction of the respiratory stigmas leading to death from water stress or asphyxia and cytotoxic effect due to binding with tyramine and octapamine receptors. Some important essential oils are *Azadirachta indica* (Neem), *Ricinus communis* (Castor oil), *Cymbopogon winterianus* (Lemon grass), *Tagetes minuta/ Tagetes patula* (Marigold), *Cinnamomum zeylanicum* (Cinnamon).

5. **Vaccines**: The first commercialised anti-tick vaccine was Bm86 based vaccines in the early 1990's. Both TickGARD (used in Australia) and Gavac (used in Latin American nations) are produced from the recombinant protein Bm86 that is membrane-bound in the midgut of *R. microplus*. When cattle are given vaccines containing Bm86, there is a favourable association between the production of antigen-specific antibodies and the decline in tick infestations and fertility resulting in reduction in tick numbers, weight and reproductive capacity of engorging female ticks. Further, trials of the Gavac vaccine on cow herds showed a decrease incidence of babesiosis and anaplasmosis. Promising vaccination options that can interfere with tick biological processes have been rationally developed using knowledge of unique physiological processes peculiar to ticks. Some of the biological mechanisms investigated to locate target antigens for developing

potential anti-tick vaccinations are reviewed. The impacts of these specific physiological processes on tick behaviour includes attachment to the host, uninterrupted host feeding, intracellular digestion of huge quantities of blood and metabolism of ingested blood into large clutches of eggs laid by the engorged female ticks. In the past few years, reverse vaccinology has been heavily utilized to find appropriate antigens for the designing anti-tick vaccines (Kasaija et al., 2022).

6. **Nanopesticides:** A nanopesticide is made up of extremely small particles with sizes ranging between 1-100 nm of a pesticide adjuvant or other uniquely designed structures with pesticidal properties (Zaheer et al., 2022). They are used to improve the effectiveness of the currently used pesticides. Emulsions (nanoemulsions), nanocapsules (with polymers), and products with nanoparticles, such as metals and metal oxides have all been proposed as formulation types. Nanopesticides affect ticks in a variety of ways, such as through modifying their lipids or proteins, inducing oxidative stress, or interfering with their metabolic processes. Green nanoparticles of ZnO have been utilized for applications against a variety of parasites and disease vectors, viz., mosquitoes, ticks, lice, and flies.

7. **Semiochemicals/Pheromones**: Semiochemicals are bioactive substances that change the physiological behaviours of other members of the same species or different species to send specific signals (Sonenshine, 2006). Ticks are purinotelic *i.e* they secrete nitrogenous bases which serves as assembly or sex pheromones. For tick control, these compounds are combined with acaricides using 'Attract and kill' strategy. Sex pheromones can also be used in tick decoys to prevent insemination of female ticks.

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