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# Collection and Dispatch of Biological Samples in Animal Disease Outbreak Investigations

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## Abstract

*Efficient management of outbreaks or emergency situations in veterinary healthcare hinges upon the accurate and timely collection and dispatch of biological samples. These processes are indispensable for swift disease identification, surveillance, and control measures implementation. However, adherence to proper sample collection protocols is paramount to maintain diagnostic accuracy and mitigate the risk of disease spread. This article highlights the critical role of efficient sample collection and dispatch in veterinary healthcare systems, emphasizing collaborative efforts among stakeholders. Failure to follow standardized protocols may compromise sample integrity, leading to erroneous diagnostic outcomes. Proper collection methods, including avoiding putrefied materials and prompt sampling from affected sites, are imperative to ensure accurate disease diagnosis. Furthermore, technological advancements like cold chain logistics and rapid diagnostic tools aid in expediting sample analysis, especially for emerging and trans-boundary diseases. To maximize diagnostic accuracy, it is crucial to adhere to standardized protocols for sample handling, packaging, and transportation. This article provides insights into various sample collection techniques for diagnosing a wide range of diseases, ensuring optimal outcomes in outbreak investigations and emergency veterinary scenarios.*

**Keywords:** *Disease diagnosis, Disease outbreak, Sample collection, Sample dispatch*

During disease outbreaks or emergency situations in veterinary healthcare, the accurate and timely collection and dispatch of biological samples play a pivotal role in swiftly identifying and managing diseases. These processes are essential for effective disease detection, surveillance, and the implementation of control measures. Failure to adhere to proper sample collection protocols can significantly impact diagnostic accuracy and delay response efforts, potentially exacerbating the spread of infectious diseases. Ochwo et al. (2023) underscored the critical role of efficient sample collection and dispatch in veterinary healthcare systems. Without standardized protocols and collaborative efforts among veterinarians, laboratory personnel, and stakeholders, the integrity of samples may be compromised, leading to erroneous diagnostic outcomes (Miltenburg et al., 2021). Improper collection methods, such as using putrefied materials or failing to collect samples promptly from affected sites, can distort test results and hinder accurate disease diagnosis (Kumar et al., 2022). Moreover, contamination during

collection or inadequate packaging and transportation can further jeopardize the reliability of diagnostic tests.

In emergency situations, technological advancements like cold chain logistics and rapid diagnostic tools become invaluable for expediting sample analysis, especially for emerging and trans-boundary diseases. However, the effectiveness of these technologies relies heavily on the quality of the samples collected and dispatched. Therefore, adhering to standardized protocols for sample handling, packaging, and transportation is paramount to preserve sample integrity and to ensure optimal diagnostic results during outbreaks or emergency situations in veterinary healthcare.

**Different Types of Sample Collection for Diagnosis of Disease**

**I. Blood Samples:** For blood sample collection, ensure to use clean and dry glass vials or tubes. In large animals, the jugular vein is the primary blood collection site. For hematological studies, collect whole blood in a specific anticoagulant.

- Heparin (Green cap tube): Use at a rate of 1 I.U. per ml of blood. Commercially available heparin-coated vials are suitable. Ideal for blood pH and acid-base balance analysis but not recommended for cell morphology studies.
- Ethylene Diamine Tetra Acetic Acid [(EDTA) - Purple cap tube]: Use at a rate of 1 mg powder per ml of blood. Commercially available EDTA-coated vials are suitable. Ideal for preserving cellular details and suitable for hematological studies.
- Sodium Citrate (Blue cap Tube): Add 1 ml of 3.8% sodium citrate solution to 9 ml of blood to prevent coagulation. It is recommended for coagulation studies.
- Sodium Fluoride (Grey cap tube): Suitable for blood glucose studies at 6-10 mg per ml of blood.

**II. Plasma and Serum Samples:** For plasma samples, collect blood with anticoagulant in appropriate tubes and separate plasma by centrifugation. Submit at least 2 ml of frozen plasma in 5 ml screw-capped vials. For serum separation, collect blood without anticoagulant and allow clot formation. Carefully collect the serum (supernatant fluid), avoiding the clot, and transfer it to clean vials. Wrap the vials in foil for protection and send them chilled to the laboratory. Avoid hemolysis of blood samples due to non-sterile containers, contamination, slow flow, or exposure to heat or sunlight.

For long-term storage of serum samples, add Merthiolate (1:10,000) or 0.1% sodium azide as a preservative. Deep freezing, dry storage on paper disks at ambient temperature, or lyophilization (freeze-drying) are suitable methods for long-term storage. Maintain a core temperature below -60°C for deep freezing.

**III. For Bacteriological Purposes**: For most bacteria, blood in nutrient broth (readily

available in most laboratories) is suitable. However, for transporting certain bacteria, especially anaerobic ones, specialized media like Amies transport medium or Cary-Blair media must be used.

**IV. For Parasitological Examination**: During blood sample collection, both thin and thick blood smears can be prepared on-site or in the laboratory for comprehensive parasitological assessments.

- **Wet Blood Film:** To detect live trypanosomes, a drop of blood is placed on a slide and covered with a cover slip. This method is particularly effective for immediate assessments.
- **Thin Blood Smear**: A small drop of blood is placed on a clean slide held horizontally. Another slide, angled at 30 to 40 degrees, is used to evenly spread the blood by drawing it smoothly and firmly. The smear is air-dried and then fixed with methanol (acetone-free methyl alcohol) for two minutes.
- **Thick Blood Smear**: For chronic infections, a large blood drop is spread over an area of about half an inch on a slide using the corner of another slide. Unlike thin smears, thick smears are not fixed before staining. They are covered with a Petri dish and allowed to dry. This technique is specifically designed for detecting chronic infections.

**V. Urine Samples**: Urine samples are crucial for various diagnostic purposes, including urinalysis, bacterial microscopy, culture, and viable bacterial count. Proper collection and handling are essential for accurate results. Samples can be obtained during normal urination or through catheterization. When catheterizing, it's vital not to over-lubricate with liquid paraffin to prevent fat globule introduction. Collect a minimum of 100 ml sample in sterile, watertight glass, or plastic bottles. Avoid old medicine vials unless thoroughly cleaned. Preserve in a refrigerator if possible or alternatively, add a drop of toluene or a crystal of thymol if immediate refrigeration isn't feasible. To preserve cell and cast structures, add a few drops of 40% formalin to 25 ml of urine. For leptospira motility, submit fresh samples within 20 minutes. For studying leptospira morphology, submit a 25 ml urine sample preserved in 0.25 ml of undiluted formalin or in 1.5 ml of 10% formalin (Balamurugan et al., 2019).

**VI. Fecal Samples**: Fecal samples are mainly collected for parasitic examination, though bacteriological and virological analyses may be necessary. Gather uncontaminated samples, ideally directly from the rectum, using screw-capped vials (Miller et al., 2017). Collect at least 30 grams of sample promptly after defecation. If examination cannot be conducted within a few hours, refrigerate the sample without any preservative. Avoid freezing, as the prolonged chilling can compromise the viability of parasitic eggs, rendering them unsuitable for larval culture for strongyle egg identification. If fecal

samples need to be transported over long distances, consider fixing and preserving them. For a short-term preservation (a few days), mix fecal sample with an equal quantity of 10% formalin. For extended periods, use 70% alcohol. Wash flukes, tapeworms, and roundworms in water before preserving them in 5% formalin. Insects and snails should be submitted in a small container filled with 70% alcohol.

**VII. Rumen Liquor**: Rumen fluid, or rumen liquor, is commonly obtained using a stomach tube, rumen fistula, or a hypodermic needle (6-inch needle) inserted into the left paralumbar fossa. It is imperative to prevent exposure to air during collection to preserve the original pH and protozoal activity. The collected rumen fluid can be stored for 3-4 days for subsequent biochemical analysis. Mercuric chloride, at a rate of 1 mg per 5 ml of rumen fluid, can be employed as a preservative. This helps to maintain the integrity of the sample for a limited period. The normal pH of rumen fluid typically falls within the range of 6.7-7.2. Monitoring alterations in pH is instrumental in diagnosing conditions such as simple indigestion, alkalosis, and acidosis (Constable et al., 2016).

**VIII. Skin Samples**: Disinfect the area with alcohol for intact pustules or vesicles. Aspirate material with a sterile syringe and needle. Pluck hair from the lesion if ring worm is suspected. Scrape the lesion's edge with a blunt scalpel blade until blood begins to ooze. Place the collected material on the paper without any preservative. For mite infestation diagnosis, collect skin scrapings in a few drops of 10% KOH. Use a blunt scalpel to scrape from deeper layers of the skin, especially in peripheral areas for extensive lesions. When suspecting Demodectic mange, apply pressure on the skin to express mites from hair follicles. Mix the collected sample in a few drops of 10% KOH using sticks or a teasing needle, breaking scabs and matted hair.

**IX Ocular Samples:** Samples from the conjunctiva or eye can be collected by gently holding the palpebra apart and swabbing the surface. The swab is then placed into the transport medium. Alternatively, scrapings may be obtained directly onto a microscopic slide for further examination.

**X. Nasal Discharge** (Saliva, Tears): For samples from nasal discharge, saliva, or tears, cotton or gauze swabs on wire handles (preferable over wood due to flexibility) can be used. Moistening the swab with the transport medium before sampling is recommended. The swab should be in contact with the secretions for up to one minute, then placed in the transport medium such as Amies or Cary-Blair and sent to the laboratory at 4$^{o}$C. Long, protected nasopharyngeal swabs are suitable for collecting samples for suspected viral infections.

**XI. Abscess**: Collect approximately 3 ml of pus in nutrient broth for bacteriological examination. Also include scrapings from the abscess wall. Note that the pus at the center of an abscess is often sterile and the best results are obtained from recently formed abscesses.

**XII. Cerebrospinal Fluid**: It can be collected through suboccipital or sublumbar puncture under aseptic preparations. Examination of its color, turbidity, protein content, total cell count, differential cell count, and bacteriological examination helps to ascertain the cause of nervous diseases.

**XIII. Milk Samples**: Milk samples are primarily collected for mastitis diagnosis and to estimate antibodies against various systemic diseases. To minimize contamination, collect samples promptly and before administering antibiotics. Thoroughly wash the udder, swab the teat orifice with 70% alcohol, and discard the first a few streaks of milk. Collect 10 ml of milk from each quarter in labeled sterile tubes, refrigerate for transport, and promptly send milk samples to the laboratory to prevent a rise in total bacterial count due to delayed transport.

**XIV. Lymph Node Smears**: For lymph node smears, clean the prescapular lymph node site with an alcohol swab. Puncture the gland with a sterilized hypodermic needle, and draw a small amount of fluid into the syringe. Eject a few drops onto a slide to create a thin smear. Impression smears can be made from removed lymph nodes or biopsy portions, cutting the node and touching the cut surface on a clean slide. These smears are dried, fixed, and stained like thin blood smears, are used for detecting trypanosomes or asexual stages of theileria.

**XV. Semen Samples**: Semen examination is crucial for maintaining fertility rates. Collect semen immediately after collection, preferably using an artificial vagina or electro-ejaculation. Semen from electro-ejaculation is suitable for evaluating motility and sperm morphology. Submit preputial material or scrapings from bulls, unchilled (at 18-30$^{o}$C) in Amies gel agar transport medium for *Trichomonas foetus*, avoiding freezing or refrigeration.

**XVI. Brain Samples**: Submit the whole brain to the laboratory. If submitting brain in parts, cut it transversely (never longitudinally) into 2-3 pieces. Transverse cutting allows examination (gross and histological) for bilateral lesions in all brain sections.

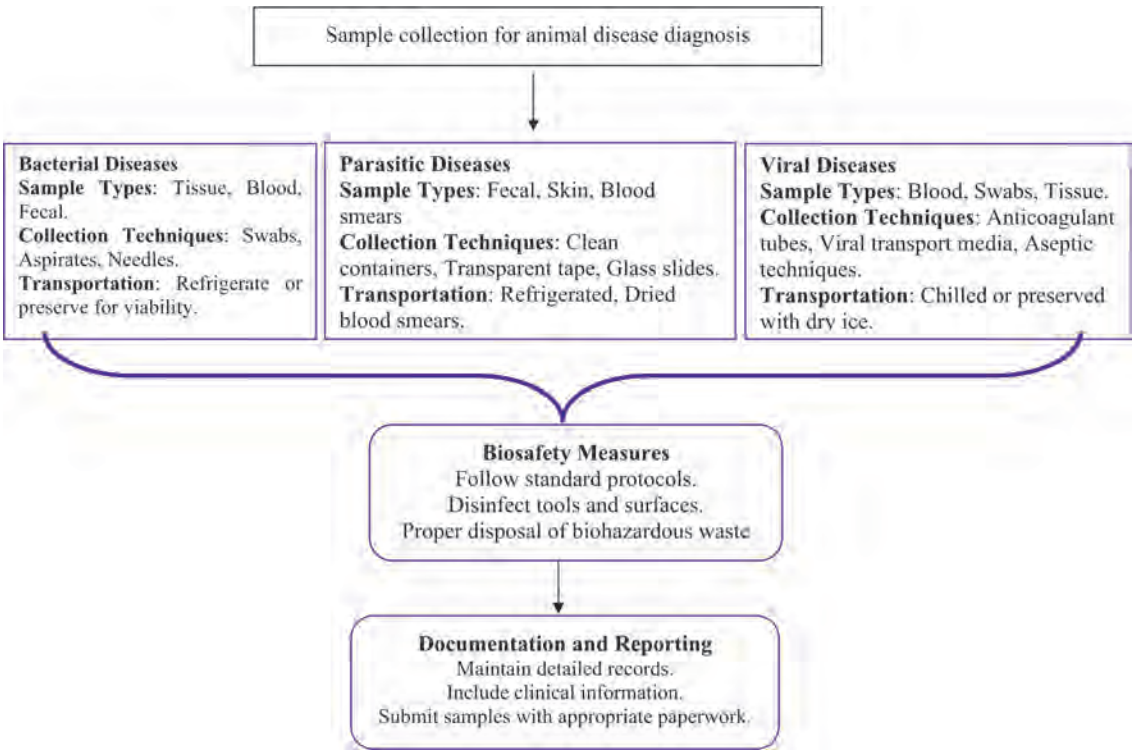
**XVII. Histopathological Samples:** Tissue pieces for histopathological analysis should be collected promptly after the animal's death or biopsy from living animals, preserving them without freezing to avoid autolysis and putrefaction. For optimal fixation, tissue pieces should not exceed 1 cm in thickness. Preferably, include both normal and diseased portions in the collected pieces. The preferred preservative for histopathological examination is 10% formalin, prepared by adding 100 ml of 40% commercial formaldehyde to 900 ml of water. The preservative volume should be at least 20 times that of the tissues. Preserve tissues in a wide-mouth container for ease of removal.

**XVIII. Cytological Examination**: For cytological examination, prepare smears from tumor tissues. Allow smears to dry immediately after collection to maintain cell

architecture. Fixation can be achieved through heat treatment or by dipping in absolute methanol.

**Samples to be Submitted during Outbreaks Investigation**

**Bacterial Diseases**: For suspected bacterial infections, affected tissue samples are collected in sterile broth media and should be sent on ice, alongside blood, pus, urine, or fecal samples for organism isolation. Surface cauterization of organs or tissues with a hot spatula is recommended before collecting deeper samples in case of the solid organs (Chandratre et al., 2023). For hollow organs like the intestine, secure both ends with a thread, cut, and place in a sterile petri dish. Transport media, such as Amies or Cary-Blair, is crucial for preserving anaerobic bacteria (Hyde et al., 2022). Avoid freezing; refrigeration at 4-10°C is sufficient to maintain cell viability and control commensal multiplication. Send paired serum samples for serological tests, and collect affected tissue samples in 10% formalin for histopathological examination (Ono et al., 2018).




**Viral Diseases**: For suspected viral infections, affected tissue samples or vesicular fluid in Hank's balanced salt solution, Tryptose Phosphate Broth, 50% phosphate-buffered glycerin or glycerin saline should be sent on ice, along with whole blood and paired serum samples for microbiological/serological tests (Kumar et al., 2022). Chill all virological specimens during transport. If submission to the laboratory exceeds 48 hours, freeze

specimens except blood samples. Also collect and submit affected tissue samples in 10% formalin for histopathological examination.

**Mycotic Diseases**: For culture examination or organism demonstration, collect affected tissue or skin biopsy samples in a sterile container without preservatives.

Sabouraud dextrose agar and Dermatophyte Test Media are commonly used for isolating fungi and dermatophytes. Submission of affected tissue samples in 10% formalin is also recommended for histopathological examination.

**Chlamydial Diseases**: Collect samples in chlamydial transport media [sucrose phosphate glutamate (SPG)] and store at 4°C. The SPG media contains sucrose potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and L-glutamic acid. Add fetal calf serum, vancomycin, streptomycin, nystatin, and gentamycin, avoiding penicillin, tetracyclines, and chloramphenicol, which inhibit Chlamydia growth.

**Protozoan Diseases**: For blood protozoan diseases, prepare thin and thick blood smears.

- For Coccidiosis, collect intestines, cecum, and fecal material to demonstrate oocysts.
- For Trichomoniasis, obtain vaginal and prepucial washings for organism demonstration.

**Parasitic Diseases**: For external parasitic infections, take deep skin scrapings in 10% KOH to demonstrate mites.

- For helminth infections, collect fecal samples without preservatives for demonstrating eggs.

**Poisoning**: Tissue samples should be at least 50g in weight and sent in chemically clean glass or plastic container. The type of poisoning suspected should be clearly written on the pack. The material should be sent immediately on ice without any preservative.

| **Suspected Poison** | **Samples to be submitted** |
|---|---|
| Nitrate poisoning | Fodder samples, water, serum |
| Cyanide poisoning | Fodder samples, stomach content, blood and liver |
| Urea poisoning | Feed, GIT contents |
| Heavy metals (lead, mercury) | Blood, liver, kidney, ruminal or stomach contents |
| Fluoride | Water |
| Organophosphate pesticides (chlorpyrifos, diazinon, dichlorvos, malathion, methyl parathion, parathion, etc.) | Suspected feed/ fodder samples, blood in anticoagulant, liver, kidney, stomach contents |

| | |
|---|---|
| Chlorinated hydrocarbon insecticides (aldrin, dieldrin, lindane, DDT, BHC, heptachlor, endosulphan, chlordane) | Suspected feed/ fodder samples, blood in anticoagulant, adipose tissue or fat, liver, stomach contents |

**Nutritional Deficiencies**

- Collect 5ml blood sample in oxalate fluoride/sodium fluoride for glucose and serum inorganic phosphate estimation.
- Collect 20-30ml blood in heparin for other constituents and serum for minerals and electrolytes estimation.
- Obtain urine samples for detecting ketone bodies.

**Abortion Cases**

- Collect a whole fetus if possible; otherwise, obtain fetal stomach contents, lung, liver, and samples of any gross lesions on or in the fetus.
- Submit a piece of the affected placenta and two or more cotyledons from cattle and sheep.
- For suspected vibriosis, collect vaginal mucus for organism isolation. If leptospirosis is suspected, preserve 20ml of mid-stream urine with 1.5ml of 10% formalin.
- Obtain serum from the dam for serological tests, preferably paired serum samples.
- Preserve placenta (cotyledons), fetal lesions, lung, and liver in 10% formalin for histopathology.

**Different Samples to be Collected in Various Infectious Diseases**

| **Infectious Diseases** | **Samples Collected** |
|---|---|
| **Viral Diseases** | |
| Rabies | Whole brain |
| FMD | Vesicular epithelium, whole blood, serum samples, esophageal pharyngeal fluid |
| IBR | Lung, trachea, liver, paired serum samples |
| Blue Tongue | Hepranized blood, serum, spleen, bone marrow, Lung, heart |
| PPR | Whole blood, serum, nasal and rectal swabs, Mediastinal and mesenteric lymph nodes, spleen and lungs |
| Contagious Ecthyma | Scabs with underlying tissue |
| Pox Diseases | Skin biopsy, lymph nodes, lung lesions, serum |

| | |
|---|---|
| Equine Infectious Anemia | Lymph node, spleen, liver, bones, whole blood, paired serum samples |
| Classical Swine Fever | Tonsils, parotid glands, lymph nodes, spleen, kidney, portion of caecum and colon, brain |
| **Bacterial Diseases** | |
| Anthrax | Postmortem should not be done; spleen, liver (If done) |
| Brucellosis | Placenta, uterine discharges, fetal stomach contents, paired serum samples from dam |
| Leptospirosis | Liver, kidney, urine, blood and paired serum samples |
| Listeriosis | Brain stem, aborted placenta, fetus |
| Tuberculosis | Affected lymph nodes or organs, tuberculous nodule |
| Johne's Disease | Caecum and colon, mesenteric lymph node, fecal samples |
| Black Quarter | Affected muscle pieces |
| Haemorrhagic Septicemia | Nasal swabs, Jugular blood (live animal), heart blood, lungs, spleen, bone marrow from long bone of carcass (Dead animal) |
| Glanders | Enlarged lymph nodes |
| Strangles | Abscessed lymph node, nasopharyngeal swab |
| Swine Erysipelas | Affected skin sample and heart tissue |
| Actinobacillosis and Actinomycosis | Affected tissue pieces and pus |
| CCPP | Lung, tracheobronchial and mediastinal lymph nodes |
| **Protozoan Diseases** | |
| Theileriosis | Blood smear |
| Babesiosis | Blood smear |
| Trypanosomiasis | Blood smear, Fresh blood |
| Coccidiosis | Intestines, caecum, colon, fecal material |
| Trichomoniasis | Vaginal and preputial washings (TF-InPouch) |
| **Mycotic Diseases** | |
| Systemic Mycotic Infection | Affected tissues |
| Cutaneous Mycotic Infections | Skin scrapping/ skin biopsy |
| **Parasitic Infestation** | |
| Mange | Deep skin scrapings |
| Helminth Infections | Fecal samples |

Therefore, a well-structured and streamlined system for gathering and sending biological samples is essential for robust animal disease surveillance and control. By giving importance to these elements, we contribute to ensure animal welfare, safeguarding public health, and maintaining the overall health of ecosystems connected to human and animal communities.

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# Critical Care Facility for Canine Patients- Need of the Hour

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**Abstract**

*Critical care in dogs requires a comprehensive approach involving medical, surgical, and supportive therapies. The article describes the need of critical care in dogs and how it can be applied practically in Indian context. It includes the assessment, monitoring, and treatment essentials in providing optimal care for dogs facing life-threatening conditions.*

**Keywords:** *Critical care, Dog, Management, Monitoring*

In dogs, conditions ranging from severe trauma and systemic diseases to post operative recovery and organ failure require critical management via a multidisciplinary approach, from veterinary medicine, surgery, pharmacology and nursing care (Mathews, 2017). This article aims to sensitize the readers about need of a critical care unit for canines and also to describe basic steps for the assessment, stabilization and monitoring of the critically ill canine patients, which can be useful for veterinary practitioners.

## Who are Critically ill Canine Patients?

Animals presented for various toxicities, hyperthermia in summers, acute kidney failure, snakebite, severe trauma, seizures, diabetes, gastric dilatation and volvulus, uroabdomen, hyperlipidemia and pneumothorax are critical ill patients.

## Facilities required

1. A designated place with air conditioner (for hyperthermia patients) and heating source (for hypothermia patient) for taking care of critical patients with all facilities ready in emergency cart
2. Oxygen delivery system
3. Face masks for oxygen delivery and endotracheal tubes of various sizes.
4. Emergency drugs like Adrenaline, nikethamide, dopamine and dexamethasone must be kept filled and marked on the cart with dose rates and route of administartion displayed in the room as a ready reckoner.
5. Cotton, bandages and tapes to handle any emergency bleeding (mention suture material for ligation).

6. Chest tubes
7. Anesthetic drugs (required for sedation in seizures)
8. ECG Machine
9. USG machine for AFAST, TFAST
10. Mobile X-ray machine
11. Acid-base monitor
12. Multipara Monitor
13. IV sets, IV Cannulas of all sizes (24-18 gauge), Central venous catheter
14. Consent note register/proforma

**Primary Assessment of Animal**

1. The presentation/mentation of the patient, itself tells about the condition of the animal. If a dog is presented dull, depressed, recumbent, panting profusely etc, it might be in need for critical care.
2. History: The history given by the owner may give the primary assessment of the condition.
3. Physical examination:
    - The dog may be examined for the color of the mucous membrane while listening to the history of the owner.
    - Touch the animal for the temperature assessment and record the temperature.
    - Look for any bleeding, respiratory distress, fracture, spinal trauma, urinary consistence, wounds etc.
    - Assess for the heart and chest with stethoscope.
4. If the dog is presented moribund, the supportive therapy/fluid line/oxygenation should be started right there and explain the possible prognosis and risk involved.
5. In case of guarded prognosis, explain the line of treatment/diagnosis that is going to follow and the risk involved in it.
6. A consent note may always be taken from the owner before proceeding any further.
7. After stabilizing the patient, go for haemato-biochemical/acid base analysis to assess the status of patient.
8. Ultrasonography if required can be performed by patient's bedside. Abdominal focused assessment with sonography for trauma (AFAST) can be performed for any free fluid in the abdomen (hemorrhage in case of internal injury). Thoracic

focused assessment with sonography for trauma (TFAST) can be performed to evaluate pneumothorax, any fluid in pericardial and pleural space.

**Cardio-pulmonary Resuscitation (CPR) Technique for Canine Brought with Apnea (Maton & Smarick, 2012)**

1. Intubate the dog with a suitable size endotracheal tube (ET) tube (smaller dogs like Pug, Shih Tzu, Pomeranians an ET of size 4.5-6, large dogs 8-10) and start IPPV (Intermittent Positive Pressure Ventilation). If ventilator is available, set the oxygen flow and breathing rate. If ventilator is not available, give oxygen manually by pressing the rebreathing bag after closing pop-off valve. Give 8-10 respiration per minute at the start.
2. While, one person is monitoring respiration, the other must check the heart rate. If heart rate is present and is bold, start with fluid therapy and do not stop IPPV.
3. If heart sound is not audible (an oesophageal stethoscope may also be placed to listen to the heart continuously), chest compression @ 100 per minute may be given.
4. Emergency drugs like adrenaline are to be given if heart rate is feeble or not present. The IPPV should not stop while drugs are being given. Other emergency drugs (table 1) may be given depending upon condition of the dog.
5. Fluid therapy is very critical at this stage; if the patient was presented in acute shock due to trauma/accident/bleeding, the fluid therapy with Normal saline may be given at fast rate, upto 90ml/Kg. But if the patient is critical due to some chronic condition and the mucous membrane colour is pale or white, a fast fluid therapy might be dangerous as it may dilute the blood further. Such cases may require whole blood transfusion.
6. A multipara monitor must be used to assess the oxygen saturation, pulse, temperature, CO2 level, ECG etc.

**Stabilization of Critically ill Patients**

1. **Airway:** Patency of airway should be maintained with the help of ET tube/mask. Take care not to apply mask to a patient which is panting, it will further block the airway. In such cases where hyperthermia is there and the animal is not allowing ET intubation, nasal oxygen pipes must be available. If there is any upper airway obstruction then tracheotomy may be performed.
2. **Breathing:** If breathing is absent, after intubation, put on ventilator or manual respiration @ 10 breaths/minute.

3. **Circulation:** Fluid therapy can be used for the treatment of shock which may be due to severe bleeding or dehydration or trauma, maintaining electrolyte and acid base balance in critical ill patients, rehydration, treating intoxications, and for renal support.
   - Fluid can be given intravenously, subcutaneously, or intra-peritoneally.
   - In birds/puppies, intraosseous route can also be tried.
   - If vein is not accessible other routes can be used. Use isotonic fluids except dextrose normal saline (DNS) through subcutaneous, intraosseous and intraperitoneal routes.
   - Isotonic crystalloids: 0.9% NSS, Ringer's lactate, DNS (Dextrose Normal Saline) etc. usually work everywhere for the first time when no assessment has been made.
   - Hypotonic crystalloid solution: D5W, 0.45% NSS are commonly used to correct electrolyte imbalances like hypernatermia in patients with less tolerance for sodium load such as heart failure and renal failure patients.
   - Colloids: Whole blood, plasma, dextrans and hydroxyethyl starch (hetastarch) etc are most commonly used when body fluid goes in third space such as in case of hypoproteinemia which leads to ascites. Colloids are used with 5-10ml/kg IV over 15 minutes. Hetastarch is most commonly used and available colloidal solution.
   - Initial fast infusion of crystalloids by 20–40 ml/kg IV for 15 minutes is recommended in cases of hypovolemic shock. Reevaluate the patient vital parameters for perfusion. If there is no improvement then colloidal solution such as hetastarch can be used.
   - If patient is stabilized then replacement and maintenance fluid volumes can be given which can be calculated as below:
     1. Replacement (in litres) = % Dehydration x Body weight (kg)
     2. Maintenance- It is normally calculated as 50ml/kg/24hr.
     3. Ongoing Losses- It includes mainly vomition and diarrhea losses

**Selection of Fluid Based on Critical Emergency Situations (Mathews, 2017)**

1. Total Blood required (ml) = 90 × Body Weight (kg) × [(Desired PCV - Patient PCV)/Donor PCV]
2. Diabetic Ketoacidosis: 25% Dextrose @ 3-5ml/Kg IV, and Ringer's Lactate @ 20-40ml/kg IV. Plasmalyte-A can also be used @ 25ml/kg IV.
3. Uroabdomen and Renal Failure: 5% dextrose in water (D5W) as CRI till potassium level is towards baseline as dextrose pushes potassium back into the cell. In

between measure the blood sugar, if exceed then insulin can be given at lower doses. To correct metabolic acidosis administer Ringer's lactate @20-40 ml/kg IV (Avoid Ringer's lactate in hepatic dysfunction patients as liver converts lactate into bicarbonate). If acid-base imbalance noticed, 7.5% bicarbonate solution @ 1ml/kg IV or equivalent can be given.

4. Head Trauma: Increased intracranial pressure and cerebral edema are common. So, colloids can be given. Mannitol @ 0.5-1gm/kg slow IV is most commonly used and it is available as 20% solution. Hypertonic saline (7.5% NaCl) @ 2-4ml/kg IV over 10-15 minutes can also be given.

**Table 1. Emergency Drugs**

| Drug | Dose Rate | Indication |
|---|---|---|
| Epinephrine | 0.01 mg/kg IV every 3–5 min | Use acceptable in most cases of Cardiopulmonary arrest (CPA); causes peripheral vasoconstriction and increased aortic pressure |
| Vasopressin | 0.8 IU/kg IV every 5 min | Use acceptable in most cases of CPA; causes peripheral vasoconstriction and increased aortic pressure |
| Atropine | 0.04 mg/kg IV every 5 min | Routine use is not recommended but may be used in animal CPA after high vagal tone (e.g., vomiting, diarrhoea) |
| Lidocaine | 2 mg/kg IV | Routine use is not recommended but may be used in shock-resistant Ventricular Tachycardia or Ventricular Fibrillation |
| Amiodarone | 5 mg/kg slow IV | Routine use is not recommended but may be used in Ventricular Fibrillation |
| Sodium bicarbonate | 1 mEq/kg IV | Routine use is not recommended but may be used in prolonged CPA or CPA due to severe hyperkalemia or severe metabolic acidosis |
| 10% Calcium gluconate | 50 mg/kg slow IV | Routine use is not recommended but may be used in CPA due to calcium channel blocker overdose, severe hypocalcemia, or severe hyperkalemia |
| Dopamine | 5 µg/kg/min CRI till normal blood pressure | Hypotension without hypovolemia |
| Diazepam | 0.5 mg/kg IV or per rectal | Epileptic seizure |
| Pentobarbitone | 5-10 mg/kg PO | Seizure |

**Pain Management:** Pain assessment and pain management in critical care patients is very crucial as pain has multiple negative effects by altering the physiology of animal. Types of drugs used to relieve pain include opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, ketamine and gabapentin. Opoids are potent analgesic but their availability is not easy being narcotics. The indications of most commonly used drug in India along with their contraindication and dose rate in dogs are given in the table 2.

**Table 2. Commonly used Drugs: their Indications and Contraindications**

| Drug | Dose | Indication | Contraindications |
|---|---|---|---|
| Butorphanol | 0.2-0.4mg/kg IM q2h | For mild pain | Head trauma patients |
| NSAIDs | Meloxicam-0.2-0.3mg/kg IM or IV<br>Carprofen- 2-4mg/kg IM or PO<br>Flunixin Meglumine-1.1mg/kg IM or IV | For pain due to orthopedic conditions such as osteoarthritis, fracture, panoseitis, hypertrophic osteodystrophy etc., for inflammatory conditions such as meningitis, cystitis, soft tissue swelling, otitis etc., for pyrexia, for postoperative pain | Renal and hepatic failure, coagulopathies |
| Steroids | Prednisolone- 0.5-1mg/kg IM or PO<br>Dexamethasone-0.5mg/kg IM or IV<br>Methylprednisolone-1-2mg/kg IM or IV | In head trauma, Spine trauma, immune mediated diseases such as neoplastic conditions | Should not be administered with NSAIDs |
| Lidocaine | A bolus of 2mg/kg IV followed by a CRI of 50µg/kg/min | If arrhythmia along with head trauma, tetracaine & proparacaine can be used as topical eye analgesics | Should not be administered at fast speed |
| Gabapentin | 5mg/kg PO tid | For neuropathic pain | Not to be administered in pregnant animal, severe hepatic dysfunction |

**Monitoring of Critically ill Patients:** Parameters such as blood pressure, heart rate, respiratory rate, temperature, and oxygenation status are crucial in evaluating the patient's status and response to interventions. Monitoring is required every 5 minutes initially,

later delaying to 15 minutes and 30 minutes as per the improvement. A multipara monitor can be used to monitor various parameters but it should always be checked in between and should not blindly rely on it.

The monitoring parameters along with their normal values for dogs are as follows:

| **Parameters** | **How to Monitor?** | **Normal Value in Dogs** | **Drugs for Abnormal Values with Dose Rates** |
|---|---|---|---|
| Blood pressure | Cuff is available with the Multi-para-monitor, which can be placed on the radius region of the dog or cat. | 80-120 mmHg | If Hypertension (>140mmHg)- Treat the root cause, If animal is not hypovolemic give Furosemide@ 1-2mg/kg IM first if not treated then amlodipine @ 0.2mg/kg PO, OD.<br>If Hypotension (<60mmHg)- treatment of the probable cause. Give fluid therapy-if not treated by it then can go for dopamine CRI @5µg/kg/min |
| Heart Rate | With the help of stethoscope or by multipara monitor | 70-120 beats/ min | If tachycardia- Lidocaine 2% by 2mg/kg as bolus followed by a CRI of 50µg/kg<br>If bradycardia- Inj. Atropine Sulphate @0.04 mg/kg IM or IV |
| Respiration Rate | By counting abdominal expansion or multipara monitor | 18-30 breaths/ min | If hypoxia then supply oxygen through face mask or ET tube |
| Temperature | By thermometer or multipara monitor | 102.5°F | If Hyperthermia, Give antipyretic drugs such as NSAIDs (Meloxicam, analgin etc.)<br>If Hypothermia, maintain the temperature by keeping the dog warm |
| Oxygenation | By Pulse oximeter | 95-100 | If below 95 then maintain proper ventilation |

**Nutritional Support:** Nutritional support is paramount in the recovery of critically ill dogs. It may include the enteral (through mouth) or parenteral nutrition (through veins) as per the condition of the animal.

**The Enteral Nutrition:** For a critically ill patient, it include the following and is also advised to the owner for home as well:

1. It is frequently possible to handfeed little amounts of easily palatable foods - like mashed potatoes and rice, boiled egg white, cooked pulses in liquid form, etc.- forcefully or voluntarily.
2. When the patient consumes a very little volume, nutrient-dense commercial critical care meals may be fed to maximize intake.
3. Other options include the use of appetite enhancers like diazepam and cyproheptadine.
4. Very ill patients may benefit from assisted enteral feeding with the implantation of different feeding tubes including orogastric, nasoesophageal, oesophagotomy, gastrotomy, and enterotomy tubes.

**The Parenteral Nutrition**

1. Parenteral feeding solutions include dextrose, emulsified lipids, crystalline amino acids, and specific vitamins and minerals.
2. The most preferred concentrations for hyperosmolar amino acid solutions are 8.5% and 10%.
3. The concentrations of hypo-osmolar emulsions are 10% and 20%. Egg yolks, glycerin, soy, and other ingredients are used to make lipid emulsions.
4. Preparation of vitamin K is injected subcutaneously or intramuscularly.
5. Slow infusion for the first 30 to 60 minutes may prevent pyrexia and vomiting related to lipid emulsion administration.
6. Diabetic patients should be monitored for blood glucose frequently.
7. Energy requirement for maintenance of dog is 132 Kcal per Kg of metabolic body weight. The requirement of protein is 2-3 grams per 100 Kcal in dogs.
8. A nonthrombogenic catheter with two or three lumens that is made of silicone, elastomer, or polyurethane should be used for parenteral feeding.

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# Trichography as a Diagnostic Tool in Canine Dermatology

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## Abstract

*Trichography is the examination of hair under the microscope in which a hair pluck is obtained using a haemostat and mounted onto a slide with liquid paraffin. It is a rapid, inexpensive and easy to perform technique. This technique when combined with history and clinical examination can aid in providing a tentative diagnosis, or an indication of the relevant differential diagnosis. It is a good diagnostic aid to determine hair growth phase abnormalities and the presence of parasites and dermatophytes in the follicles.*

**Keywords:** *Diagnosis, Dogs, Skin Diseases, Trichography.*

Diagnostic approach for skin diseases includes detailed history with thorough physical and dermatological examination. Skin scrapings, trichography, fungal and bacterial culture, cytological evaluation and skin biopsy are important diagnosis techniques in dermatology. Skin scrapings are utilized for mite detection, with the depth of the scraping depending on the suspected type of mite (Beale, 2011). Specific tests, and sometimes therapeutic trials, can also be conducted.

**Indications and Methodology**: Hair examination serves as a valuable tool in the differential diagnosis of skin and hair disorders. Trichography involves plucking hairs from the skin and examining them under a microscope. The direct examination of these hairs, known as a trichogram, entails pulling hairs from the affected area and evaluating them under a microscope. Trichograms have been proposed as an alternative to deep skin scrapings (Beco et al., 2007), especially beneficial in areas that are challenging to scrape, such as periocular and interdigital regions. It is a useful technique for the analysis of fungal diseases, parasitic diseases, congenital or/hereditary diseases and to differentiate self-trauma from other causes of hair loss/damage.

This method enables the identification of abnormalities in hair growth phases as well as the presence of follicular parasites and dermatophytes (Beale, 2011). Hairs are extracted from affected skin using forceps, pulled in the direction of hair growth (Fig. 1), and then carefully deposited onto a slide within a drop of either mineral or paraffin oil. To enhance the likelihood of obtaining a positive trichogram result, it is advisable

to pluck a substantial quantity of hairs, ideally ranging from 50 to 100. Trichography is performed by grasping a small number of hairs with hemostatic forceps, epilating them completely. The hair is laid on microscope slide (with mineral oil) with hair oriented in the same direction, or glass microscopy slides are prepared by previously sticking a double-faced adhesive tape on the upper surface (25.4×76.2 mm) in a longitudinal direction. The collected hairs are positioned separately in parallel, perpendicular to the tape surface, to facilitate hair bulb analyses. The hairs are examined for morphology, concentrating on hair bulb, integrity of the shaft, stage (anagen, catagen, or telogen), and pigmentation. The hairs are evaluated by light microscopy at magnifications of 40× and 100× and classed as anagen or telogen by the appearance of bulbs (Scott et al., 2001).

In typical adult animals, there exists a blend of anagen and telogen hairs, with the proportion fluctuating due to seasonal changes, management practices, and various other factors. Hairs are categorized as anagen phase when their bulbs display characteristics such as roundness, flatness, shininess, occasional curvature, and pigmentation (Fig. 2). Conversely, hairs are identified as being in the telogen phase when they exhibit features like a club-shaped appearance, wrinkling, lack of pigmentation, and typically straightness. Since catagen hair roots are morphologically similar to telogen roots (Fig. 3), hairs suspected of being in the catagen phase are classed as telogen (Diaz et al., 2004). The percentage of hairs in each phase can be determined by classifying the bulbs of the first 100 hairs with whole roots (Scott et al., 2001), always counting from right to left side of the glass slide. An additional count can be similarly undertaken to determine the percentage of damaged hairs, i.e. those with a broken hair shaft or a damaged root, by counting the number of damaged hairs within the first 100 hairs. All hairs should have a clearly discernible cuticle and a sharply demarcated cortex and medulla (Muller, 2001).

Fig. 1. Haemostat with solid locking mechanism. When performing hair plucks, grasp the hair shaft close to the skin.

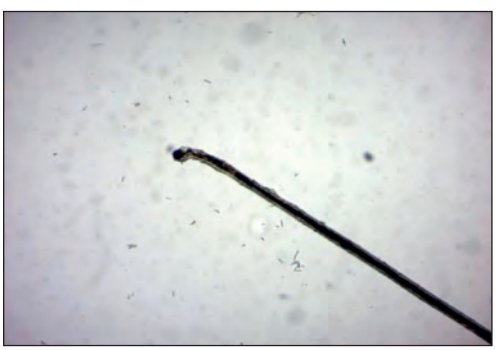

Fig. 2. Anagen hair bulbs are smooth and pigmented, tend to bend when plucked looking like a club. (100X)

When a hair bulb is present, it is important to document whether the follicles are predominantly in anagen, telogen or catagen. Anagen bulbs are round shaped, while telogen bulbs are often spear shaped. Predominance of telogen follicles can be indicative of endocrinopathies, nutritional disorders and metabolic diseases. Further, there is a great deal of breed variability.

After assessing the hair bulbs, the examination proceeds to inspect the hair shaft. Any hairs exhibiting abnormal curling, misshaping, or malformation may indicate an underlying nutritional, metabolic, or congenital/hereditary disorder (Muller, 2001). Conversely, hairs with an intact shaft but showing clean breaks or longitudinal splits typically suggest external trauma resulting from excessive licking, scratching, or overly vigorous grooming (Fig. 4). Hair casts are commonly observed in conditions characterized by abnormalities in follicular keratinization, such as sebaceous adenitis, demodicosis, primary seborrhea, and follicular dysplasia. The trichogram can provide diagnostic information in cases of demodicosis (mites seen around the hair bulb), dermatophytosis (spores and hyphae seen on the hair shaft), endocrine alopecias (all the bulbs are in telogen), colour dilution alopecia (macro-melanosomes in the hair shaft) and sebaceous adenitis (follicular casts seen around the hair shaft). Hair examination may be beneficial in cases of demodicosis or *Malassezia* dermatitis (Muller, 2001). A trichogram is used to visualize the hair for evidence of pruritus in case of self- inflicted alopecia (broken hairs), dermatophytosis, endocrine alopecia, pigmentation defects, and growth phase. If most of the hairs have been sheared off, this is likely the result of licking, as seen in cats that excessively groom.

Hair breakage is also observed in conditions like coat-dilution alopecia and traction alopecia. Several uncommon conditions can lead to damage to the hair shaft, with dermatophytosis being the most prevalent cause. In cases where trichograms yield negative results, it's advisable to perform deep skin scrapings before ruling out

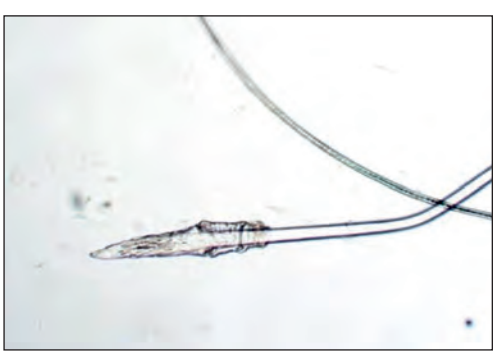

Fig. 3. Telogen hair bulbs have an arrow or spear shaped appearance. (100X)

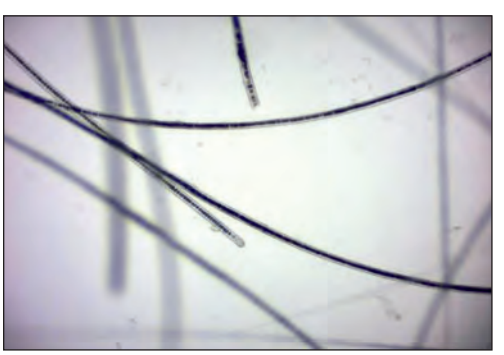

Fig. 4. Broken hairs are associated with over grooming. (100X)

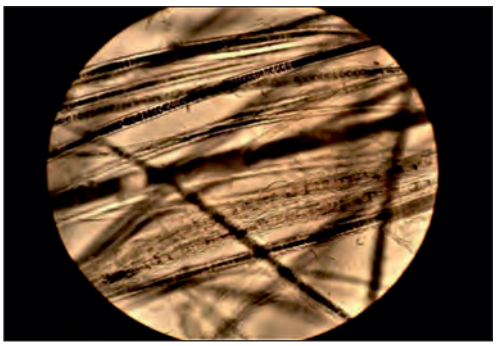

Fig. 5. Arthrospores on hair shaft by M canis (100X)

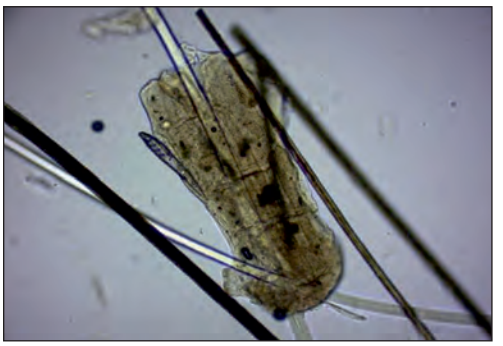

Fig. 6. Demodex mite in a follicular cast (100X)

demodicosis. It's worth noting that positive trichograms in otherwise healthy dogs are uncommon (Fondati et al., 2010).

**Advantages**: Trichography technique offers several advantages as it is simple, cost-effective, and accurate, unlike alternatives such as biopsy and fungal culture, which can be expensive. It serves as a valuable tool for diagnosing a wide range of disease processes, spanning from infectious and parasitic conditions like dermatophytosis and demodicosis to various forms of alopecia such as color-dilution alopecia and alopecia areata (Fig. 5 & 6). When diagnosing dermatophytosis, trichography complements a positive culture by providing evidence of active infection, as false positive cultures can occur due to contamination. A fur pluck may be more readily collected than a scraping from certain patients and certain areas of the body viz., face or feet and may be adequate to diagnose (but not to rule out) demodicosis. Trichography may be of value in generalized and complicated demodicosis (Saridomichelakis et al., 2007).

The major disadvantage of the technique is time consuming. Besides for dermatophytosis, it does not provide information about the fungal species involved. This technique requires practice, but once skilled, it is a very useful tool especially in field conditions either in making a list of the most likely differential diagnosis or a tentative diagnosis.

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# Significance of Toxic Neutrophils in the Peripheral Blood of Dog

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## Abstract

*Neutrophils are the most abundant circulating leukocytes that play a fundamental role in the innate immune response and acting as the first line of cellular defense against microbial infection. Toxic change is a common and important morphologic abnormality of neutrophils. Toxic change in neutrophils is not necessarily because of the toxic effects of neutrophils or toxemia. These changes are due to cytoplasmic alterations resulting from accelerated production in the bone marrow and include cytoplasmic basophilia, cytoplasmic vacuolation, Döhle bodies, and toxic granulation.*

**Keywords***: Cytoplasmic basophilia, Cytoplasmic vacuolation, Döhle bodies, Neutrophils, Toxic granulation*

Neutrophils are the most abundant circulating leukocytes in animals and humans and play a fundamental role in the innate immune response and acting as the first line of cellular defense against microbial infection. There also is a growing body of evidence to support neutrophils' substantial contribution to the adaptive immune response by modulating both cellular and humoral immunity, particularly by synthesizing and releasing immune-regulatory cytokines. Unsurprisingly, neutrophil kinetics and their role in disease have been extensively studied. Toxic change is a common and important morphologic abnormality of neutrophils, used as an inflammation biomarker.

**Neutrophil Kinetics:** In healthy animals, the rate of neutrophil production and release equals the rate of neutrophil egress from the circulation. At normal utilization rates, the marrow neutrophil reserve in dogs contains approximately a 5-day supply of cells. When there is increased demand for neutrophils, the marrow storage pool of mature neutrophils gets depleted. Band neutrophils, or even earlier granulocytic precursors, then are released into circulation, and the leukogram is referred to as left-shifted. A high neutrophil count with a left shift suggests that the bone marrow can respond to an inflammatory stimulus. In contrast, left shifts with normal or low neutrophil counts, despite stimulated granulopoiesis, suggest the inability of the bone marrow to meet increased demand. A degenerative left shift is defined by the number of immature neutrophils exceeding the number of mature neutrophils in circulation and implies that demand for neutrophils from an inflammatory nidus exceeds granulopoietic capacity. If there is a degenerative

left shift, then the prognosis is grave mostly (Leal et al., 2023).

**Role of Neutrophils in Disease Control:** Neutrophils are recruited rapidly to sites of inflammation, where their primary role is to kill invading bacteria and certain fungi through phagocytosis by release of preformed granular enzymes and proteins, and by the production of a range of oxygen species. Neutrophil homeostasis is maintained by a fine balance between granulocytopoiesis, bone marrow storage and release, intravascular margination, clearance, and destruction. Tissue inflammation results in the release of multiple inflammatory mediators and subsequent neutrophil priming and mobilization.

There are two components of the peripheral blood granulocyte pool; Circulating and Marginating (adherent to the endothelium of small venules and capillaries).

Neutrophilia may occur by three mechanisms; a). Mobilization of marginating cells, b). Increased rate of maturation, c). Increased rate of mitosis

The earliest identifiable neutrophil precursor is the myeloblast, usually found in small numbers in the bone marrow, but absent from the peripheral blood in healthy individuals, except in myeloid leukemia.

**Functions of the Neutrophils:** The lifespan of neutrophils in the marrow is 11 days. When neutrophils enter the peripheral pool, they only survive for hours. Survival in tissues is, however, 1-2 days. The neutrophils serve following functions:

- Migration to the site of infection or inflammation
- Phagocytosis
- Killing microorganisms by oxygen-dependent mechanisms. This involves the production of hydrogen peroxide and the superoxide anion by the enzyme NADH oxidase
- Killing microorganisms by oxygen-independent mechanisms - intracellular acid pH, or enzymes lysozyme and lactoferrin that are contents of the secondary granules.

**Toxic Changes in Neutrophils**: Toxic changes in neutrophils are not necessarily because of the toxic effects of neutrophils or toxemia. These toxic changes in neutrophils were first noticed in human patients with gram-negative sepsis and endotoxemia, so they are called toxic changes. These changes are morphologic abnormalities acquired during maturation when there is increased demand for neutrophil production in the bone marrow in response to inflammation, secondary to cytokine stimulation (Lambert et al., 2016). Animals recovering from bone marrow injury can also show accelerated maturation in neutrophils or toxic change.

Most of the toxic changes are due to asynchrony of maturation between the nucleus and cytoplasm. When there is normal granulocytopoiesis, the lengthening and pinching of the nucleus are coordinated with progressive condensation of the chromatin and loss of cytoplasmic protein synthetic machinery. But when there is accelerated maturation, nuclear divisions may be skipped (resulting in larger cells than normal) and the cells retain immature features, including increased amounts of rough endoplasmic reticulum or ribosomes in the cytoplasm and lighter chromatin than normal.

**Features of Toxic Neutrophils**: In a blood smear, there are five main features of toxic neutrophils:

- **Cytoplasmic basophilia**: Cytoplasmic basophilia is a severe form of toxic change that gives a blue, pale appearance to the cytoplasm (Fig. 1). It is due to the presence of polyribosomes and rough endoplasmic reticulum or their remnants in the band or mature neutrophils.

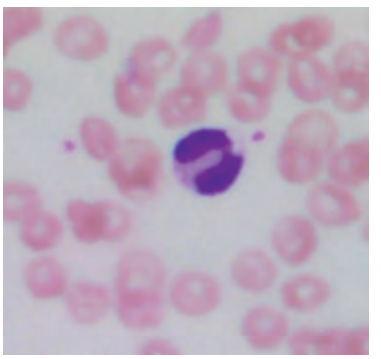

Fig. 1. Cytoplasmic basophilia

- **Döhle bodies**: Dohle bodies are the mildest form of toxic neutrophilic change. These are pale round to linear blue/grey aggregates in the cytoplasm, caused by whorls of degenerated rough endoplasmic reticulum or rarely free ribosomes. Dohle bodies always indicate a systemic effect of inflammation in dogs but are occasionally seen without significant inflammation in cats.
- **Cytoplasmic Vacuolation**: The foamy cytoplasmic vacuolation is an indication of severe systemic toxicity and occurs due to abnormal lysosomal formation and intracellular release of autolyzing enzymes. The toxic change appears as vaguely defined vacuoles throughout the cytoplasm (Fig. 2). The clear punctate vacuoles are usually not attributed to toxic change, but are frequently another storage-related artifact (these vacuoles can occur within 4 hours of blood collection).

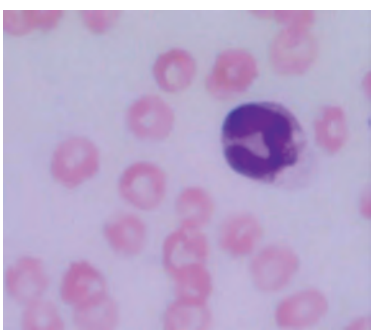

Fig. 2. Cytoplasmic vacoulation

- **Nuclear Immaturity / Giant Neutrophils**: The giant neutrophils represent skipped mitotic divisions of rapidly developing neutrophil precursor cells. Giant neutrophils are similar in appearance to, but about twice the size of, band or mature neutrophils and are generally found more often in cats than dogs.

- **Toxic Granulation:** Toxic granulation that occurs with severe abberant granulopoiesis is seen in Infection and inflammation or sepsis conditions, but burns cancers and non-infectious causes may also induce it. These are distinct granules in the cytoplasm due to the primary granules taking up stain manifested by the presence of large granules in the cytoplasm of segmented and immature or band neutrophils in the peripheral blood. The color of these granules can range from dark purplish blue (usually) to an almost red appearance (rarely) (Fig. 3). Toxic granules are azurophilic granules that contain peroxidases and hydrolases. These granules are normally seen in early myeloid forms but are not seen at the band and segmented stages of neutrophil maturation (Valenciano and Cowell, 2019). Increased basophilia of azurophilic granules simulating toxic granules may also occur in normal neutrophils with prolonged staining time or decreased pH of the stain.

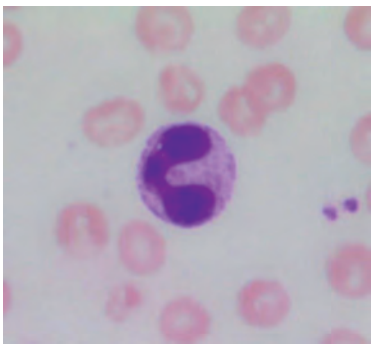

Fig. 3. Toxic granulation

On the standard hemogram, the presence of toxic change is reported while examining Romanowsky-stained blood smears. Each type of toxic change was assigned to 1 of 3 final grade scores of morphologic abnormality: mild, moderate, or marked (toxicity scores 1, 2, and 3, respectively) based on cytoplasmic toxic change and then adjusted for the percentage of cells affected (<10, 10–30, or >30%). The overall toxic grade is the sum of the 3 scores for each morphologic feature. A toxic grade of 0–3 is considered to be mild and clinically irrelevant, whereas a toxic grade of 4 or more was considered to reflect clinically relevant toxic change. The final toxic grade is the sum of the grades for each criterion, for a maximum grade of 9. A grade of 0–3 is considered mild and clinically irrelevant while a grade of 4–9 is considered clinically significant (Aroch et al., 2005).

Neutrophil toxic change indicates an early acute systemic illness. In dogs, toxic neutrophil change is most commonly caused by circulating bacterial toxins. However, tissue necrosis, a variety of drugs, and numerous nonspecific toxins such as lead are all capable of interfering with neutrophil development. Because toxicity indicates maturation arrest, the stage of development affected may be important in assessing prognosis or gauging response to therapy. In cases where both mature neutrophils and band cells are equally affected, no such interpretation is possible. However, in cases where mature neutrophils are toxic but bands are normal, morphology would suggest that the systemic toxemia is resolving. In contrast, in cases where bands are toxic but mature neutrophils are normal, morphology suggests a worsening condition. Morphologically,

toxic neutrophils exhibit a variety of alterations that reflect either cytoplasmic or nuclear developmental arrests or both (Al-Gwaiz & Babay, 2007).

The most common form of toxicity in dog neutrophils is foamy basophilia of the cytoplasm. This is a reflection of the retention of cytoplasmic RNA and the failure of the affected cells to form their normal complement of protein and cytoplasmic granules. Döhle bodies, a relatively minor sign of toxicity in neutrophils in most species is rather a reasonable sign of toxicity in dog neutrophils. Toxic neutrophils in dogs are extremely large; this is a reflection of nuclear maturation arrest characterized by the failure of developing cells to divide properly. Bizarre nuclear shapes and even multiple nuclei also are indicative of nuclear maturation arrests (Kabutomuri, 2000).

Thus toxic changes play an important role in diagnosing the severity and prognosis of a disease.

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# Potential Application of Artificial Intelligence for the Diagnosis of Parasitic Diseases

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**Abstract**

*Parasites are the major cause of various diseases in the livestock resulting into serious health risks, production and economic losses. Traditional diagnostic techniques have major constrains related to longer turnaround time, high costs, use of bulky equipments and need of trained staff in laboratories. In order to avoid these hindrances for proper diagnosis of various parasitic agents, accurate and rapid growth in artificial intelligence research is the crucial weapon in order to improve the veterinary practice. This article highlights the potential role of artificial intelligence in veterinary practice to diagnose various parasitic infectious for easy and quick diagnosis.*

**Keywords:** *Artificial intelligence, Diagnosis, Parasitic diseases*

Artificial intelligence (AI) is the ability of computerized systems to emulate cognitive functions such as learning and problem-solving in humans and animals. AI is basically a simulation of human intelligence into machines to do things that we would normally rely on humans. In today's world of revolutionary technological advancement, there is remarkable progress of AI in human and animal healthcare. The use of AI in livestock health management has transformed the dairy production industry. Artificial intelligence can be applied for diagnosis and prediction of various parasitic diseases like malaria, toxoplasmosis, cryptosporidiosis, giardiasis, toxoplasmosis, ticks/vector borne diseases etc.

The disease technology company Techion has developed and tested new machine learning tools that automatically identify the parasites using images from a camera. The recent study demonstrated that the artificial intelligence device 'VETSCAN IMAGYST' have also been accurately assisted by veterinarians to qualitatively identify parasites. IMAGYST is a verified learning system that classifies intestinal parasites with high sensitivity and specificity to help veterinarians make an accurate and timely diagnosis.

Commonly used AI-related key terms are as follows:

- **AI:** The field of computer science focused on intelligent computer programs that

can sense, reason, act, and adapt.

- **Machine Learning:** A subset of AI in which algorithms can improve in performance over time when exposed to more data.
- **Neural Network:** A series of algorithms used as a process in machine learning that can recognize patterns and relationships in large quantities of data. Neural networks use a logic structure inspired by the human brain and are the foundation for deep learning algorithms.
- **Deep Learning:** A subset of machine learning in which multilayered neural networks learn from vast amounts of data.

AI using deep learning and convolutional neural networks (CNNs) is increasingly becoming one of the important components of clinical parasitology diagnostics among all the AI techniques. Different artificial intelligencetechniques used to identify some of the important parasitic infectious agents infecting animals as well as humans are:

**Malaria**

*Plasmodium* parasites exhibit essentially the same but complex lifecycle stages that involve two major hosts, i.e., a vertebrate host (human or animals) and a vector host (mosquito), of which intraerythrocytic stages (trophozoite, schizont, and gametocyte stages) cause malaria. eg; *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and P. *knowlesi.* Although microscopy of stained blood smears is the standard method of malaria diagnosis but is difficult in distinguishing young ring-stage parasites and gets frequent misdiagnosedreports in areas of co-endemic for *P. malariae* with *P. knowlesi*. The intraerythrocytic stages vary significantly in morphology, the different stages of this parasite can be recognized easily by stained blood smear images, which can serve as the image sets used for machine learning (ML) based diagnosis analysis. In terms of malaria diagnosis, some critical solutions in ML, including image collection, image preprocessing, parasite and cell segmentation, feature selection, feature extraction, and cell classification (Poostchi et al., 2018). An AI-based object detection system called AIDMAN for malaria diagnosis from Smartphone thin-blood-smear images. This system employs a deep learning algorithm for the detection of *Plasmodium* spp. in thin-blood-smear images. In this system, the YOLOv5 and transformer model are combined to perform image analysis for malaria diagnosis. In this for each blood-smear image, a heat map of the most characteristic cells is generated and used for diagnosis especially by reducing interference caused by false positive cells model in order to detect cells in a thin blood smear. An attentional aligner model is then applied for cellular classification that consists of multi-scale features, a local context aligner, and multi-scale attention. Finally, a convolutional neural network classifieris applied for diagnosis using blood-smear images.

**Toxoplasmosis**

Toxoplasma infects almost all warm-blooded vertebrates and has multiple divergent life cycle stages. Two lifecycle stages – tachyzoites (invading red blood cells) and tissue cysts (invading brain or muscle tissue) – are correlated with the intermediate host, while another stage – the oocyst – is linked to the felid host and is released by faeces into the external environment. The toxoplasma oocysts in faecal, water and environment can be separated by filtration or centrifugation for examination bylight microscopy and the tissue cysts can be stained, which helps to distinguish the parasites from host cells either by Giemsa, Haematoxylin and Eosin staining or Periodic acid Schiff which stains amylopectin granules in bradyzoites. As these methods are relatively time consuming and require considerable skill to obtain reliable detection results. Thus, a deep learning method for *T. gondii* recognition is used to identify the cyst which looks similar to the aggregated parasites resembles the images of a bunch of bananas, where host cells are significantly different. It employs the fuzzy cycle generative adversarial network with transfer learning utilizing knowledge gained in order to identify *Toxoplasma* in banana or crescent shaped (Li et al., 2020).

**Cryptosporidiosis and Giardiasis**

*Cryptosporidium* spp. and *Giardia* spp.are the zoonotic enteric protozoans which are implicated as a cause of diarrhoea in dairy calves with wide range of vertebrates. Infected dairy calves excrete high numbers of cysts or oocysts for weeks and both diseases can potentially reduce the growth, performance and development of ruminants. These infectious agents can be transmitted by contaminated food and water. Therefore, detection of its infectious stages (oocyst and cyst) in the environment bears particularly critical significance for the prevention and control of infection. Diagnosis by microscopic examination of *cryptosporidium* oocyst by acid-fast stain and *giardia* cyst by lugols iodine staining technique is considered as gold standard for screening in laboratories but due to time consuming process, labor intensive and lack in sensitivity, one of the AImethods called as Artificial Neural Networksmethod is used to detect immunofluorescent labelled *Cryptosporidium* oocysts. It is ANN which is algorithms based on brain function and is used to model complicated patterns and forecast issues. Widmer et al. (2002) utilized ANN methods to identify *Cryptosporidium* oocysts and *giardia* cysts in a large-scale image set, and the correct rates of detected oocysts and cysts.

**Ticks**

Tick infestation not only causes blood loss in ruminants but also serve as vectors for various diseases especially babesiosis and theileriosis thus causing significant economic losses to the livestock causing. Besides, ticks can also transmit a variety of other pathogens in the livestock such as Lyme disease (Xu et al., 2021). Smartphone-

based deep learning (DL) algorithm (termed "TickPhone app") is available for tick identification. The deep learning model can identify more than 2000 tick images at a time and is usually optimized by different parameters, including normal sizes of images, deep learning architectures, image styles, and training–testing dataset distributions.

**Prediction of parasitic disease outbreaks**

Predictive disease modelling is becoming popular for forecasting of important diseases of economic importance especially for vector borne diseases. Raizada et al. (2020) used a convolutional neural network (CNN) algorithm trained with 2013–2017 data for vector-borne diseases (chikungunya, malaria, and dengue) with predicted disease outbreak accuracy of 88%. Predictive algorithms were also developed for forecasting malaria outbreaks (Oguntimilehin et al., 2015) and for mapping cutaneous leishmaniasis (Shabanpour et al., 2022). Early predictions of potentially important diseases offer valuable time for early public health interventions.

This article states about the application of artificial intelligence and its tremendous benefits in processing a large number of images for blood smears, stool samples, and tissue biopsies. Using ML tools such as CNNs, the AI has perfectly identified and classified parasitic stages such as eggs, larvae, and adult worms. The diagnostic power of AI has been well explored in detecting different intestinal parasites from faecal samples. The problem of timely detection and limited medical counter measures for parasite management can be decreased by use of AI in the field of veterinary parasitology. It limits the detection by conventional strategies and reduces human error and workload, especially in high-burden areas. AI-powered tools which are incorporated into smart phone-based applications, making parasite diagnostics readily available, even in remote areas with limited health-care infrastructure. Early prediction of parasitic diseases helps in implementation of prompt preventive and control measures. Despite several benefits of AI in healthcare, there is also disadvantage of potential for ethical and privacy concerns. There is a need to ensure that this data is collected, stored, and used in a secured manner. AI technology cannot replace human professionals but their role is going to change in the coming era of artificial intelligence, especially for the diagnosis of complicated diseases.

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# Epidemiology, Diagnosis and Control Strategies of Anaplasmosis in Bovines

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## Abstract

*Bovine anaplasmosis (gall sickness), is one of the predominant tick-borne diseases caused by Anaplasma marginale, an obligate intra-erythrocytic bacteria belonging to the order Rickettsia and family Anaplasmataceae. The disease affects various species of ruminants but it is more pathogenic in adult cattle and is clinically characterized by anaemia, icterus, fever, weight loss, dark brown urine, lethargy, production losses and death in neglected cases. The diagnostic techniques employed for the direct detection of the parasite are conventional microscopic examination of Romanowsky stained blood smears, nucleic acid-based assays and demonstration of antibodies by serological tests. The treatment of choice for anaplasmosis is long-acting oxytetracycline at a dosage of 20 mg/kg b.wt. and Imidocarb dihydrochloride at 1.5 mg/kg or imidocarb dipropionate at 3 mg/kg subcutaneously in acute cases. Under field conditions, typical necropsy lesions such as severe jaundice, enlarged gall bladder and splenomegaly are indicative of anaplasmosis. The integrated approaches for prevention of Anaplasma infection are chemotherapy, vector management and measures to prevent mechanical transmission.*

**Keywords:** *Anaplasmosis, Buffaloes, Cattle, Control Measures*

Anaplasmosis (gall sickness), caused by an obligate intra-erythrocytic bacteria of the genus *Anaplasma* belongs to order Rickettsia and family Anaplasmataceae, found exclusively in the membrane-bound vacuole within the cytoplasm of the host cell and usually affects the ruminants. In 1894, Salmon and Smith first time observed inclusion bodies in erythrocytes of calf, but the detailed description of *Anaplasma* was provided by Sir Arnold Theiler in 1910 in the erythrocytes of cattle in South Africa. Anaplasmosis is an economically important haemoparasitic infection in animals especially of cattle in the tropical, subtropical and temperate countries. The projectile economic losses incurred on the control of tick and tick borne diseases are USD 787.63 million per annum in India (Singh et al., 2022).

The different species of *Anaplasma* affects the dairy animals across the globe are *Anaplasma marginale, A. centrale, A. bovis, A. phagocytophilum* and *A. caudatum*. Among all the five species, *A. marginale* is the widely prevalent worldwide and is most pathogenic. *Anaplsama. centrale* causes mild disease in cattle, and is often used as a vaccine

to protect from *A marginale* infections in cattle (Abdala et al., 1990). *A. marginale* is endemic in India, while *A. centrale* is less pathogenic and its occurrence is low in the region. Morphologically *A. centrale* and *A. marginale* species are distinguished by the location of the inclusions in erythrocytes; more often found in the center of erythrocytes rather than in a marginal location.

**Life Cycle and Transmission:** The transmission of anaplasmosis is mainly implicated by the 20 different ixodid tick species, but *Rhipicephalus (Boophilus) microplus* is the primary transmission agent. Ticks transmit *A. marginale* biologically after extensive multiplication in several ticks by stage to stage (transstadial) or within a stage (intrastadial) transmission in several tissues of the tick like midgut cells and acini cells of the salivary gland. The rickettesial organisms multiply in the tick membrane by binary fission and grow in the form of reticulated (vegetative) body forming huge colonies that comprise of hundreds of the organism. The reticulated form matures and converts into the dense form which is the infective stage to the host (Fig. 1). The other modes of infection either mechanical via any blood-contaminated fomite, including contaminated needles, dehorning saws, nose tongs, tattooing instruments, ear tagging devices, and castration instruments or transplacental also reported. After many cycles of multiplication, the dense forms from the salivary glands of the ticks are inoculated into the mammalian host.

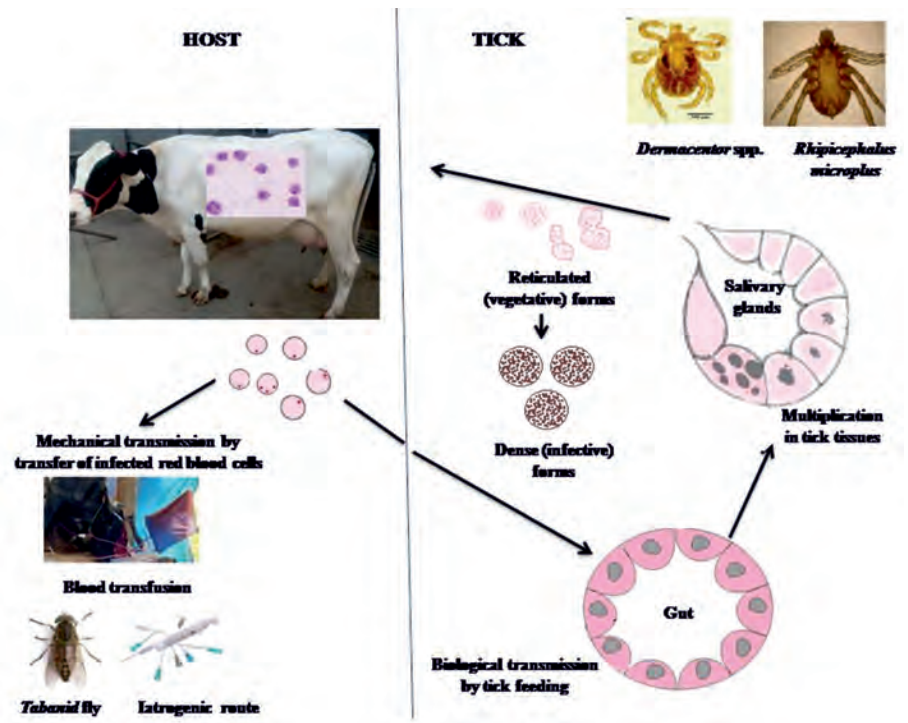


Fig 1: Life cycle of *Anaplasma marginale* (modified from Kocan et al., 2004)

Upon ingress in the erythrocytes, the *Anaplasma* bodies replicates by simple cell division and forms 8 to 12 initial bodies which exit from the erythrocyte without rupturing of the host cell (Kocan et al., 2004).

**Pathogenesis and Clinical Signs:** The clinical symptoms depend on the strain of *Anaplasma*, host susceptibility and varying level of parasitaemia. The adult cattle of age more than 2 years often have severe illness and possible high mortality than the animals less than 1 year of age that tend to either be resistant to infection or have very mild signs of illness. The resistance of young animals to infection may be explained partially by passive antibodies obtained from colostrum. The incubation period is 15-36 days after the initial infection in cattle (Kocan et al., 2004). Generally 10 to 90 percent of the parasitized erythrocytes results in the acute phase of the infection (Radostits et al., 2007). The main symptoms of anaplasmosis are anaemia and icterus result from the massive phagocytosis of infected erythrocytes by the bovine reticuloendothelial system, fever, weight loss, abortion, lethargy, reduced milk production and death in extreme cases (Kocan et al., 2004). Carcasses of animal that die from anaplasmosis are usually anemic and jaundice. The spleen is significantly enlarged and soft, with prominent follicles. The liver may be mottled and yellow-orange.

The recovered animals acquire the immunity to re-infection but these carrier animals serves as the main reservoir of infection for mechanical or biological transmission, and thus, contribute markedly to the spread of anaplasmosis.

**Diagnosis:** Routine diagnosis of anaplasmosis is based on microscopic examination of Giemsa stained thin blood smears which is considered as a gold standard test. *Anaplasma marginale* organism appear as dense, spherical, rounded red to dark red, deeply stained intraerythrocytic bodies of approximately 0.3–1.0 μm in diameter without cytoplasm present at or near the margin of the erythrocyte in acute cases or sub acute cases. In field conditions, typical post-mortem lesions of severe jaundice and spleen enlargement are indicative of disease which is confirmed by conventional microscopic examination. The microscopy based conventional tests inherits the limitation of required expertise personnel and organism may be misidentified with Howell Jelly Bodies. Initially a series of serological tests developed for the diagnosis of anaplasmosis were the complement fixation (CF) and card agglutination (CA) tests, with high specificity and low sensitivity for the identification of acute cases. Later several variants of the Enzyme-linked immunosorbent assay (ELISA) were developed. But in mid 1990 onwards, competitive ELISA (cELISA) with high sensitivity and specificity was widely accepted globally because of low cost and ability to screen large numbers of samples for epidemiological studies. Serological tests owe the limitation that it detects the presence of antibodies not the presence of pathogens. Specific and sensitive polymerase chain reaction (PCR)

is commonly used to detect parasite DNA from animal blood and tick and especially useful to confirm the latent cases and species confirmation of *Anaplasma* or other related haemoprotozoan parasites (Ntesang et al., 2022).

**Epidemiology:** The data on the prevalence and associated risk factors of the anaplasmosis in diary animals of India and world showed high heterogeneity due to diverse variables; different criteria of samples, breed, age, animal species and health status of the host, agroclimatic conditions, farm management practices and diagnostic technique(s). The review based on meta-data analysis of anaplasmosis depicted 11 percent prevalence in India and 39 percent prevalence throughout the world (Paramanandham et al., 2019). Based on the technique employed for the detection of *Anaplasma* spp. the prevalence at national level by serology, PCR and blood smear examination is 34, 32 and 7 percent (Paramanandham et al., 2019). Northern Indian states including Punjab have the tropical climate conditions which are ideal for the growth of the vector and vector borne pathogens including *Anaplasma* spp. in animals. The molecular prevalence of bovine anaplasmosis in the adjoining states of Punjab is 38% in Haryana (Bhanot and Jindal 2022), 42.07% in Rajasthan (Bhatnagar et al., 2015), 16.5% in Jammu (Kaur et al., 2021), 51.52 percent in Gujarat (Gohil et al., 2018) while the prevalence from the neighboring country Pakistan is 19.79% (Badshah et al., 2023). The published report on prevalence from different districts of Punjab state varied from 11.25-63.64 percent by microscopy (Ashuma et al., 2012; Bal et al., 2017) and 32.48-40.83 percent by PCR (Ashuma et al., 2012; Jaswal et al., 2014; Ntesang et al., 2022).

**Treatment and Control:** The control measures employed globally are arthropod control by application of acaricides, administration of antibiotics, preimunization with live or killed vaccines. The tetracycline (tetracycline hydrochloride, chlortetracycline, oxytetracycline, and doxycycline) are widely accepted chemotherapeutics for bovine anaplasmosis. The commonly used therapeutics is long-acting oxytetracycline at a dosage of 20 mg/kg, intramuscularly. Imidocarb dihydrochloride at 1.5 mg/kg or imidocarb dipropionate at 3 mg/kg subcutaneously is also found effective in acute cases. Chemotherapy of carrier animals require multiple doses over long periods that may result in antibiotic resistance. The regular screening of the previously infected and treated animals is very important to prevent the transmission of the infection to healthy animals. A blood transfusion is also required in severely anaemic animals. The prime objective for the control of *Anaplasma* infection is the control of vectors (ticks, flies and mosquitoes) through the adoption of the integrated approaches comprising of chemical, bio-control, vaccination, management and herbal medicine. In endemic countries, in absence of the vaccination programme, chemoprophylaxis with low doses of the tetracyclines can be prescribed for several months to prevent the infection. Under field scenario the mechanical transmission of the anaplasmosis by iatrogenic route is more common. The use of disposable needles

and syringes for treatment and mass vaccination, proper sterilization of the surgical instruments and equipments used for the castration, dehorning and other operative procedures is recommended and is very important in prevention of disease in field conditions.

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# Recent Advances in the Diagnosis of Rabies - A Review

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**Abstract**

*Rabies is the oldest known zoonotic disease and exclusively affects warm-blooded mammals. Although rabies is a vaccine-preventable viral disease but still it remains as a significant public health concern in most of the developing nations and territories. India alone is responsible for 35% of rabies deaths worldwide and close to 60% of rabies deaths in Asia. Uncontrolled spread of the virus through bite of rabid animals poses significant risks to the humans, resulting in death. Diagnosis of rabies can be made using a variety of conventional, immunological, serological and molecular techniques. The disease can be prevented and controlled via pre-exposure and post-exposure management.*

**Keywords***: Diagnosis, Rabies, Zoonotic.*

Rabies comes from the latin word ‘rabere’ meaning ‘to be mad’ or “the rage”. In sanskrit it is called as rabhas (to do violence). In the 1880s, Louis Pasteur discovered the neurotropism nature of the virus and was the first person to develop an effective rabies vaccination using rabbit spinal cord.

**Epidemiology:** Rabies is the oldest known zoonotic disease and exclusively affects warm-blooded mammals. As per the definition of World Health Organization (WHO), a country may be regarded as rabies-free,if it has no documented case of human or animal rabies that were contracted indigenously within two years period due to either import regulation or improved surveillance. Countries can be divided into three categories based on rabies occurrence- Rabies-free countries; Rabies-controlled countries and High risk countries (Table 1)(World population review, 2023)

**Table 1. Categorization of Countries on the Basis of Rabies Occurrence**

| Sr. No | Categories | Description | Countries |
|---|---|---|---|
| 1 | Rabies-free countries | They have no documented case of human or animal rabies within two years period i.e. entirely eliminated rabies from both domestic and wild animals. | American Samoa, Barbados, Australia, Belgium, Fiji, England, Ireland, Hawaii, Jamaica, Ireland, Malta, Japan, New Zealand, Scotland, Sweden, Singapore, the UK, and Vatican City. |

| | | | |
|---|---|---|---|
| 2 | Rabies-controlled countries | They have the rabies disease existing in the wild but, in general have a very low incidence of infection. | Bahrain, Belgium, Bulgaria, Belarus, Chile, Canada, Hong Kong, Kuwait, Hungary, Qatar, Latvia, Taiwan, Slovakia, UAE, Trinidad and Tobago, UK, USA |
| 3 | High risk countries | High frequency in either domesticated or wild animals. | Developing countries from Asia, Latin America, and Africa, where it is mostly endemic. |

Although rabies is a vaccine-preventable viral disease; it remains as a significant public health concern in most of the developing nations and territories. In Asia and Africa, children under the age of fifteen account for forty percent of the deaths it causes annually (WHO, 2023). Over 59,000 people worldwide die from rabies each year, with 45% of the global burden coming from South Asian Association for Regional Cooperation countries. India alone is responsible for 35% of rabies deaths worldwide and close to 60% of rabies deaths in Asia. Every part of India is endemic for rabies except Andaman and Nicobar and Lakshadweep islands.

**Viral Structure:** Rabies is caused by a bullet-shaped, negative-sense, single-stranded, neurotropic RNA virus of the genus Lyssavirus, belonging to the Rhabdoviridae family. It has a 180 nm length and 75 nm width, with a ribonucleoprotein core and an envelope. The genome contains five genes (3'-N-NS-M-G-L-5'), with the N gene coding for the capsid structural protein and the NS and L gene codes for non-structural components i.e. phosphoprotein and RNA dependent RNA polymerase respectively. The M gene codes for matrix protein and helps in viral assembly and release. The envelope has knob-like spikes composed of Glycoprotein encoded by G gene, which helps to form virus-neutralizing antibodies and induces protective immunity (Schnell et al., 2010). There are seven different rabies virus genotypes, with genotypes 1 causing rabies worldwide, while genotypes 2 (Lagos bat virus), 3 (Mokola virus), 4 (Duvenhage virus) widely distributed in Africa and genotypes 5 (EBLV-1), 6 (EBLV-2) are limited to European countries. The 7th genotype i.e. Australian bat Lyssavirus (ABLV) is limited to Australia. Rhabdoviruses are sensitive to heat, UV irradiation, and disinfectants.

**Transmission:** Rabies is typically transmitted to humans by a rabid animal's bite and a small percentage of rabies cases can be caused by licking of rabid animal on scratches or open wounds. In India approximately 91.5% are though dog bites of which 60% are strays and 40% pets (Menezes, 2008). Human mucosa or open skin wounds may also get contaminated if comes in close contact with infected animal saliva. Other rare routes of transmission are through organ transplantation, by the inhalation of virus-containing aerosols, consumption of raw animal flesh or milk. In India, dogs, cats account for 98%

of transmission and foxes, wolves, jackals accounts for rest 2% of rabies transmission.

**Pathogenesis:** Rabies has a variable incubation period ranging from 2 weeks to 6 years and it depends on virus inoculation site (greatest at head, neck, hand),viral load at the inoculation site, and density of nerve innervations. Following biting, the virus binds to G protein receptors at the target myocytes and amplifies in muscle cells and in macrophages. It then travels within the peripheral nerves via a retrograde axonal transport at a speed of 3-4 mm/hr towards the CNS (centripetally) (Mazarakis et al., 2001). The rabies virus infects neurons in the brain, causing neuronal dysfunction and resulting in clinical symptoms. The virus affects functioning of sodium-potassium ($Na^+$-$K^+$) ion channels, production and release of serotonin, GABA, acetylcholine, nitric oxide neurotransmitters (Singh et al., 2017). Rabid animals have lesions in the neurons of limbic system leading to altered behaviour and increased biting tendencies. The virus then spreads centrifugally (away from the CNS) via the parasympathetic fibres to salivary glands, also to many other organs. The rabies viruses is secreted in vectors' saliva and transmitted to new hosts via biting.

**Rabies in Dogs:** Furious form of rabies mostly seen called as "mad-dog syndrome". This form lasts for 1-7 days. Animal goes into rages, snaps at inanimate objects/ sand/ wood, shows biting tendency towards humans/animals, jaw champing, excess salivation, hyperaesthesia to light and sounds. Animal shows alert look and barking changes to coarse form. The dogs eventually develop muscle incoordination and quadriplegia along with paralysis of respiratory muscles leading to difficulty in breathing and finally it dies within 10 days due to respiratory failure. Paralytic/dumb form of rabies characterised by peculiar starring expression of animals and they show a hiding tendency. Paralysis of muscles of mastication leads to characteristic dropped jaw condition, profuse froathy salivation, difficulty in swallowing, tongue protrusion. Soon paralysis of whole body occurs and the animal dies.

**Rabies in Other Animals**

**Large Ruminants:** In the furious form of rabies animal is restless and hyperaggressive and has clinical signs such as anorexia, pruritis, ataxia, lameness, tenesmus, hypersalivation, nymphomania. Rabid cattle may appear choking, prompting owners and veterinarians to remove foreign bodies. This form lasts for 24-48 hrs after that the animal become paralytic and dies. The paralytic form is characterised by soundless bellowing movement, swaying of hindlimb, knuckling of fetlock ultimately collapse and death.In camel, furious form is common and shows signs of restlessness, salivation, and an attacking and biting behaviour, and terminally paralysis seen with characteristic flexion of four limbs (Kasem et al., 2019).

**Small Ruminants:** Clinical signs are similar to cattle and it includes excessive bleating,

hypersalivation, muscle tremor, hyperexcitability and hyperaesthesia. However sick sheep does not show excessive bleating unlike that of goats.

**Pigs:** Clinical signs in rabid pigs were aggressiveness, violent grunting along with profuse froathy salivation, head twitching, hyperexcitation, terminal paralysis, convulsions and lateral recumbency.

**Wildlife:** Resemble that of furious form of rabies in dogs with initial loss of fear of natural enemies and animal attacks humans and other wildlife. Later the animals become paralytic and die.

**Detection and Identification of Virus**

- **Based on History and Clinical Signs:** If an animal is showing abnormal behaviour, it should be quarantined for ten days to prevent it from biting people.
- **Collection of Sample:** For the antemortem diagnosis the samples obtained can be saliva, serum, cerebrospinal fluid (CSF), blood, urine, nasal swab, hair follicles, eye and throat, corneal impression smear. For post mortem diagnosis tissues from the brain (brainstem, cerebellum and hippocampus) are the preferred specimen (Warrell and Warrell, 2004).
- **Transport of Sample:** If refrigeration is not possible other means of sample preservation can be used such as 10% neutral buffer formalin, 50% glycerol.
- **Laboratory Tests**

**1. Immunochemical Identification of Rabies Virus Antigen**

***i. Direct Fluorescent Antibody (DFA) Test:*** A routinely used gold standard technique to show rabies antigen in clinical specimens that is widely recommended by both WHO as well as OIE for diagnosis of rabies is the direct fluorescent antibody (DFA) test (WHO, 2016). This test utilises certain monoclonal antibodies for detecting viral antigen in the clinical specimens. It can be used directly on a brain impression smear giving an apple green fluorescence (Fig. 1). The test is 100% sensitive in fresh brain tissue, and it is possible to receive a confirming diagnosis in two hours.

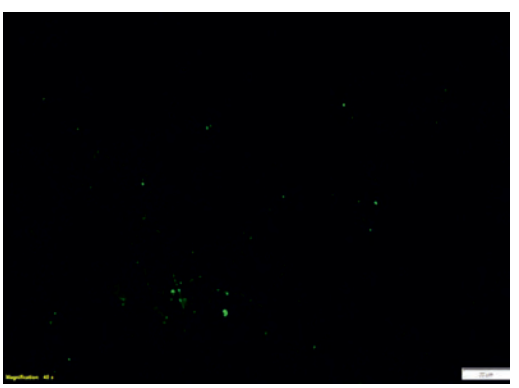

Fig. 1. Impression smear of brain showing characteristic apple green fluorescence indicative of rabies antigen (dFAT, Bar=20µm)

The brainstem showed the highest FAT sensitivity of 99.6%, followed by the cerebellum (99.3%), cerebrum (98.9%), and hippocampus (98.7%).

ii. ***Direct Rapid Immunohistochemistry Test (dRIT*)**: As the dRIT has similar sensitivity and specificity to that of DFA it can be used as an alternative in routine rabies diagnosis. The principle is almost similar to the DFA except that the here streptavidin–biotin peroxidase staining is used here.

iii. ***Reverse-Transcription Polymerase Chain Reaction (RT-PCR)*:** RT-PCR tests have similar specificity and sensitivity to that of DFT & dRIT, so it can be used as an alternative to these tests for routine rabies diagnosis. Advantage of this test is that instead of using live virus, lyssavirus-derived ribonucleic acid (RNA) is used. The RT-PCR test that target 3' proximal viral gene gives most sensitive result. Among different types of RT-PCR reactions, conventional pan-lyssavirus hemi-nested RT-PCR assay (hnRT-PCR) and real-time pan-lyssavirus RT-PCR assay (based on a fluorescent DNA stain) are most commonly used. The nucleoprotein gene (N gene) is widely used for molecular characterization and phylogenetic analysis of RABVs (Mauhay et al., 2023). RNA-dependent RNA polymerase or large protein (L) also has been targeted (Nakagawa et al, 2017). Both N and P gene, while N and G gene has also been targeted (Huaman et al., 2023).

iv. ***LN34 Assay:*** Recently a novel molecular method, the LN34 real-time RT-PCR assay, can detect the RABV genome in formalin-fixed tissues. The LN34 amplicon is a 164 base pair (bp) DNA fragment present at the 3′ end leader region of the RABV genome and it also covers a portion of nucleoprotein gene (Minozzo et al., 2022).

v. ***Rapid Immunodiagnostic Assay (RIDA):*** A monoclonal antibody-based technique has been introduced for rapid diagnosis of rabies. In this, a swab is soaked in 10% brain homogenate sample, transferred to a proprietary RIDA kit buffer. Then 100 µl of this aliquot is added to the sample well. A positive result indicated by two lines formed after 5 minutes, while a negative result indicated by one line formation (Ahmad & Singh, 2016).

vi. ***Rapid Immunochromatographic Tests (Lateral Flow Devices):*** These rapid immunochromatographic tests are commercially used for detecting viral antigen quickly.

**2. Histopathological Identification of Negri bodies**

Demonstration of Negri bodies by microscopy is the pathognomic histological feature of rabies. Negri bodies are found in the cytoplasm of pyramidal cells of the hippocampus in case of dogs and also in the Purkinje cells of the cerebellar cortex in case of cattle (Fig. 2). With haematoxylin-eosin staining, Negri bodies appear as acidophilic,

oval or round and highly refringent, with Seller's staining it appears as magenta color with small (0.2–0.5 mm) and dark blue interior basophilic granules and with Mann's staining it appears red. Viral inclusions and virus particles can be found using electron microscopy.

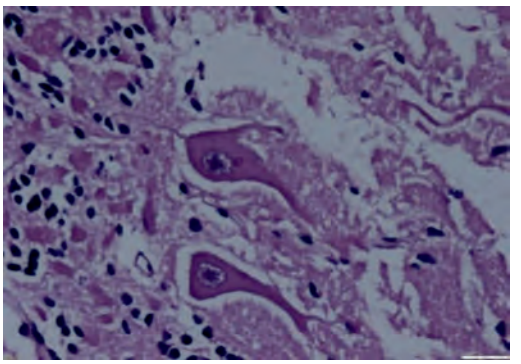

Fig.2. Cerebellum brain: Intra cytoplasmic inclusion Negri bodies in the Purkinje cells (H&E, 40X)

**3. Virus Isolation**

Virus isolation in cell culture should be given more preference than the mouse inoculation test (MIT), as the cell culture tests avoid use of animals, are equally sensitive as that of mouse inoculation test, are rapid and less expensive.

**i.** **Rabies Tissue Culture Infection Test (RTCIT)**: Different cell lines such as baby hamster kidney (BHK) 21/C13, mouse neuroblastoma cell line – Neuro2a/CCL 131, American type culture collection (ATCC), Vero cell, McCoy cell, chicken embryo related (CER) cell line are being used for culture. Direct fluorescent antibody test (dFAT) and monoclonal rabies antibodies labelled with fluorescein iso-thiocyanate is used to detect viral replication (the antigen).

**ii.** **Animal Inoculation**: The preferred laboratory animal is mice. For this 3-10, 3-4 weeks old mice are inoculatedintra-cerebrally with supernatant of brain tissue homogenate. After 28 days of injection or if any mice dies before that, the brain tissues are examined under a microscope for the presence of Negri bodies or for viral antigen using the DFA or dRIT test.

**4. Serological Tests**

These tests are mainly used to know the response to vaccination particularly during the international travel, or for monitoring mass vaccination campaigns. The viral neutralization test is the most significant serological test. Rapid fluorescent focus inhibition test (RFFIT) and fluorescent antibody virus neutralization test are the procedures currently advised by WHO for calculation of RABV neutralizing antibodies. The most frequent applications of RFFIT are for assessing seroconversion after prophylactic immunization and for assisting with ante-mortem rabies diagnosis in suspected cases**.** Now-a-days ELISA is also used for the purpose of rapid detection of antibodies.

**Prevention and Control:** In 2015, the World Organization for Animal Health (OIE), the Food and Agriculture Organization, and the Global Alliance for Rabies Control jointly called for action to eliminate rabies by the year 2030 'Zero by 30'. This prevention and control can be broadly categorized into pre-exposure and post-exposure management.

**Pre-exposure Vaccination and Management:** The primary components of rabies control are managing the removal of stray dogs and cats, administering timely vaccinations to dogs and cats, monitoring the movement of pets and following quarantine measures whenever introducing to a new place. The WHO advises all staff members who handle contaminated materials to get preventative immunizations. The immunization protocol follows 0, 7, and 28 days protocol. It is followed by a booster shot at one year, then revaccination every one to three years. For domestic animal the vaccine is administered via parenteral route and oral vaccine is used for immunization of wild animals. The injectable inactivated viral vaccines are used mostly for companion animals and livestock and injectable recombinant vaccines are used for cats. In 2004, the WHO stopped recommending modified live rabies vaccine strains, namely Evelyn-Rokitnicki-Abelseth (ERA), Flury high egg passage, Flury low egg passage, and Street-Alabama-Dufferin (SAD) for parenteral inoculation in animals.

**Post-exposure Management:** It is mandatory to strictly follow quarantine for 6 months for any animal exposed to a confirmed or suspected rabid animal (Table 2). Upon entry into isolation or 1 month before release, administration of rabies vaccine should be done. Cattle and horses upon exposure, there is requirement of revaccination (On days 0, 3, 7, 14 and 30, I/M route) immediately followed by observation for 45 days. It is suggested to euthanize animals developing signs of rabies and the brain sample must be shipped for testing. For travellers as well as veterinarians and researchers pre-exposure vaccination (two 1ml doses, on each on 0 and 7 days) is appropriate. In humans post-exposure immunization followed by injection of immunoglobulins specific for RABV either of equine or human origin. Collectively this is called as PEP, which can be decided by the exposure level.

**Table 2: Post Exposure Management**

| Category | Type of Contact | Recommended Post-Exposure Prophylaxis |
|---|---|---|
| I | Animal contact or feeding Licks on unbroken skin | None |
| II | Minor scratches or abrasions without bleeding | Immediate vaccination + Wound management. Stop treatment if the animal remains healthy after 10 days or is confirmed to be negative |
| III | Skin bites or scratches, licks on broken skin/ mucous membrane / bat bite | Immediate vaccination + Wound management + Rabies immunoglobulin |

## Gaps in the Research and Future Directions

Despite vaccination, there is increase in the cases of rabies in animals. This may be due to either vaccine inefficiency or mutation in virus strain. Again, rabies vaccinated animals should be checked periodically for the effective titre (not less than OIE approved titre of 0.5 IU/ml) of the antibodies. Further study is needed for genotyping of the rabies virus to know the population dynamic of rabies virus strains in a particular area.

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# Lumpy Jaw in Bovines

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**Abstract**

*Actinomycosis (Lumpy Jaw) in bovines commonly affects mandible or maxilla and rarely other skull bones. When the bone is involved; it may interfere in prehension of feed and/or rumination. Presence of abscess like lesion(s) near the mandibular region is the common findings, with thick granular pus coming off and on. This disease condition is transmissible to other bovines with weak immunity. The treatment rarely involves surgery and is usually treated medicinally with a variable success rate. This article describes the diagnosis and treatment of actinomycosis in bovines.*

**Keywords***: Actinomycosis, Buffalo, Cattle, Chronic abscess, Mandible*

Actinomycosis in bovines is caused by *actinomyces bovis*, a gram-positive, anaerobic, non-acid fast, filamentous bacterium. Actinomycosis in cattle and buffaloes usually affects skull bones with common predisposition to the mandibular or maxillary bones. It is commonly known as lumpy jaw as the mandibular region or mandible bone is most commonly affected in this disease condition (Constable et al., 2016). This article describes the clinical features, diagnosis and management of actinomycosis in bovines.

**Clinical Pathophysiology:** The bacterium, *Actinomyces bovis,* is normally present in the oral cavity of the bovine and the Infection occurs through abrasion in buccal mucosa due to coarse feed material or also through dental alveoli at the time of teeth eruption. There are cavities and sinuses formed in affected bone which contain thick yellow pus with small granules (sulphur granules). The organism is most commonly found in the centre of crushed granule. The teeth embedded in affected bone become loose, displaced and become painful which cause difficulty in mastication and prehension. The swellings are very hard, immovable and, in the later stages, painful to the touch. They usually break through the skin and discharge through one or more openings. An anti-fibrotic agent is required in the treatment protocol to let the antibiotic enter upto the bacteria.

**Clinical Signs:** Usually affected animals do not show any clinical signs except when bone is involved that interferes with prehension of food and mastication. Major clinical findings include presence of localized, chronic, and progressive lesion with hard, painless granulomatous abscessation in the mandibular, maxilla and occasional other skull bone

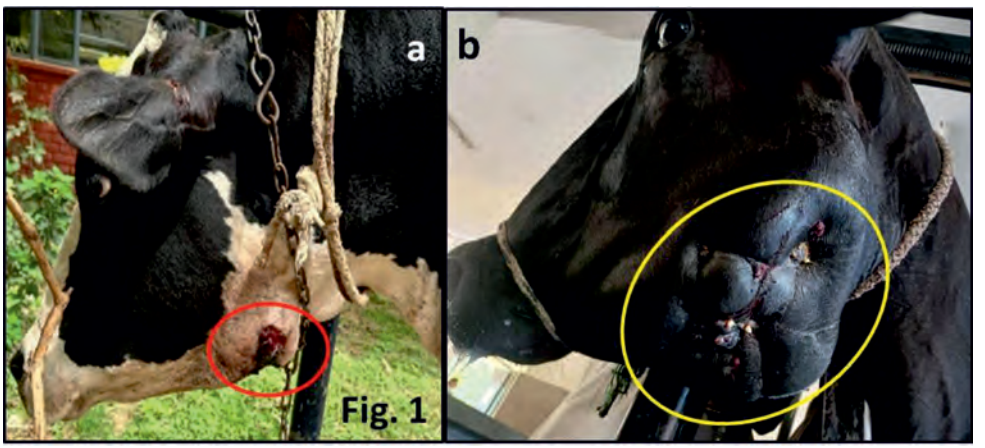
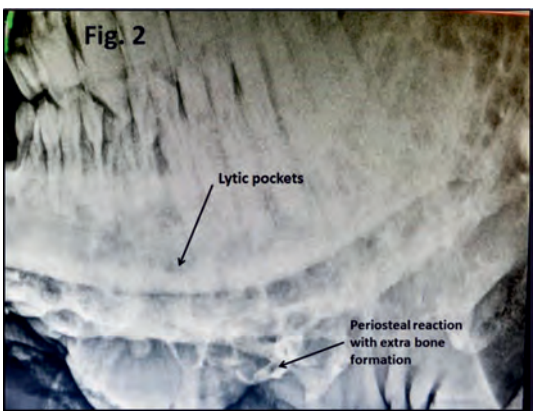



Fig. 1. Photograph showing the clinical presentation of a cow with lumpy jaw condition but no bone involvement (a) and another cow with bone involvement (b) on radiograph.

regions. The small abscess has yellow pus with sulphur granules in it and is surrounded by large amount of hard fibrous tissue (Fig. 1a). The swelling is usually diffuse and hard along the bone if, it is involved (Fig. 1b).




**Diagnosis**: The Lumpy jaw condition can be diagnosed based on

- Typical gross clinical lesions
- Gram staining of pus granules, which will show branching filamentous (sulphur granules) organism. For collection of pus, a swab can be obtained from the discharge and is smeared on the slide for staining. Although, the bacterial isolation is rarely successful as it requires anaerobic conditions for the growth of the bacteria, however, cytology can help with filaments visible in it.
- A lateral oblique radiograph of the mandible/maxilla (by keeping the mandibular lesion lower and towards the cassette) which may show rarefication with multiple lytic pockets and periosteal reaction (Fig. 2). If bone is more severely involved, loosening of teeth or bony deformity may also be seen.

**Treatment**

- Inj. Sodium Iodide (70mg/Kg of a 10-20% solution), intravenously, at weekly interval, several times can be given. If signs of iodine toxicity develop, (eg

diarrhoea, dandruff, anorexia, coughing, excessive lacrimation), discontinue medication for a few weeks or the interval may be increased. Sodium iodide is considered safe in pregnant cows with little risk of abortion (Aiello et al., 2016).

- Antibiotics like Penicillin {Penicillin G + streptomycin}, gentamicin @ 4mg/kg, terramycin should also be added for 5-7 days, twice daily, along with an anti-inflammatory for 3 days.
- The wound should be managed to prevent maggot infestation by regular wound dressing and spraying fly repellent. Powder magnesium sulphate may also be used after flushing the wound with Normal saline, if required.
- An alternative to Sodium Iodide is Powder Potassium iodide which can be administered orally @6-7gm to adult bovine for 15 days. If improvement is present the treatment may be extended, provided toxicity signs of iodine are not present. Potassium iodide is available as a 20 gm preparation, which can be divided as per the animal body weight. A commercial preparation with the trade name SPE-15 (with Potassium Iodide Serratiopeptidase Niacin Silymarin Antioxidant) is also available, which contain 5 sachet, one each for an adult bovine, orally. The period of administration may be adjusted as per the response to treatment.

In summary, most of the cases of lumpy jaw in bovines with mandibular involvement can diagnosed based on typical lesions / clinical presentation and can be managed successfully with medicinal treatment.

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# Technique of Burdizzo Castration in Farm Animals

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## Abstract

*Burdizzo castration is a bloodless method of castration for farm animals and is routinely used to castrate calves, lambs and kids at field level. It is done using Burdizzo castrator which is available in different sizes for both large and small ruminants. This method causes ischemia of the testicles leading to degenerative changes in the testicular parenchyma and subsequent atrophy. This article describes indications, important steps, guidelines and precautions to be exercised for optimum outcome during Burdizzo castration in farm animals.*

**Keywords***: Burdizzo, Castration, Spermatic cord*

Castration in young farm animals is an age old practice, which mainly aims at taming the aggressive behavior of males in order to use it for draught purposes and to make them docile for easier handling. Various methods of castration have been adopted over time, each with its own advantages and disadvantages (Gilbert et al., 2017). The most popular methods of castration used presently are Burdizzo castration and surgical method. Use of elastrators and chemicals have also been reported, but are not used in current practice as they are considered inhumane (Denooy, 1992). Surgical method of castration requires expertise and sterilized instruments, Burdizzo method of castration on the other hand is easier to perform under field conditions. However, if not performed using the correct technique, Burdizzo method can lead to severe complications. Therefore, it is very important for veterinary practitioners working under field conditions to keep in mind certain important points explained in the subsequent sections while castrating animals using Burdizzo castrator.

## Indications for Burdizzo Castration

- To reduce aggressive behavior and tame animals for draught purposes
- To improve carcass quality
- To prevent unwanted mating

## Age for Castration

- Calves: 8-10 weeks
- Lambs and Kids: 2-4 weeks

This method of castration is suitable only for young animals as cremaster muscle in older animals is large enough to prevent proper crushing of the spermatic cord (Capucille et al., 2002) thus making this procedure ineffective in adult animals.

**Restraining:** Animal is restrained in lateral recumbency with hind limb on top pulled apart so as to approach the testicles (Fig.1).

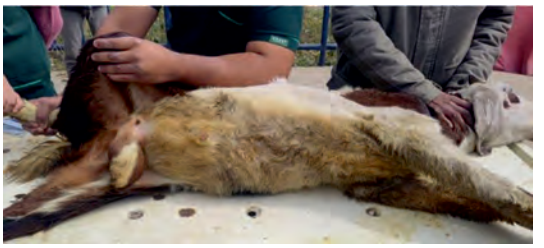

Fig.1. Photograph showing restrain of a kid for castration

**Anaesthesia:** Local infiltration of 2% lignocaine into each testicular parenchyma (not more than 1 ml per testicle, depending on the weight of the animal). The anesthetic drug will diffuse in to the spermatic cord ensuring analgesia during crushing of the spermatic cord.

**Procedure**

- Choose the appropriate size of castrator according to the animal, as undersized castrator will not have enough force to crush large amount of tissue and the bigger sized castrator may cause more tissue trauma and skin wounds which can lead to complications.

Fig. 2. Photograph showing isolation of spermatic cord

- Pull the scrotal sac so as to gain access to majority of the length of the spermatic cord and to avoid urethra in the field of operation, which might be mistakenly crushed otherwise and can lead to urinary obstruction (Gupta & Sangwan, 2023). Isolate the spermatic cord of one of the testicle and pinch it to the lateral wall of the scrotum (Fig. 2).
- Place a gauze piece or a thin piece of muslin cloth over the spermatic cord to prevent directly placing the jaws of the Burdizzo castrator on the scrotal skin. This will prevent damage to the superficial vessels of the scrotal skin and hence preventing necrosis (Fig. 3).
- Place the jaws of the castrator on the isolated spermatic cord, 3 cm from the testicles correctly, so that the jaw with the projection is on the underside, with projections facing the operator. This helps in preventing slipping of the spermatic cord (Fig.3 a & b)

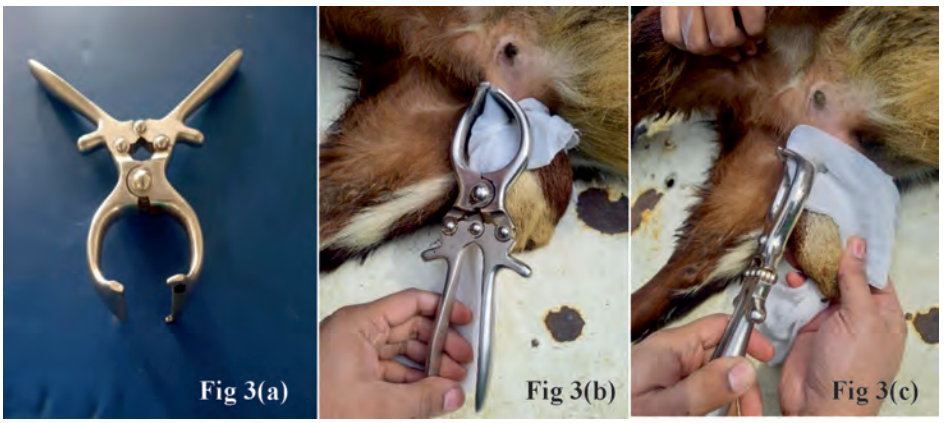


Fig 3. Photographs showing correct use of Burdizzo Castrator (a) white arrow points towards the jaw of the castrator which should be kept underside ; (b) and (c) show the 2 sites of placement of castrator, with first site being more proximal than the second. Also note the placement of a gauze piece.

- Close the jaw of the castrator while ensuring that the cord has not slipped out. Keep the jaws closed for approximately 30 seconds.
- Crush each spermatic cord at 2 sites. Release the jaws from the first site and move to the second site, 1 cm below the first and repeat the procedure. Second site is chosen below the first one to minimize pain as nerve supply has already been severed to a great extent by first crush. (Fig. 3 c)
- Repeat the procedure on the other side.
- The castrator should be placed only in the area of spermatic cord and not on the entire scrotum. Also both the spermatic cord should be crushed separately. This is done to prevent damage to the vessels to the scrotal sac and hence, necrosis and sloughing of the scrotal skin.After the procedure, move both the testicles up and down in scrotal sac, once or twice to create gap at the site of crushing (Fig. 4), to prevent ischemia of the scrotal sac that otherwise can lead to sloughing off the scrotal skin and testicular evisceration (Gupta & Sangwan, 2023).
- Apply povidone iodine or any other antiseptic at the site of procedure.

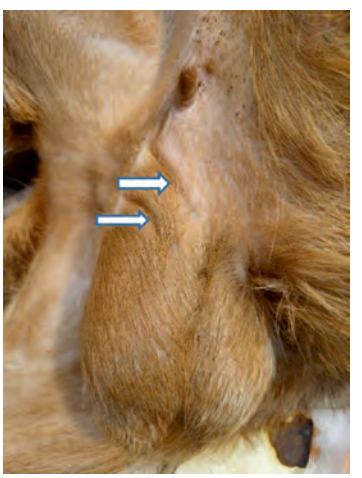

Fig. 4. Note the two sites after crushing. If the gap is not created at these sites, it might lead to ischemic necrosis of scrotal sac.

- Advise cold or tap water application on the scrotum for a few days after the procedure.
- The size of the testicles reduces within a few weeks after the procedure.

**Complications:** Previous article reported various potential complications associated with the Burdizzo Castration (Gupta & Sangwan, 2023). Following complications are the result of faulty castration such as Scirrhous cord, hydrocele, pyocele, orchitis, urethral damage leading to acute urinary obstruction, failure of the procedure, sloughing of the scrotal skin and testicular evisceration.

**Prevention of Complications**

- Do not span the castrator along the entire scrotum to prevent Scirrhous cord and hydrocele.
- Maintain asepsis during the procedure.
- Pull the scrotal sac and take care while placing the Burdizzo castrator to not involve urethra / penile tissue.
- Chose appropriate size of the castrator and practice this procedure only in immature animal.

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# Crossword Depicting Inflammatory Conditions of Various Organs, Tissues, or Cells

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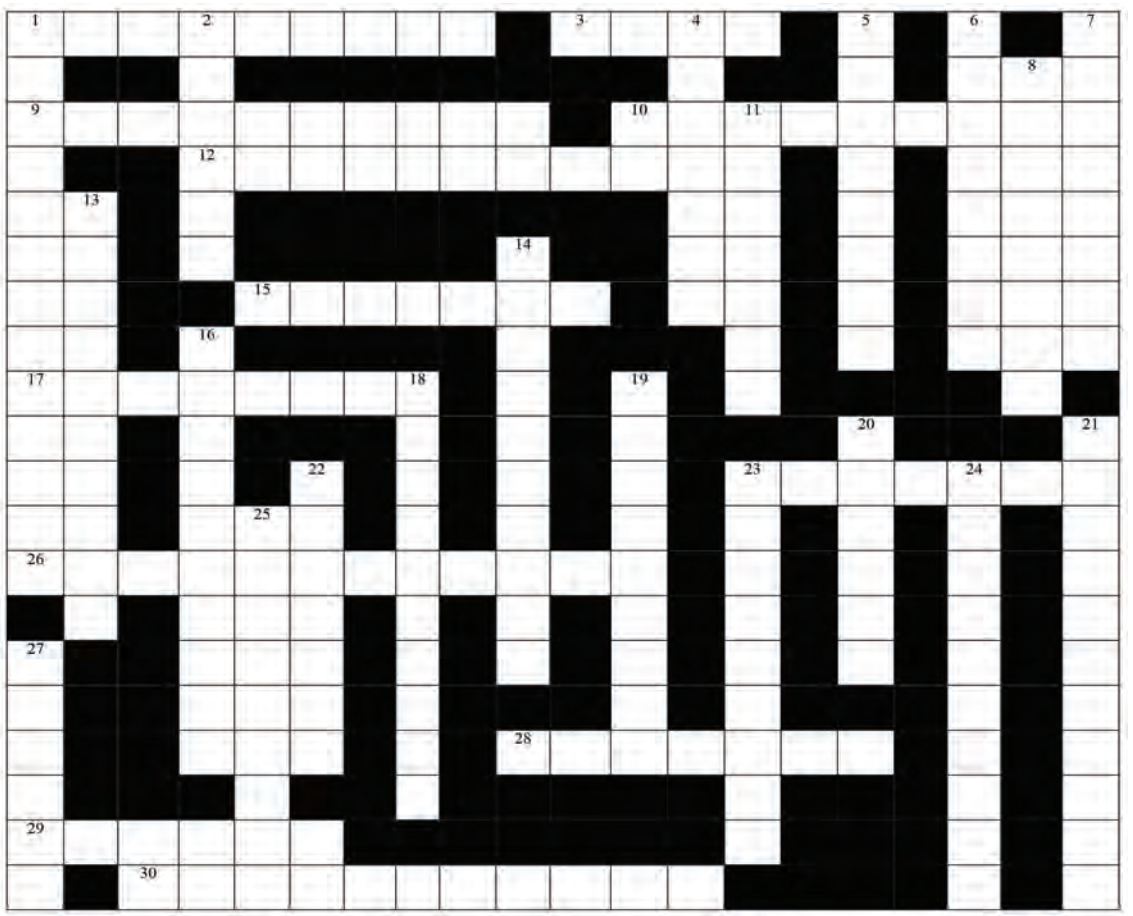

**Note:**

1. Spellings for this crossword are according to American English.
2. The keys to words are simply inflammatory condition of a single or multiple organs, tissues or cells. On most of the occasions, it is the name of organ, tissue or cell only that has been depicted in the key (ACROSS as well as DOWN).

**ACROSS:**

1. Laminar corium (9) -
3. Suffix for inflammatory condition (4)
9. Protective membranes covering the brain and spinal cord (10)
10. Kidney (9)
12. Tonsils (11)
15. Middle layer of eyeball (7)
17. Peripheral nerves (8)
23. Colon (7)
26. One or more salivary glands (12)
28. Vas deferens (7)
29. A voluntary muscle (6)
30. Seminal Vesicles (11)

**DOWN:**

1. One or more lymph nodes (13)
2. Coloured part of the eye (6)
4. Ileum (7)
5. Small fluid-filled sacs that cushion the bones, tendons and muscles near joints (8)
6. Bone (8)
7. Udder (8)
8. Chorionic villi (8)
11. Psoas muscle or its sheath (7)
13. Inflammation of sites at which tendons or ligaments attach to bones (10)
14. Gums around base of teeth (10)
16. Acute miliary tuberculosis (10)
18. Sound box in birds (10)
19. Umbilical cord (9)
20. Necrotizing ulcerative inflammation of gingivae (6)
21. Food pipe (11)
22. Lowermost part of bile and pancreatic duct (7)
23. Lips (9)
24. Tendon (10)
25. Glans penis (9)
27. Pia mater (6)

(Crossword answers on page no. 109)

# Managing Fatty Liver Hemorrhagic Syndrome in Laying Hens: Key Approaches

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## Abstract

*Fatty Liver Hemorrhagic Syndrome (FLHS) is a metabolic condition that affects poultry, particularly laying hens, on a widespread basis. It is a serious health hazard that leads to an excessive buildup of fat in the liver, resulting in liver dysfunction, decreased egg production, and increased mortality rates if not treated immediately. Early identification of FLHS is crucial and can be done by recognizing the symptoms of diminished egg production, wasting listlessness, and abdominal swelling. To effectively prevent FLHS, maintaining a balanced diet, controlling feed intake, ensuring optimal environmental conditions, conducting routine surveillance, and seeking expert veterinary advice are essential steps. By implementing these proactive measures, poultry farmers can safeguard both the well-being and productivity of their flocks, thus securing the sustainability of their operations.*

**Keywords:** *Fatty Liver Hemorrhagic Syndrome, High energy diets, Metabolic disease, Pale friable liver, Sudden death*

Fatty Liver Hemorrhagic Syndrome (FLHS) known as Hepatic Lipidosis or Fatty Liver Syndrome is a metabolic disorder primarily affecting laying hens that causes excessive fat buildup in the poultry liver leading to liver dysfunction, decreased egg production, and increased mortality rates if left untreated. This condition poses a significant threat to the health and productivity of poultry flocks worldwide. It occurs higher in commercial layers with 40-70% mortality and occasionally in broiler breeders (Shini et al., 2019). Further, FLHS is considered as the most frequently occurring non-infectious cause of backyard layers' mortality (Trott et al., 2014). It is crucial to understand the causes, symptoms, and preventive measures to effectively combat this condition. This article aims to provide comprehensive insights into FLHS, with the knowledge to safeguard their flocks and ensure optimal welfare and productivity.

**Causes of FLHS:** In poultry birds, liver is the main organ of fat synthesis. Fatty liver occurs when increase in fat synthesis surpasses the ability to synthesis and secrete lipoproteins (Zaefarian et al., 2019). There is no clear single entity that caused FLHS, however various factors may combine and caused this disease (Crespo & Shivaprasad, 2003). These factors include the nutritional, hormonal, environmental, genetic and

managemental factors (Table 1).

**Table 1. Summary on the Causes of Fatty Layer Hemorrhagic Syndrome**

| S No. | Causes | |
|---|---|---|
| 1. | Overfeeding | Excessive consumption of high-energy diets, particularly those that are rich in carbohydrates and fats, can overload the liver's capacity to metabolize and store fat |
| 2. | Nutritional | Diets deficient in essential nutrients, such as methionine, choline, and vitamin E, can disrupt lipid metabolism and predispose birds to FLS |
| 3. | Hormonal | Fluctuations in estrogen levels, particularly during the onset of lay, can influence lipid deposition in the liver. |
| 4. | Obesity | Sedentary behavior |
| | | Genetic factor |
| 5. | Age | Older birds over 35 weeks are more likely to develop this condition, mostly high-producing and overweight birds. |
| 6. | Management | Poor housing conditions |
| | | Heat stress |
| | | Overcrowding |
| 7. | Environmental | High environmental temperature |
| 8. | Genetic | Strains and breed differences |

**Nutritional**: The birds that died of FLHS are usually in good to fatty body conditions indicating that nutrition plays an important role in the pathogenesis. The nutritional causes may be

1. Overfeeding: Excessive intake of either high energy or high concentrated diets and/or excessive intake of feed with imbalance required energy can results in fat deposition in the body. Ideally in laying hens the dietary energy concentration ranges from 2,550 to 2,700 kcal ME/kg. On an average one laying hen with 90% egg production requires 16-18 g protein and 285 to 290 kcal ME per day (NRC, 1994). Variation in the energy-to-protein ratios (either too high or too low) can cause FLHS in layers (Wang et al., 2020).
2. Deficiency of essential amino acids (methionine, valine, isoleucine) which are necessary for transportation of fatty acid. Deficiency of lipotropic agents (choline, methionine and Vitamin B12) which are required to mobilize lipids from the liver.

3. Feed composition and types of energy sources: When carbohydrates rich foods (corn, wheat) are given to the birds, these carbohydrates are utilized in place of fat for fatty acids synthesis leading to metabolic stress in the liver. Further cereal grains or corn-based diet are usually low in choline (which are required for secretion of very low-density lipoprotein) and aggravate the development of FLHS in layer hens.
4. Mycotoxins contaminated feed (maximum permissible limit is 0.02 ppm) consumption can cause accumulation of fat and liver injury.

**Environmental:** During hot or summer seasons, due to the high environmental temperature, there is less activity and less heat production to maintain bird's body temperature and thus less energy is required for maintenance (Shini et al., 2019).

**Hormonal:** Estrogen is produced in active ovaries at sexual maturity (18-20 weeks), from a few weeks prior to first egg laying, and has as major function stimulating fat synthesis in the liver for egg yolk production. There is an increased risk of FLHS laying hens around peak production when plasma estrogen concentration is higher (Shini et al., 2020).

**Genetic:** Various strains of poultry are genetically susceptible to FLHS (Zaefarian et al., 2019). Heavy and high producing laying hens are more susceptible to FLHS. Rhode Island Red are more susceptible than White leghorn hens (Takahashi & Jensen, 1985). Strain of single comb White Leghorn laying hens (UCD-003) are reported to be susceptible to FLHS (Schuman et al., 2000). Lipid metabolism genes (ACACA and MTTP) are up-regulated in FLHS (Liu et al., 2016).

**Managemental:** As compare to other housing systems, birds in cage housing system have less activity and thus less maintenance energy required. Overcrowding and heat stress may also contribute to this disease (Shini et al., 2019).

**Miscellaneous**

*Age*: Birds of 35 weeks or high producing birds are more prone to this disease.

*Obesity*: It leads to sedentary behavior. Increased body weight had a major impact on hen mortality and, in many cases, was associated with fatty livers and FLHS.

*Concurrent Diseases*: Some studies reported the concurrence of collisepticaemia along with FLHS (Dey et al., 2018) though the exact mechanism is not clear.

Some antibacterial drugs such as tetracycline can cause fatty liver as it reduced hepatic lipoprotein synthesis thereby causing dysregulation of lipid metabolism and thus fat deposition leading to FLHS. Further, heavy metal (mercury, lead and arsenic) toxicity can impede protein synthesis leading to fatty liver (Schuman et al. 2000).

**Clinical Signs:** Clinical signs of FLHS vary ranging from mild anorexia and inactivity, to acute hemorrhage and sudden death. Further;

- A noticeable decline in egg production or the production of soft-shelled or misshapen eggs (due to impaired calcium metabolism in the bird) (Shini et al., 2019).
- Emaciation despite adequate feed consumption. Birds may appear lethargic, weak, and reluctant to move.
- Swollen or distended abdomens due to liver enlargement or ascites (fluid accumulation).
- Sudden death, without premonitory signs.
- Birds are found dead with pale comb and wattles (due to blood loss, and hypovolemic anemia) (Fig. 1).

Fig. 1: Pale comb and wattle of a bird died of FLHS.

**Necropsy Findings:** In commercial layers, due to its sporadic occurrence and over an extended period, it is very difficult to follow the stages of starting, development and resolution of FLHS. Thus, necropsy is the only possible way for the diagnosis of this disease. In necropsy, grossly the liver appears enlarged, pale and friable along with presence of hemorrhagic clots in the abdominal cavity (Fig. 2). In most of the birds, excessive fat deposition around the visceral organs in the abdomen and fatty changes in the liver with pallor areas are observed. Due to fatty deposition, the liver becomes friable leading to liver rupture and hemorrhages, and finally death.

Fig 2. Liver of FLHS showing pale friable liver along with hemorrhages and fatty deposition in the abdominal cavity.

**Preventive Measures**

*Balanced Nutrition*: Ensure a well-balanced diet for poultry, tailored to their nutritional requirements at different stages. Control excess energy and protein consumption at 22-30 weeks to avoid excess positive energy balance. Further, inclusion of appropriate concentrations of selenium (at least 0.3 mcg/g of feed) and vitamin E (up to 100 IU/kg of feed) or an antioxidant such as L-tryptophan (1000 mg/kg of feed) can help prevent the occurrence of FLHS. Calcium and vitamin D3 need to be incorporated in the diet plan.

*Controlled Feeding*: Limiting the presence of mycotoxins @ 0.20ppm. Avoid overfeeding the poultry and restrict access to high-energy feeds (maize/corn) through the use of low energy diet or change the feed composition (instead of maize, barley may be added), especially during periods of reduced activity. Restricted feeding for two (1 and ½ hours periods) daily also results in body weight loss. Dietary carbohydrates can be replaced with supplemental fat that depressed the new fatty acids synthesis thereby less fat production by the liver. Care should be taken that dry and fresh feeds are provided to the layers; prevent moisture and feed mildew. And also, probiotics can be added to the diet to improve the intestinal flora and prevent the occurrence of FLHS. Natural polyphenol having anti-oxidant property such resveratrol may be supplemented (Wang et al., 2020).

*Optimal Environment*: Create a conducive environment with adequate ventilation, temperature control, and sufficient space to minimize stress and promote healthy growth.

*Regular Monitoring*: Keep a close eye on the performance of the flock, including egg production, body condition, and behavior, to detect early signs of FLHS.

*Water Quality*: Lack of water is another stress factor that cause production of glucocorticoids, that stimulate gluconeogenesis and promote of fat formation. Further water quality has also been associated with FLHS. The relative hardness of water due to presence of more calcium, magnesium, sodium, copper has been associated with FLHS (Jensen et al., 1976). Thus, good water quality should be provided.

*Genetic Selection*: Selection of less susceptible strain of poultry birds.

*Veterinary Consultation*: Collaborate with a poultry veterinarian to develop a comprehensive health management plan and seek professional advice if FLHS is suspected.

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# Revolutionizing Goat Breeding: Harnessing the Potential of Artificial Insemination in Goats

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## Abstract

*Goat breeding is experiencing a revolutionary transformation with the advent of Artificial Insemination (AI). This cutting-edge technique ushers a new era of scientific precision, unlocking a plethora of possibilities for improving genetic traits and propagating desirable traits within goat populations. The traditional methods of goat breeding often face limitations in terms of genetic diversity and controlled mating. AI, however, breaks these barriers by offering a controlled and meticulous approach to breeding, allowing for the strategic selection of semen from elite sires. This article delves into the benefits, methods, and successes associated with AI technology in goat breeding.*

**Keywords:** *Artificial insemination, Goat, Laparoscopy, Reproduction*

Artificial Insemination (AI) is a well-established method involving the deliberate introduction of sperm from superior males into the female reproductive tract using specialized tools such as an AI gun. This age-old practice stands as the first and best-utilized form of reproductive biotechnology, playing a central role in driving global genetic enhancement efforts. While AI offers notable advantages, its efficacy is balanced by challenges stemming from limited male availability, reduced genetic diversity, potential transmission of genetic anomalies, and an increased risk of inbreeding, which can impact maternal traits. The integration of semen preservation techniques, such as chilling or cryopreservation, for sperm from genetically superior male enhances the effectiveness and adaptability of AI within breeding programs (Kumar & Honparkhe, 2023). This article delves into the benefits, methods, and successes associated with AI technology in goat breeding.

## 1. Procedure of Semen Preservation

Semen collection from sexually mature bucks is typically achieved via artificial vagina or electro-ejaculation, with the former being preferred. Following collection, a physical examination assesses consistency, volume, and color, discarding any contaminated samples. Sperm concentration is measured using a spectrophotometer /

haemocytometer, while motility is evaluated under a phase-contrast microscope. Semen is then centrifuged to remove seminal plasma, diluted with tris buffer, and centrifuged again to determine sperm concentration. Diluted semen is mixed with Tris-egg yolk glycerol medium, cooled gradually to 5°C, loaded into straws, and frozen by exposure to liquid nitrogen. Thawed samples are evaluated for post-thaw motility after being warmed in a water bath at 37°C for 30 seconds.

**2. Caprine AI Techniques**

AI in goats comprises an array of techniques like intravaginal, trans-cervical, and laparoscopic, etc.

- **Vaginal AI (Peri-cervical Insemination):** Semen is deposited into the cranial vagina (fundus) without targeting the cervix or using visual guidance. Ideally performed 12-18 hours after the onset of estrus in female goats. Recommended semen parameters for optimum result include a volume of 0.2 mL and a minimum of $400\times10^6$ progressively motile spermatozoa. Intravaginal AI is effective with fresh semen, but has a low success rate with extended or frozen semen. Peri cervical insemination is suited for natural breeding season when estrus detection is easier.
- **Trans-Cervical AI (Intra-cervical Insemination):** Favoured for small ruminants, this technique involves locating the cervical *os* using illumination. It can be utilized with fresh, chilled, or frozen semen, with the best results achieved 15-17 hours after estrus onset is detected. While frozen semen can be used, it is linked to reduced conception rates due to decreased sperm viability, resulting in fewer viable sperm reaching the fertilization site. Optimal parameters include an inseminate volume of 0.2 mL and a minimum of $200\times10^6$ motile spermatozoa.
- **Trans-Cervical Intra-Uterine AI (Guelph System):** Transcervical AI (TCAI), was developed at the University of Guelph and is referred to as the "Standardized Guelph system for TCAI" (Buckrell et al., 1994). This method involves positioning of doe in dorsal recumbency using a cradle. A lubricated vaginal speculum is then gently introduced into the vagina, allowing visualization of the external *os* with the assistance of a penlight. Subsequently, forceps are utilized for grasping and retracting tissue near to external os. Following this, the insemination device is cautiously maneuvered over the spiral folds of the cervix, ultimately depositing semen into the uterine body, typically near the uterine bifurcation (Candappa et al., 2009). Using this technique a 0.2-0.5 mL inseminate volume with a minimum of $60\times10^6$ motile spermatozoa can be deposited into the uterus is required for obtaining optimum conception rate. While this method is typically aimed at the uterine body, however, deposition of semen into the cranial cervix has also produced acceptable results.

- **Laparoscopic Intrauterine AI:** Recent advancements have led to the improvement of the laparoscopic intrauterine AI technique. With this technique, semen is introduced directly into the "uterine horn's lumen", circumventing the cervical barrier and reducing the required semen volume. Intrauterine AI is commonly conducted with the help of a laparoscope. This procedure involves creating two incisions under local anaesthesia: one for inserting an endoscope and the other for introducing an inseminating pipette into the uterine lumen. With this technique, a minimum of $20\times10^6$ motile spermatozoa per dose is recommended. Conception rates attained with this technique using frozen semen, exceed those of intracervical insemination. However, challenges include the need for costly laparoscopic equipment, surgical risk, and specialized technical expertise.

**3. Success Rate of AI in Goats**

Generally, the success rate of AI in goats can range from 50% to 70%, but it can be higher or lower depending on the specific circumstances. In peri-cervical insemination and intracervical in small ruminants a success rate of 5-15% and 40-80%, in terms of conception was recorded by Nuti (2007). However, in laparoscopic and trans-cervical intrauterine insemination a conception rate of 60-80% and 71% was observed by Youngquist & Threlfall (2006); Sohnrey & Holtz (2005), respectively. The total pregnancy rate of frozen-thawed caprine semen varies from 7 to 79% as reported by Arangasamy et al., 2018. The success rate of AI in goats can vary based on several factors such as the timing of AI, quality of semen, technique, and expertise of the inseminator, health and nutritional status of the animal, breed, environmental, and management factors.

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# Advancing Swine Genetics: The Indispensable Role of Artificial Insemination and Semen Processing Units

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**Abstract**

*In the domain of animal breeding, especially in pig farming, optimizing reproductive productivity is crucial. However, the swine sector is actively seeking ways to enhance efficiency, with a particular emphasis on improving artificial insemination methods. Establishing boar semen laboratories is necessary to amplify the spread of boar genes and to reach a larger female population compared to natural mating. This article elucidates about boar semen characteristics, equipments and processing of semen.*

**Keywords***: Artificial insemination, Boar semen, Dummy, Semen processing*

The reproduction of livestock holds a crucial position in maintaining the sustainability and productivity of global agricultural practices, thereby contributing to the economic growth of countries. One of the key factors influencing successful reproduction, especially in the swine sector, is the quality of semen. It's common for rural Punjab farmers to resort to natural mating for their animals, likely due to limited access to extended boar semen and a lack of artificial insemination techniques. Consequently, they stick to the traditional method of raising boars for natural mating. Establishing a specialized laboratory dedicated to processing boar semen can significantly support breeding programs, enhance genetic diversity, and ultimately boost the efficiency of pig farming. The utilization of preserved semen for artificial insemination (AI) in pigs has seen a notable increase over the past 15 years. However, it is vital to comprehend the unique aspects of semen collection and processing in boars, as they differ from other domestic animals. Boar spermatozoa are extremely vulnerable to cold shock or sudden cooling immediately after collection. This article deals with the semen collection and processing techniques in boars.

## Equipment for the Collection of Boar Semen

1. Using an adjustable dummy sow can help prevent size discrepancies between males, protect sows from injury, and ensure the oestrus sow supports the boar's weight until ejaculation is complete.
2. For semen collection, plastic insulated flasks or polystyrene cups with a minimum

capacity of 250mL are recommended.

3. A semen filter, such as surgical or bandage gauge, should be placed over the collection flask to filter the semen.
4. A polythene graduated measuring cylinder or flask is necessary for accurate measurement of semen volume.
5. It's important to use a quality, calibrated thermometer to monitor the temperature of the semen storage container.
6. Small polystyrene boxes cooled with frozen glacial acetic acid can be used to store diluted semen, or alternatively, a specific temperature refrigerator maintaining between 14°C to 18°C is suitable.
7. A temperature-controlled water bath and cabinet are essential for warming equipment and slides that will be in contact with fresh sperm during evaluation and processing.
8. Semen diluent is required for diluting semen before storage or insemination.

**Characteristics of the Boar Semen Collection Site**

1. The place should be in a quiet area.
2. The sperm processing laboratory should be less than 30 meters away from this place.
3. The floor should be clean, dry and non-slippery. The floor should be sloped towards the drain.
4. There should be adequate space in the pen so that the pig can roam comfortably before coming into the pen.
5. The collection area should be shady as the quality of semen deteriorates with exposure to ultraviolet light, sudden temperature changes and water contamination.
6. The farm staff which help in semen collection process and artificial insemination should be trained.

**Semen Collection Methods**

1. Move the pig comfortably from its pen to the dummy pen.
2. Trim the hair around the pig's penis and wash it with clean water and towel.
3. Dry it so that no dirt gets into the semen.
4. Give the pig a short time for adjustment (about 30 seconds to a minute).
5. After alerting, let the pig place both of its front legs on the dummy and hold the dummy tightly so that the pig sees the dummy as a hopeful sow.
6. After placing both legs on the dummy, make a fist shape of your hand in front of the skin (preputial sheath) covering the penis of the pig so that when the pig pulls out its penis, the fist of your hand will touch the penile prepuce of the pig. This technique is called 'Hand Gloved Technique'. In this, the interaction between the

person catching the pig, the person collecting the semen, and the animal is very important.

7. Let the pig push hard with its hind legs to pull out its penis. In this way, the pig keeps moving its penis in and out of the skin several times.
8. When the pig pulls out the penis completely, hold the glans penis with your hand, which has a spiral shape.
9. When the curved part of the penis fits well in your hand, apply pressure on it but never pull out the penis. By doing this, the penis may break.
10. Applying pressure causes the pig to ejaculate in liquid-gel form. Different pigs require different pressures depending on experience.
11. Boar semen comes out in four stages and it takes about 5-10 minutes to fully collect the semen. In the first stage (Pre-sperm fraction) gel, dead sperm, and clear water-like fluid are released. It contains a lot of germs. Pre-sperm fraction should not be collected. In the second stage (Sperm-rich fraction), sperm count is high and its colour is creamy-white. Its volume is approximately 50 ml. In the third stage, total volume is 80 ml and spermatozoa are less in quantity in this phase. The amount of sperm in this stage is approximately 80 ml. In the fourth stage (Post-sperm fraction), there is a large quantity (about 250 ml) of gel-like clear and seminal fluid (seminal plasma) that is released from the accessory glands. While it's probable enough for some people to collect both the sperm-rich and post-sperm fractions, others argue that storing the entire post-sperm fraction could shorten its shelf life. Basically, what's being collected is a "modified, sperm-rich" fraction, which includes second and third fractions avoiding the gel like fluid. Normally, for collection of sperm rich fractions and to avoid the gel like portion with seminal fluids, a filter/sieve is attached on the top of the funnel while collecting the semen as different semen fractions cannot be differentiated properly during collection. However, many researchers have proved that the presence of all ejaculate fractions within the seminal doses does not impair the reproductive performance and accelerated embryo development (Luongo et al., 2021; Párraga-Ros et al., 2024)
12. After collecting the semen, water the pig, feed it, and let it go back to its pen.
13. Immediately transport the collected semen to the laboratory at 37°C in a thermos for testing and preparation for semen dose preparation.

Other methods of semen collection are – Artificial vagina (AV) method and Electro ejaculation method. In artificial vagina method, an AV of 12.5 cm length and 4.5 cm diameter is used to collect the semen. The temperature of AV should be about 45-50$^{o}$C. Artificial vagina of pigs should be such that it can adopt the shape of corkscrew penis of boar. An automated system having a dummy as well as artificial vagina is available for collection of boar semen. Some companies like imv technologies are selling

such automated system named as 'Collectis'. Collectis includes one control unit with management software system and six artificial vaginas. It has twice the efficiency of manual collection with an isothermal vagina for collections under the conditions of optimal cleanliness. These type of automated system can be used at large droves otherwise for small number of animals gloved hand method is used. In electro ejaculation method electric current of 12-20 volts at 5 to 10 second interval is passed to obtain ejaculation.

**Semen Processing Lab:** Boar semen processing laboratory serves as a specialized facility equipped with technologies and expertise dedicated to the collection, evaluation, processing, and preservation of boar semen. Microscope, semen cooling unit, sperm counting equipment (photometer/hemocytometer), glasswares, plasticwares, thermometers, gloves, thermos, water bath, adequate dedicated workspace, i.e., clean, dry, temperature controlled should be available in the laboratory. After the collection of semen, evaluation of semen is done. Various semen tests applicable to bulls can be done to know the quality and fertilizing potential of semen (Singh and Nain, 2023).

**Attributes of Boar semen:** Semen should not be collected more than 2 times a week from one pig. Collecting semen more often has a negative impact on its quality. A good semen sample should have following properties:

- Volume of pure semen: 150-350 ml
- Pure sperm motility: +++ or more
- Sperm motility in pure semen: 80 percent or more
- Sperm count in pure semen: 200-300 million per ml or 30-60 billion sperm/ ejaculate (Jainudeen and Hafez, 2000)
- Defective sperm rate in pure semen: less than 20 percent
- Volume of one prepared dose: 60-80 ml
- Quantity of sperm in prepared dose of semen: 3000-5000 million (3-5 billion approx.)
- Sperm motility in prepared semen should be more than 50 percent
- Other tests like eosin-nigrosine staining (liveability), giemsa staining (acrosomal integrity) and hypo-osmotic swelling tests (functionality of plasma membrane) can also be done to evaluate the semen. Some advanced tests like Computer assisted semen analysis, cervical mucus penetration test, sperm zona pellucida binding assay can also be performed (Singh and Nain, 2023).

**Semen Processing:** As a first step, concentration of spermatozoa in total semen is measured using a photometer or haemocytometer. Semen volume is also another important parameter to calculate the number of semen doses.

1. For on-farm AI programs, 1 part of sperm rich fraction of semen is added to 4 or 5 parts diluent (e.g. 20mL of good quality semen is made up to 80mL or 100mL with

diluent). Accurate estimations of the total number of live and normal spermatozoa in the semen sample are required for greater dilutions. Semen extender/diluent is a liquid diluent which is added to semen to preserve its fertilizing ability. It serves as a buffer that safeguards sperm cells from the harmful byproducts they produce on their own and from osmotic stress and cold shock that might occur during the shipping and chilling processes. Various diluents are being used in pig semen industry, these are grouped as short term extenders [Beltsville Liquid (BL-1), Beltsville Thawing Solution (BTS), Kiev, Vital®] and long term extenders (Zorlesco, ZORPVA, Acromax, Androhep). New commercial semen extenders available at imv technologies are PRIMXcell Ultra, TRIXcell ultra, NUTRIXcell +, SAFEcell etc. A semen extender should be carefully selected; if semen is to be stored for 72 hours use short term extenders and if the semen need to be stored for more than 4 days, long term diluents should be used. The best performance (in terms of fertility and litter size) must be considered while choosing the semen extender, and this is dependent on the particular circumstances of each pig farm. Important components of Beltsville Thawing Solution (BTS) dilutor are Glucose, sodium citrate, EDTA, sodium bicarbonate, potassium chloride (Gadea, 2003).

2. After preparation of the semen doses, keep them in an incubator (BOD incubator) at a temperature of 16-18°C and use them for artificial insemination for 72 hours as required. Farrowing rate will be reduced if semen will be used after 72 hours if short term extender has been used.

3. Interestingly, the inclusion of the last part of the ejaculate (seminal fluid) for artificial insemination (AI) purposes is still under debate due to its controversial effects (Luongo et al., 2021). However, some of the researchers found out that seminal plasma also plays an important role in the conception as after the semen dose deposition by artificial insemination, seminal plasma interacts with tissues in the female reproductive tract and induces molecular and cellular changes that increase the chances of conception and pregnancy (Bromfield, 2016). But another study revealed that spermatozoa from sperm-rich fraction show superior resistance to preservation both in refrigeration and freezing than the mean resistance of spermatozoa from the whole ejaculate (Saravia et al., 2009). The explanation for this phenomenon is that these spermatozoa are mainly exposed to the epididymal fluid and prostatic secretion, avoiding the protein-rich secretions of the vesicular and bulbourethral glands secretions which have been found to cause detrimental effects on spermatozoa.

**Some Important Instructions While Semen Collection in Pigs**

- Often the boar becomes adult in 5-6 months and after 1-2 months of good training on a dummy, semen can be collected from them.

- To train a pig to mount a dummy, semen from any pig or the urine of female may be rubbed on the nose of the pig, which stimulates the pig to mount the dummy.
- Never mistreat the pig during training as it may discourage them.
- The height, length and width of the dummy should be suitable for the pig so that it feels comfortable while mounting.
- Wash the dummy with clean water before and after semen collection.
- Try to collect and prepare the semen 8-12 hours before artificial insemination in sow.
- Always store semen doses at 16-18°C. Make sure that the temperature should never be below 15°C and above 20°C as this adversely affects the viability of the spermatozoa and thus conception rate.
- Do not use the semen for artificial insemination if the prepared semen has less than 50 percent sperm motility which can be checked under a microscope.
- It is recommended to replace the breeding pig after 3 years.

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# Computer Aided Drug Designing and its Implications in Drug Discovery and Development

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## Abstract

*Discovery and development of a new drug is generally known as a very complex process which takes a lot of time and resources. So now-a-days computer aided drug design approaches are used very widely to increase the efficiency of the drug discovery and development course. Various approaches of Computer-Aided Drug Discovery are evaluated as promising techniques according to their need, in between all these structure-based and ligand-based drug design approaches are known as very efficient and powerful techniques in drug discovery and development. These both methods can be applied with molecular docking to virtual screening for lead identification and optimization. In the recent times computational tools are widely used in pharmaceutical industries and research areas to improve effectiveness and efficacy of drug discovery and development.*

**Keywords:** *Computer aided drug discovery, Drug design, Ligand-based drug design, Molecular docking structure-based drug design*

Drug discovery process is often frowned upon because of consuming lot of time approximately 10 to 15 years and being a very expensive process (Hoque et al., 2017). Introduction of *in silico* method of drug designing where we can design the molecule, predict the pharmacokinetics and pharmacodynamics of the molecule, test its toxicity and do high throughput screening of the molecule in the computer, made the process of drug discovery easy, less time consuming (approx. 5-6 years) and cost-effective. This communication highlights that computer aided drug designing has evolved rapidly in current years, improving the understanding of multifaceted and complex biological processes and thus new pharmacologically active agents can now be established in a short period.

**Process of Computer Aided Drug Designing (CADD)**: Computer aided drug designing process were initially confined to the visualization of potential drug interactions in targeted enzyme binding. But later these techniques are now used by many research groups worldwide as a powerful tool to define the relationship between biological activities, binding geometries and mechanisms of action in physiological or pathological processes. Many drugs and vaccines including for Covid 19 were discovered by this

method. (Murgueitio et al., 2012). Burning concerns like antimicrobial resistance, cancer chemotherapy, antiviral drugs etc. can be addressed by this computer aided drug designing process. Identification of new drugs as well as construction of 3D target molecules by homology modelling with prediction target sites and its possible agonists and antagonists can be predicted with high accuracies.

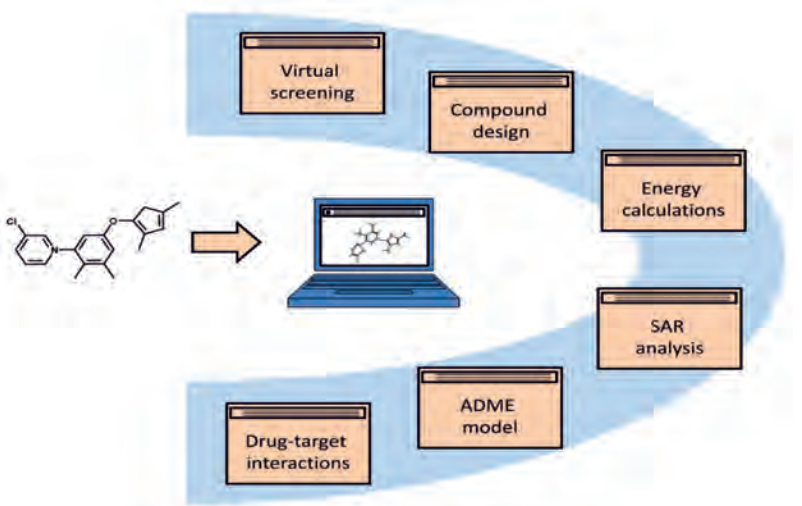


Fig. 1: Computer aided drug designing

**Types of CADD**: There are two types of computer aided drug designing methods: Ligand based drug designing (LBDD) and Structure based drug designing (SBDD). LBDD is used in the absence of 3D structure of the target molecule. Modification of the structure of the compound existing earlier by using structure activity relationship to find different configurations and conformations of the existing compound with a molecule with lesser side effects and more potent can be predicted. Later the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) can be predicted and thus indicated for the particular disease. In SBDD, 3D structure of the target molecule is available either through NMR spectroscopy or by X-Ray crystallography which can be converted to protein data bank files from where the data can be extrapolated and then various available ligands from the library can be screened against the 3D structure of the target. Structure-based and ligand-based drug design methods along with molecular dynamics simulation studies are the backbone of modern CADD processes (Maithri et al., 2016)

Scoring function in the process “Docking” is most important in SBDD where the ligands that bind with the target molecule with low binding energy are being selected as the lead molecules here they are further sent for the molecular simulations. The selected lead compounds with the target molecules are subjected to stability tests through high throughput screening and the most stable compounds are selected for the *in vitro* testing

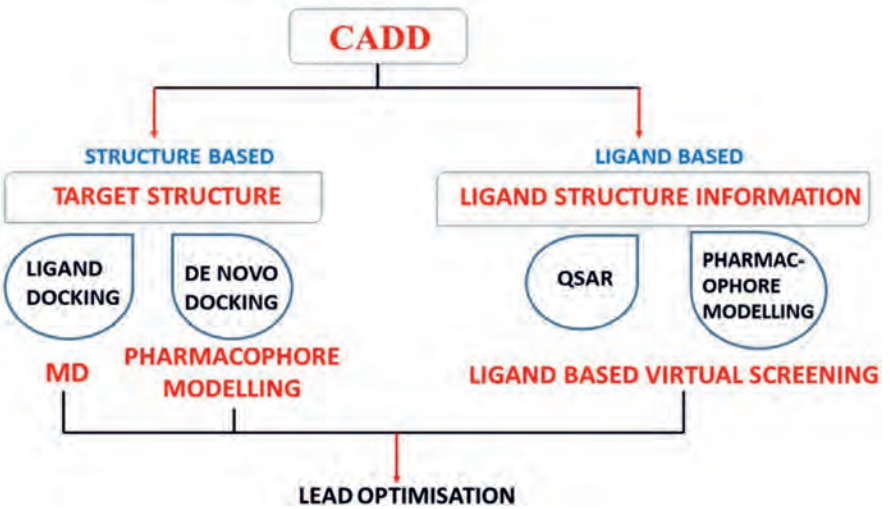


Fig. 2. Categories of CADD for lead optimization

**Advantages**: To list the major advantages of the CADD are recent discoveries of drugs and vaccines including HIV, Flu and Covid 19 vaccines. Reduction of a significant time and money for drug discovery are two major factors contributed by the CADD. The CADD's role in drug discovery is first to characterize the target molecules in lakhs into small identical clusters of compounds according to functional groups from where the lead compound can be selected and optimized, screened for absorption, distribution, metabolism, excretion and toxicity. Computational algorithms have been developed in such a way that the clusters of the similar compounds according to their potency and ligand efficiency can be screened.

Advances in computational tools now can define and elaborate the strength of interaction between ligands and target proteins. It helps to elucidate the molecular basis of therapeutic activity and possible derivatives, and those variables that could be applied or improved for generating an optimal drug compound, thus leading to prioritization of the actives without requiring extensive development and validation prior to use, as in the


Fig. 3. Molecular docking

case of assay high throughput screening. It has been applied during various stages in drug discovery: target identification, validation, molecular design, and interactions of drug candidates with targets of interest. (Taft et al., 2008).

**Disadvantages**: One of the major disadvantages of CADD are it cannot replace *in vitro* testing or clinical trials as the human beings/ animals have very complex molecular structure and are governed by various factors. There is a lack of synergistic computational model because of which drug-drug interaction or food drug interaction cannot be studied. Synergistic studies are needed to study protein functions in development, metabolism and cell signaling, molecular genetics and development, synergy among communities of organisms. Receptors and ligand molecules are highly soluble in the bodily fluid because of their conformational changes where in the CADD we deal with the rigid structures of the receptor though the ligands are highly flexible. Therefore a rigid receptor structure may render wrong results.

Drug discovery through computer aided drug designing needs a reliable database for prediction of target sites for ligands. Lack of standardization is also there in this process. One of the major drawbacks in LBDD is that it depends on pre-computed database which may be insufficient to render a perfect result (Veselovsk et al., 2003). Missing different conformations may be there that can lead to ambiguity in the results. Molecular dynamics have several limitations among which one is the method is computationally very demanding and dependent on the size of the system simulation, with times limited to hundreds of nanoseconds or a few microseconds. It is too short for analysis since the complete folding of a protein requires a time period ranging from milliseconds to seconds. Accordingly, this limitation can lead to inadequate sampling of conformations.

**Scope of CADD in Indian Veterinary Research**: Canine distemper virus (CDV) and canine parvovirus (CPV) are two significant pathogens that affect domestic dogs, causing severe and often fatal diseases. The development of effective drugs against these viruses is crucial for preventing and treating these infections, as well as for controlling their spread within canine populations. Both CDV and CPV are RNA viruses, which are known for their ability to mutate rapidly. This characteristic poses a challenge in drug development as the virus can evolve potentially leading to resistance against antiviral drugs. The severity and rapid progression of these diseases necessitate the development of drugs that can act quickly and effectively to mitigate the clinical signs and reduce mortality rates. The need for safe and well-tolerated drugs is essential to avoid adverse effects, especially in the often-vulnerable populations of puppies and immunocompromised dogs. *In silico* drug discovery or computer-aided drug, designing can fast-forwarded the drug discovery process for these animal diseases.

Computer aided drug design is an efficient tool in the area of drug discovery and development, through it we can find the most promising drug candidate in a very cost-effective way. It always provides a hope for betterment in drug discovery area. In the past years through Computer aided drug design many impressive researches are achieved so it will play a very much important role in the near future. With the current achievement's, there is a promising future of computer aided drug design to aid drug discovery of many more curatives in future. Overall, use of the modern techniques of CADD for drug design and development is the need of the hour and also irreplaceable for its high throughput screening methods and less expensiveness.

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# Comparison of Glycerine and Thermocol Plastination Techniques for the Preservation of Forelimb Specimens of Cattle

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## Abstract

*In veterinary education, preserving animal cadavers and organs is crucial for teaching anatomy effectively. Plastination has emerged as a modern method to preserve organ specimens, offering advantages over traditional preservation methods like formalin fixation. Plastinated specimens provide invaluable teaching tools for anatomy education while challenges such as cost and maintenance exist. This study compared glycerine and thermocol plastination techniques to preserve cow's distal forelimb specimen. Glycerine plastination yielded superior results, preserving natural appearance, flexibility, and odourlessness, facilitating enhanced educational experiences. Thermocol plastination too yielded odourless specimens but with desiccation-induced rigidity, aesthetic deterioration, and structural deformities. Overall, glycerine plastination was adjudged as superior method for producing high-quality, durable specimens, underscoring its importance in veterinary anatomy.*

**Keywords:** *Bovine, Glycerine, Plastination, Specimens, Teaching aid, Thermocol*

In veterinary institutes, learning about animal anatomy is important and for this purpose the animal cadavers and organs are used. Availability of the fresh organ specimens and their rapid decay are two important issues in veterinary and medical anatomy teaching. There are different preservation methods that keep anatomical specimens usable for longer, such as mummification, embalming, or plastination (Amin & Yesmin, 2015; Menaka & Chaurasia, 2015). Plastination is a modern method that preserves specimens using special materials to make them hard, dry and odourless (Weiglein, 1997). Instead of using fluids, plastination replaces them with unique materials such as silicone, epoxy resins and polyesters that stop decay while keeping the natural look and flexibility of tissues (Von Hagens, 1979). It is commonly used to preserve human cadavers for exhibits but it's also important for saving animal specimens. We hypothesize that both glycerine and thermocol plastination techniques will effectively preserve the forelimb specimens of cattle. However, we anticipate differences in preservation quality, durability, cost-effectiveness, and ease of handling between the two methods. Specifically, we expect that glycerine plastination will yield specimens with better flexibility and anatomical detail

due to its ability to maintain tissue hydration, while thermocol plastination may offer advantages in terms of longevity and cost-effective in specimen preparation.

**Methodology**

This study compared two plastination techniques using the distal forelimb of a cow that died naturally due to reasons unassociated with the musculoskeletal system, and the samples were obtained from the necropsy hall of the institute. Initially, the specimens were cleaned thoroughly to remove any blood clots. Then, cut into two halves using a hacksaw machine. These specimens were fixed in a 10% neutral buffered formalin for three months before testing two plastination techniques on each half specimen.

The common steps in both plastination techniques were explained by Bansal et al. (2022) and as follows:

1. **Dehydration:** After fixation, the specimens were dehydrated by gradually immersing them in (100%) acetone (3 changes for 7 days each) to remove water content from the tissues.
2. **Fat Removal:** The specimens were then subjected to a fat removal process using chloroform (100%) (3 changes 7 days each) to dissolve lipid content.

**Glycerine Plastination Protocol**

3. **Impregnation:** Glycerine, known for its refractive index similar to biological tissues, was used as the impregnating medium. The specimens were immersed in glycerine for 15-20 days to replace the solvent and ensure preservation of structural integrity and flexibility.
4. **Curing:** Excess glycerine was removed from the specimens and kept for drying inside an incubator maintained at 40$^{o}$C for 24-48 hours to reduce leakages, enhance transparency and reveal intricate anatomical details.

**Thermocol Plastination Protocol**

3. **Impregnation**: Instead of glycerine, polystyrene-based thermocol sheets were used as the impregnating medium. The specimens were subjected to impregnation directly by placing specimen in the melted thermocol in chloroform (2:1 ratio) done for 2 to 3 days that filled interstitial spaces, replacing water and other fluids (Bansal et al., 2022). Thus, it took lesser time of for impregnation as compared to the Glycerine method and thus a quicker method.
4. **Curing:** Once impregnated, the specimens were cured, allowing the thermocol to harden and solidify by air drying for 3 days, preserving the structural integrity of the tissues within the specimen and later coated with varnish to improve the aesthetics.

## Results

**Glycerine Plastinated Specimen:** Upon visual examination, the glycerine plastinated specimen presented itself with a remarkable and pleasing aesthetic appeal (Fig. 1). The muscular bellies, tendons, and ligaments demonstrated exceptional suppleness, devoid of any rigidity, thereby facilitating facile handling for educational purposes both within laboratory settings and classroom environments. Notably, the absence of any foul odour emanating from the specimen indicated a successful preservation process. Furthermore, the articulating joint spaces, including those of the fetlock, pastern, and coffin joints, were discernible and notably flexible, affording students a comprehensive understanding of joint biomechanics (Fig. 1a). The integrity of the hoof portion remained intact, displaying a smooth surface devoid of any structural deformities attributable to the dehydration process (Fig. 1b). The samples were evaluated physically for its physical properties once in a month for 12 months. After one year, it was observed that the physical appearance of the limb was as good as just after the curing process. Similar to our experiment with this method, Hassan & Sawad (2021) also used the glycerine method of plastination for the preservation of the pelvic region of dogs and found it a better method than the hard plastination method. The use of glycerine as a preservation material has been suggested instead of silicon and other polymers by Mohsen et al. (2013). This method does not require a vacuum pump to impregnate. In most of the silicon or other polymers base impregnation, a forced impregnation with a vacuum pump is a prerequisite (Hayat et al. (2016).

**Thermocol Plastinated Specimen:** Contrastingly, the thermocol plastinated specimens, post-impregnation and clearing stages, manifested a desiccated appearance, characterized by a pallid hue. This rendered the anatomical structures, such as muscle bellies, tendons, and ligaments, exceedingly rigid and bereft of their inherent flexibility (Fig. 2). The aesthetic allure of these specimens notably paled in comparison to their glycerine counterparts, betraying signs of dehydration-induced shrinkage. This phenomenon notably exacerbated the spaciousness of joint cavities, owing to the retraction of surrounding tissues (Fig. 2a). The hoof region, in particular, exhibited conspicuous desiccation, marked by a white, flaky texture on the plantar aspect (Fig. 2b). In this method glycerine was not used as impregnating agent and instead of the same waste from the packaging material (thermocol) was used for the same. Thus, the cost of glycerine was added to the cost of the development of plastinate with the glycerine method. The only advantages of this technique are the odourlessness of the specimen and the reduced cost of specimen preparation. The visual and physical examination showed that the brittleness of the plastinated limb increased with the passage of time. Additionally, certain regions along the cut surfaces revealed instances of bone brittleness, further compounded by observable bending of osseous segments, underscoring the deleterious effects of the

plastination process (Fig. 2a). Thus, it was concluded that the plastinate was less durable than the plastinate with another method. Concerns while specimen preparation includes hazardous effects on the health of individual due to exposure to the fumes that were released during thermocol melting process in chloroform.

**Discussion**

Plastinated specimens can enhance public awareness and medical outreach programs. Glycerine plastination preserves tissue texture and colour, making it an excellent teaching tool. Students can explore joint movement and relationships in a realistic manner. The lack of odour enhances the learning experience.

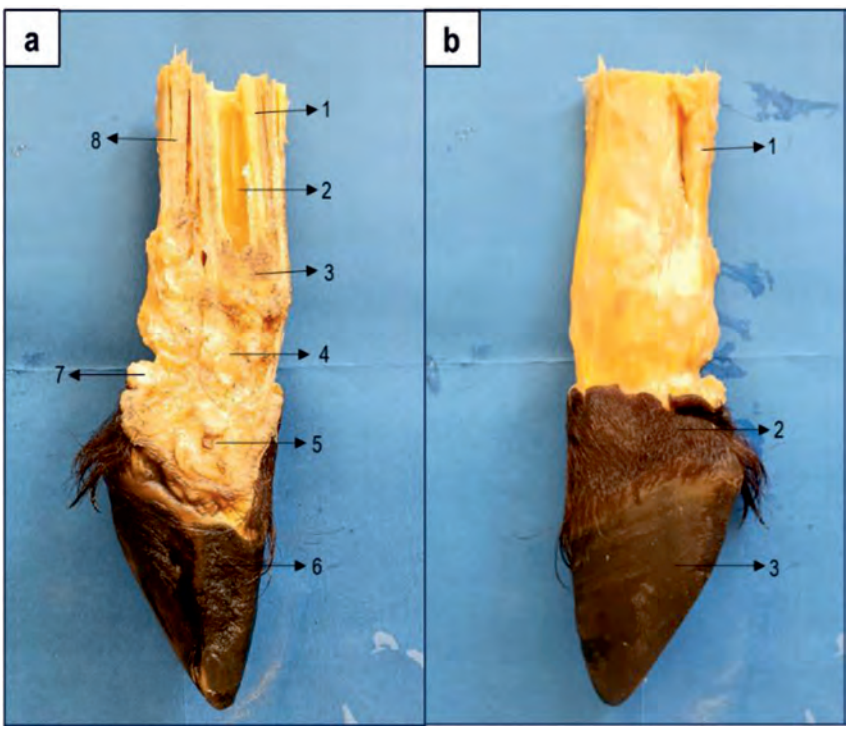


Fig. 1. Glycerine plastinated distal forelimb specimen of bovine. (a) Medial sided cut surface of limb showing 1. Diaphysis of metacarpal bone, 2. Bone marrow cavity of metacarpal, 3. Condyle region of metacarpal, 4. 1st phalanx, 5. 2nd phalanx, 6. Hoof (3rd phalanx), 7. Dewclaw, 8. Cut surface of superficial digital flexor tendon; b) Lateral surface of limb showing 1. Superficial Digital Flexure (SDF), 2. Skin, 3. Lateral surface of the hoof.

Plastination replaces traditional formalin-based preservation methods. It offers several advantages, including reduced toxicity, improved durability, and realistic representation. They allow repeated handling without deterioration, making them ideal for practical sessions. Challenges may include cost, maintenance, and ensuring proper consent for specimen donation (Jain & Ranjan, 2018).

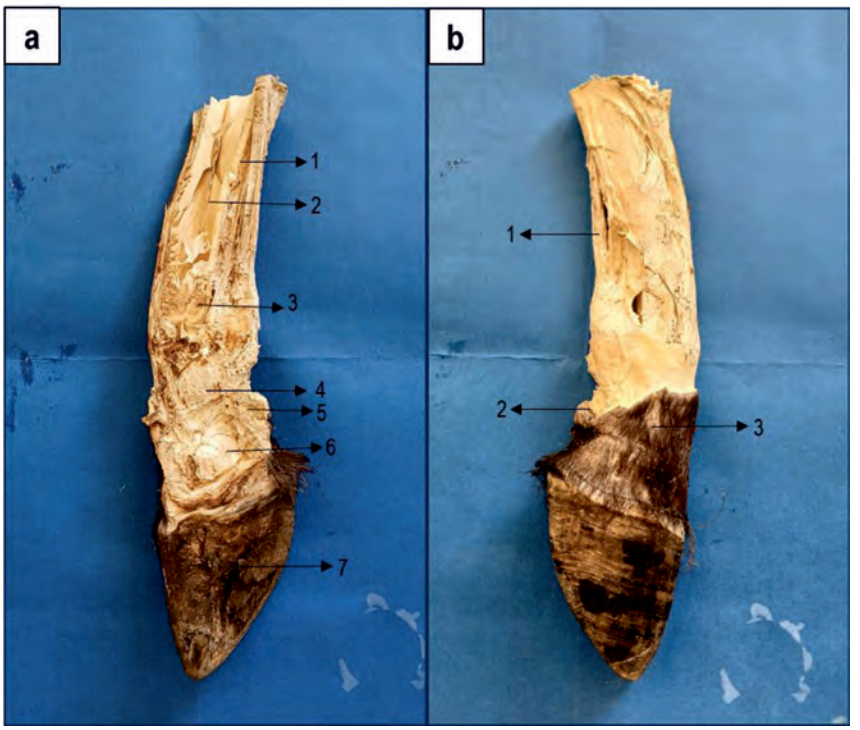


Fig. 2. Thermocol plastinated distal forelimb specimen of bovine. a) Medial-sided cut surface of limb showing 1. Diaphysis of metacarpal bone, 2. Bone marrow cavity of metacarpal, 3. Condyle region of metacarpal, 4. 1st phalanx, 5. Dewclaw, 6. 2nd phalanx, 7. Hoof (3rd phalanx); b) Lateral surface of limb showing 1. SDF, 2. Dewclaw, 3. Skin

Thermocol plastination involves using polystyrene foam (thermocol) as the impregnating material. While it provides structural stability, it sacrifices tissue suppleness and natural appearance. The pallid hue and rigidity affect the educational experience. Dehydration-induced shrinkage impacts joint spaces and overall aesthetics (Brown et al., 2002). Bansal et al. (2022) observed that this procedure is far less expensive than the alternative approaches and led to the clean, odourless, non-toxic, dry to the touch, processed plastinated visceral organs and most of them still had their original form and appearance. Plastination has influenced medical curricula, research, and artistic expression. Plastination of glycerin-fixed specimens produced adequate results, which preserved the morphoanatomical aspects, color, and dimensions of the specimens. Challenges include balancing scientific rigor with artistic presentation and addressing misconceptions about plastination (Ottone, 2023). In conclusion, the glycerine plastination technique yielded specimens of superior quality, boasting natural appearance, flexibility, and odourlessness. Conversely, thermocol plastinated specimens displayed significant drawbacks including desiccation-induced rigidity, aesthetic deterioration, and structural deformities. These

findings underscore the efficacy of glycerine plastination in preserving anatomical integrity and facilitating enhanced educational experiences. Thus, glycerine plastination emerges as the preferred method for producing high-quality, durable specimens for veterinary anatomy education.

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# Transforming Animal Bones from Waste into Teaching Aid

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**Abstract**

*The study aimed to repurpose waste animal bones into valuable resources for veterinary anatomy museum specimens and clinical studies. Unmacerated cattle femur bones were collected and processed through standard maceration, degreasing, bleaching, varnishing, and categorization procedures. The resulting specimens were then utilized in the osteology laboratory for identifying specific characteristics of the bone as well as in teaching and training in clinical diagnosis and management of bone affections.*

**Keywords:** *Bone, Veterinary Education, Waste*

Osteology is an integral part of veterinary education. It is the foundation of animal biology helping with the diagnosis and treatment of disease. There is a gap in the availability of animal bones for students due to the unavailability of the specimen or high cost. Frequently, animals perish unnoticed due to natural causes, leaving their remains untouched and subsequent degradation. Waste bones from these animals can undergo processing, converting them into valuable specimens. These specimens can serve as practical teaching tools for veterinary anatomy in undergraduate education and museum displays. This process aligns with modern practices of salvaging or repurposing materials for informative reuse or recycling (Ajayi et al., 2016).

In this study, we randomly found some bones in the surrounding area, but only one bone was complete and identified as a femur (Fig. 1). The bone was processed by following these processing steps.

1. **Maceration of Bones:** Animal carcass undergone maceration of the tissue to obtain the bone. There are four maceration methods i.e., Physical by burial, hot water and ordinary water method, chemical method, biological method and enzyme maceration method. The basic burial method was utilized since the bones were somewhat macerated. After one week, the bones were removed from the ground and cleaned using a paintbrush and water to eliminate dust and debris (Modi et al., 2014).
2. **Degreasing:** This process eliminates animal fat, which can be done by immersing

the bones in detergents, ammonia solution, xylene, or chloroform. In this study, degreasing was achieved by immersing the bones in a soapy solution for 24 hours (Mairs et al., 2004) followed by washing with water. The bones were then left to dry for several days at room temperature in the shade.

3. **Bleaching:** This step whitens and brightens the bones, eliminates odor, and removes any remaining debris. Bleaching was achieved by soaking the bones in 3-6% hydrogen peroxide for two days, though the duration may vary based on size and shape (Savitri et al., 2023). In this study, the bones were bleached for 2 days, then washed with clean water to remove any bleach residue, and finally dried at room temperature (Fig 2).

4. **Varnishing:** The dried bones were then coated with varnish mixed with turpentine oil to enhance their brightness and prolong their durability. This coating prevents the growth of mites, insects, fungi, mold, etc. (Savitri et al., 2023).

Fig. 1. Unmacerated femur of cattle

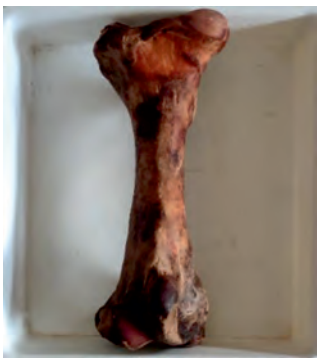

Fig. 2. Femur of cattle after bleaching

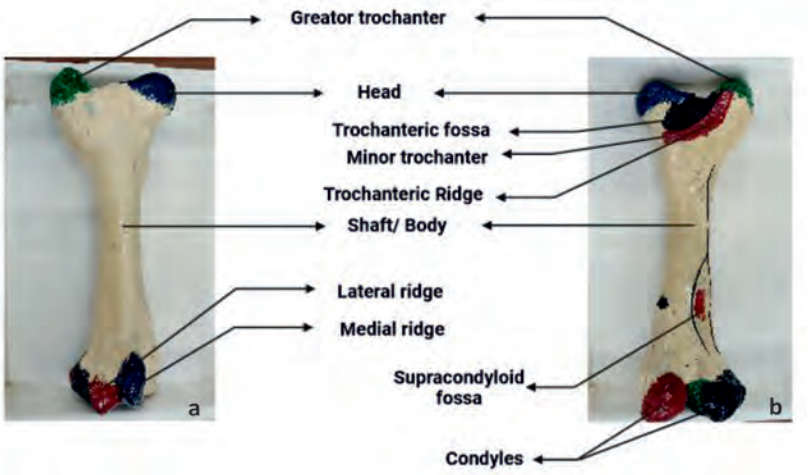


Fig. 3a & b. Processed femur of cattle with highlighted features

5. **Bone Categorization:** As the bones were randomly collected and only one bone was complete and identified as the femur of cattle. The femur (round bone) of cattle was identified in the present work as it consisted of greater and lesser trochanters connected by an oblique trochanteric ridge with trochanteric fossa at the proximal extremity and trochlea and condyles at the distal extremity (Fig. 3).

6. **Colouration of Characteristic Features:** Highlighting the specific features of the bone with different colors that aids in identification of various anatomical structures. In this step, all the significant features were accentuated with distinct colors, as depicted in Fig. 3. Synthetic enamel paint was used to protect from fungal problem and to increase the durability of bone. Following these procedures, the femur bones can be displayed in osteology laboratory. Roop et al. (2023) have also reported using the following procedure for preservation of Emu bone.

For this whole processing, the approximate cost was Rs. 385 per bone which is too less than the bone available in the market which may reach up to thousands. By this cost-effective preservation and processing method, the unprocessed bone was processed and preserved to make The specimens thus processed can be utilized in the osteology laboratory for studying the anatomy of the bone by the undergraduate and postgraduate students as well as correlate with the bone lesion in a radiograph for diagnosis and management of fracture or other bone affections by the Veterinary Radiologist or Clinician.

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# Goat Milk: Nutritional Value, Therapeutic Benefits and Market Trends

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## Abstract

*Goats are often considered poor man's cows. These animals can provide superior quality milk and meat to the farmers' families along with the substantial income from the sale of these products. Nowadays goat farming is considered an important entrepreneurial venture and the Government of Punjab is also promoting goat farming and production. Goat milk has also gained popularity as a nutritional powerhouse, captivating the attention of health-conscious consumers and medical professionals alike. Goat milk consumption offers a wide range of health benefits such as antioxidant, antimicrobial, and immune-boosting properties. Additionally, it is an ideal food for persons with lactose intolerance and its potential role in preventing diseases such as dengue fever. This article highlights the nutritional and health benefits of the inclusion of goat milk in the diet.*

**Keywords:** *Antimicrobial, Dengue, Farmers, Health, Production.*

Dairy goats, often called as the 'cow of the poor,' offer rich nutrients through milk and meat at low initial investment and maintenance costs. Goat milk, prized for its high nutritional value, is easier to digest than cow's milk, making it suitable for lactose-intolerant individuals. Goat's adaptability to diverse climates makes them widely accessible, especially to small-scale Indian households. Indian small-scale farmers manage most dairy goat populations sustainably, due to less space and resource requirements. According to the FAO, in 2021 the global goat population was approximately 1.11 billion, yielding about 20.7 million metric tonnes of milk annually. Primary producers of goat milk include Asia, Africa, and America, contributing 12.37, 4.4, and 0.82 million tonnes, respectively. India leads as the largest producer of goat milk, accounting for 6.07 million tonnes, which is nearly half of Asia's total production. Asia has the largest goat population (57.7%), followed by Africa (35.7%), accounting for 93.4% of the global goat population. Global goat milk output has doubled from 10.0 million tonnes in 2010 to 20.6 million tonnes in 2020. Dairy goats account for 20.8% of the world's goat population and produce 1.3% of the world's total milk production (Food, 2018).

**Status of Goat Farming in Punjab:** In the year 2023 India ranks 1$^{st}$ in goat population of around 148.88 million followed by China (133 million) globally. About 27.8% of the

total livestock is contributed by goats with an increase in 10.14% from 135.17 to 148.88 million but only 5.93% compared to 2007 (BAHS, 2019). India is the largest goat milk producer, with 20.17% contribution globally. Goats contribute about 9% of livestock GDP due to their multipurpose use for meat, milk, skin, hair, and manure, etc (Singh et al., 2023).

Goats contributes around 4% of total milk and 13.74% of total meat produced in India. The highest goat population is in Rajasthan, and the highest goat meat production is in West Bengal. As per the 20$^{th}$ livestock census of the Government of India, the goat population in India and in Punjab is 148.88 million and 3.48 lakhs, respectively, showing an overall increase of 10.1% over the previous census. In Punjab, the goat population is highest in Firozpur, followed by Bathinda, Muktsar, and Ludhiana. The breed which is specific to Punjab's agricultural landscape is beetle goat. Their capacity to thrive in a variety of climatic conditions strengthens their reputation as a reliable livestock choice.

**Composition and Nutritional Value of Goat Milk:** Goat milk has a unique composition and nutritional value that makes it important for human consumption. It contains elevated levels of short and medium-chain fatty acids and a reduced content of αS1-casein Additionally, goat milk contains naturally homogenized fat globules measuring less than 0.35 µm (Chen et al., 2022). This smaller fat globule size contributes to its easy digestibility, aided by a higher concentration of medium-chain triglycerides (MCTs) in its fat content. These MCTs, characterized by saturated fatty acids with shorter carbon chain lengths, are readily absorbed and metabolized for energy, making goat milk a readily available source of nourishment (Van Immerseel et al., 2004). Goat milk also aids digestion by neutralising acids and has therapeutic qualities such as anti-inflammatory, and antioxidant activity (Al-Kaisy et al., 2023).

Along with high water content of 87%, it also contains a moderate amount of fat (4%), carbohydrates (4.5%), protein (3.5%), and ash (1%). The presence of medium and short-chain fatty acids produces a distinct aroma, often described as a "goaty flavour." This odour is attributed partially to the presence of medium and short-chain fatty acids (Haenlein, 2004). The lipase enzyme in goat milk aids in its easy digestibility, while its softer, more pliable curd structure allows for efficient breakdown by stomach proteases. Furthermore, goat milk is low in allergens, making it more similar to human milk and hence more easily tolerated by sensitive persons. Its enhanced buffering capacity, which is due to elevated amounts of nitrogen, non-protein nitrogen, and phosphorus pentoxide, improves its nutritional profile.

Additionally, goat milk contains alpha hydroxy acids like lactic acid, which contribute to skin health by removing dead cells and promoting rejuvenation. Furthermore, its lactoferrin content facilitates the bioavailability of iron and copper, aiding in the

prevention of anaemia and the reduction of total cholesterol and LDL fractions (Kell et al., 2018). The distinctive composition of goat milk renders it not only a highly nutritious option but also one with potential health benefits ranging from improved digestion to enhanced skin health and cardiovascular support.

**Health Benefits of Goat Milk:** In recent years, goat milk has gained popularity due to its numerous health benefits as it is rich in essential nutrients such as proteins, carbohydrates, minerals (zinc, calcium, potassium, magnesium) and vitamins C, B, and A (El-Hatmi et al., 2015). Goat milk is considered superior for its ability to boost the immune system and promote stronger bones, making it a vital component of overall well-being. The oligosaccharides found in goat milk are more common than those found in cow's milk, and also share certain structural similarities with human milk. As a result, goat milk may have special functional properties that can be used in a range of commercial milk products, such as formula for infants (Van Leeuwen et al., 2020). Researchers have shown significant interest in producing functional dairy products from goat milk containing oligosaccharides (Van Leeuwen et al., 2020) that serve as a potential source for maintaining intestinal health.

Furthermore, its striking similarity to human milk makes it an excellent choice for infants and those seeking a more natural alternative to human milk. Moreover, its higher proportion of medium-chain fatty acids confers antiviral properties, aiding digestion and promoting heart health by dissolving cholesterol deposits. With elevated levels of iron, copper, Vitamin B, zinc, and selenium, goat milk further enhances the immune system and supports overall health. Goat milk also have very good anti-inflammatory properties and promoting overall wellness.

**Consumption during Dengue Fever:** It is often recommended that regular consumption of goat milk during dengue fever is beneficial. It aids in maintaining body fluid balance, particularly when platelet transfusions are not feasible for all patients. Compared to cow milk, goat milk contains 27% more selenium (Se), a vital micronutrient known for its role in controlling the human immune system, especially in controlling autoimmune diseases. Higher selenium concentration in goat milk results in platelet regeneration when suffering from dengue fever. Selenium also plays a crucial role in preventing the replication of dengue virus strains (DEN 1, 2, 3, and 4) (Panta et al., 2021). In the treatment of dengue fever, goat milk and its derivatives provide significant aid by directly modifying the human immune system and hastening recovery.

**Goat Milk's Antimicrobial Properties**: Studies have highlighted the role of Alpha-S2 Casein protein present in goat milk, for its antimicrobial activity. This caprine protein exhibits bactericidal properties against pathogenic bacteria. Additionally, it also contains short-chain fatty acids and medium chain fatty acid including capric, caproic, and

caprylic acids, which have been proven to possess antimicrobial activity (Van Immerseel et al., 2004). The goat milk is a potential source of a natural antimicrobial agent, offering promising applications in food preservation and health maintenance.

**Immunomodulatory Potential of Goat Milk**: Proteins like lactoferrins and caseins found in goat milk not only possess antimicrobial properties but also demonstrate the capability to regulate the immune system. A study by Mathipa-Mdakane et al. (2022) found that probiotic *Lactobacillus rhamnosus* bacteria isolated from goat milk had immunomodulatory effects on Swiss-albino mice's intestinal and respiratory systems.

**Goat husbandry in India - Economic Significance and Global Market Dynamics:** In the Indian context, goat husbandry contributes significantly to the Livestock Gross Domestic Product (GDP), surpassing a notable 9% (Singh et al., 2023). India holds the highest population of goats globally around 148.88 million. Astonishingly, this population has been on an increasing trend with a growth rate of 3.5% between 2007 and 2019, despite a staggering 56% annual slaughter rate. The presence of 34 registered breeds of goats in India shines a light on the diversity within the species. Among these breeds, six breeds namely Beetal, Jakhrana, Jamunapari, Surti, Zalawadi and Gohilwadi are specifically recognized for their suitability in dairy production. This diversity underscores the potential for specialized breeding programs catering to dairy-centric objectives.

India ranks among the top producers of goat milk globally, alongside Bangladesh, Sudan, Pakistan, and France, with Turkey, Spain, South Sudan, Niger, and the Netherlands following closely. Together, these nations contribute significantly to the richness and diversity of the global goat milk market, reflecting its increasing demand worldwide. Market projections suggest substantial growth, with a forecasted Compound Annual Growth Rate of 7.45%, indicating a rise from $8.5 billion in 2018 to an estimated $11.4 billion by 2026 (Data Bridge Market Research). Particularly, the goat milk powder market is witnessing a surge, expanding at a rate of 7.1% in North America and internationally, propelled by the growing demand for food and beverages.

According to Volza's India Export data, India has become a major player in the goat milk export market, sending significant quantities of its production to the US, Singapore, and Spain. This highlights India's pivotal role in meeting the global demand for goat milk and playing its key position as a major influencer in the global goat milk trade.

**Goat Breeds in India and the Emerging Goat Milk Market**: In India, goat breeds are categorized into dairy and dual-purpose breeds. Among these the dairy goat breeds are Beetal, Jakharana, Jamunapari, Surti, Zalawadi, and Gohilwadi. On average, these breeds produce milk ranging from 150 to 280 liters during a total lactation period of 130-180 days. For breeds that serve both meat and milk purposes include Barbari, Sirohi,

Marwari, Kuttchi, Mehsana, Kahmi, Rohil Khandi, Sangamneri, Osmanabadi, Malabari, and Berari. The average lactation length and milk yield for these dual-purpose breeds fall within the ranges of 90-130 days and 75-120 liters, respectively (Singh et al., 2008).

The market for goat milk products encompasses various segments and distribution channels, reflecting the diverse consumer preferences and purchasing habits. The products in this market are categorized into different types, including liquid milk, cheese like Chevre, and milk powder, flavoured milk, probiotic yogurt, cheeses, ice-cream and bioactive peptides all these product type caters to distinct consumer needs and usage preferences, offering options for both immediate consumption and culinary applications and creating opportunities in rural development, employment and sustainable income generation.

The goat milk market in India is supported by various goat farms and dairy companies. Notable ones include Aai Goat Farm in Pune, Maharashtra, Gadariya based in Rajasthan, Sonchandra Goat Milk Dairy located in Belgaum, Karnataka, and Vanarai Goat Milk Dairy situated in Chinchodi-Landewadi, Tal-Ambegaon, Dist-Pune. Some key players operating in India Goat Milk Market include Nandini Dairy Products Pvt Ltd., Amul Dairy Products, Mother Dairy Fruit & Vegetable Pvt Ltd., Danone S A, Schreiber Dynamix Dairies Private Limited and Pashu Sandesh Agro Foods (P) Ltd. Overall, the goat milk market dynamics are shaped by a combination of product variety, distribution channels, and sectoral organization, reflecting the evolving preferences and demands of consumers seeking nutritious and alternative dairy options.

**Scope and Opportunity of Goat Dairying in India:** Goat dairying in India presents a significant opportunity for socio-economic development, primarily by addressing unemployment among educated rural youth. This sector not only generates employment but also fosters entrepreneurship, as individuals venture into goat farming and dairy product marketing. Moreover, goat dairying enables the utilization of non-conventional resources like solar energy, rainwater harvesting, and agricultural by-products, making it environmentally sustainable. Entrepreneurs in this field often introduce new technologies and products, driving innovation and market expansion. Furthermore, exporting functional dairy products is becoming more popular, which strengthens India's position in the international market. Smallholder dairy production is integrated into the value chain by ensuring fair benefit distribution by confirming fair benefit distribution and contributes to the nation's overall commercial dairy production sustainability.

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# Temperament Matters: The Surprising Link Between Personality and Production in Dairy Animals

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## Abstract

*The personality traits of dairy animals can significantly influence various aspects of productivity within a dairy farm. Calmer, more docile animals tend to exhibit higher milk yields, better reproductive success, and longer productive lifespans compared to their more anxious or aggressive counterparts. Furthermore, temperament affects behavioural issues, stress levels, handling ease, and overall welfare, impacting not only production but also farm efficiency and worker safety. Recognizing the importance of temperament in dairy farming, opened up avenues for improved breeding practices and management strategies aimed at optimizing both productivity and animal well-being.*

**Keywords***: Aggressive behaviour, Bovine, Efficiency, Productivity*

Since the early days of domestication, the temperament of cattle has been a subject of interest. Over centuries, efforts have been made to selectively breed for calmer animals. Temperament, defined as consistent behavioural and physiological variations in response to stressors or environmental stimuli, primarily refers to how animals react to human handling. Its significance lies in welfare considerations, as calmer animals tend to experience less stress and fewer injuries during handling and restraint. Interestingly, temperament has emerged as a notable factor influencing milk production in dairy cows, alongside genetics and diet (Chang et al., 2020). A cow's disposition, ranging from placid to reactive, can have a substantial impact on milk yield. This article highlights the importance of an animal's temperament influencing the production performance and methods adopted to judge the temperament.

## Behavioural Characteristics that Comprise Temperament

i. Shyness-boldness: How cows react to humans while being milked, ranging from being easily startled to remaining calm.

ii. Exploration-avoidance: Adaptation to new environments, distinguishing between those easily stressed by new stalls and those who readily adjust.

iii. Activity: Levels of daily movement and grazing behaviour, varying from high

activity to more sedentary behaviour.

iv. Sociability: Preference for interaction with other cows, indicating whether a cow prefers isolation or seeks companionship within the herd.

v. Aggressiveness: Behaviour related to competition for resources such as feed or space, spanning from being easily agitated towards other cows to being more passive.

**How to Assess the Temperament?**

Assessing temperament in dairy cows involves observing their behaviour across various scenarios (Jaskowski et al., 2023), with common methods including (Fig. 1):

- **Observation During Milking**

i. Reactivity Assessment: Evaluate the cow's response to milking procedures such as the attachment of milking equipment, noting signs like kicking, vocalization, or excessive movement, which signify a more reactive temperament.

ii. Milking Speed Observation: Note the speed at which cows are milked, as calmer cows typically exhibit less resistance, allowing for faster milking.

- **Employing Standardized Tests**

i. Flight Speed and Distance Measurement: Assess how quickly and how far a cow moves away from perceived threats, such as humans approaching in a handling facility. Faster flight speeds and covering longer distances indicate a more fearful temperament.

ii. Novel Object Test: Introduce a new object into the cow's environment and observe her reaction. A bolder temperament is indicated by curiosity and calm exploration.

iii. Open-Field Test: Allow the cow to explore a new, enclosed area and record behaviours like vocalization frequency, movement patterns, and urination. Higher levels of these activities suggest nervousness.

**Most Common Measurements for Assessing Cattle Temperament**

**I. Chute Score (**Schiller et al., 2020)

*Approach:* A subjective assessment conducted by observing a cow's behaviour while confined in a handling chute.

*Scoring System:* Utilizes a numerical scale, usually ranging from 1 to 6, where 1 signifies a tranquil, gentle animal, while higher scores indicate increasing levels of agitation, vocalization, and resistance.

*Advantages:* Simple to implement and does not necessitate specialized equipment.

*Limitations:* Reliant on the observer's expertise and consistency in scoring.

**II. Pen Score**

*Approach:* Similar to chute scoring, but the cow's conduct is assessed within a confined enclosure rather than a chute.

*Scoring System:* Utilizes comparable rating scales as chute scoring, focusing on movement, vocalization, and interaction with handlers.

*Advantages:* Offers an alternative to chute scoring and may be less stressful for the animal.

*Limitations:* Subject to similar subjectivity concerns as chute scoring, and behaviour may be influenced by pen size.

**III. Exit Velocity (Flight Speed)** (Curley et al., 2006)

*Approach:* Quantifies how rapidly a cow exits a handling chute upon release.

*Measurement:* Typically gauged using a stopwatch and predetermined distance from the chute exit. Swifter exit times indicate a more reactive temperament.

*Advantages:* More objective compared to chute or pen scoring, potentially less stressful for the animal.

*Limitations:* Requires additional equipment and does not encompass all facets of temperament, such as sociability.

**Milking Temperament Based on Automated Milking Systems (AMS)**

As AMS or milking robots gain popularity worldwide, with over 38,000 currently in operation on dairy farms, there's a concern that traditional methods of assessing milking temperament may become less accurate due to reduced interaction between animals and farmers. Consequently, there's a need for more reliable temperament indicators.

Temperament indicators in AMS include metrics such as the number of rejected and incomplete milkings, kickoffs, and instances of teats not found. Failures in milking and poor milkability can significantly affect the capacity and efficiency of AMS systems, impacting farm profitability.

Assessing temperament based on AMS information offers several advantages such as:

- It seamlessly integrates into farm routines without requiring additional labor.
- AMS generates standardized and objective measures across individuals, providing a large number of repeated records per animal.
- Compared to subjective scoring systems, AMS data exhibit greater phenotypic variability, making them promising indicators for improving milking temperament.

**Factors Affecting Cattle Temperament**

*Breed* - Zebu breeds typically exhibit poorer temperament compared to European breeds, notably struggling with adaptation to machine milking, particularly when compared to cows of European descent. Additionally, some purebred Zebu may require the presence of their calves during milking.

*Age* - Generally, younger cattle display more temperamental behavior than older counterparts. With age, cattle behavior tends to become more consistent over time, influenced by individual traits and habituation.

*Production System* - Cattle kept extensively, such as those in free-range systems, have less frequent human interaction and thus are less receptive to handling compared to cattle in intensively housed dairy systems. Consequently, there is a wide range of temperamental variability toward human contact due to adaptation to different production and housing conditions.

*Fear* - In behavior tests involving humans, cattle exhibit more noticeable fear responses compared to tests conducted without human presence. The level of fear or avoidance of humans is often indicated by measures such as flight distance or flight speed.

*Sex* - Studies on beef cattle have indicated that cows tend to have higher temperament scores compared to steers.

*Hormonal* - Increased cortisol levels during milking in unfamiliar environments may inhibit the release of oxytocin, negatively impacting milk production.

Assessing the temperament of cattle offers valuable insights into their physical, physiological, and psychological well-being, impacting immunity, stress levels, and metabolic functions. The temperament displayed during milking significantly affects milk production, flow rate, ease of milking, lifetime productivity, and somatic cell count. There is a clear trend in milk yield, with docile animals yielding more than slightly restless, restless, aggressive, and nervous ones. Measures like temperament score, exit velocity, and pen score have been linked to stress indicators such as cortisol and epinephrine levels. The lower milk yield in nervous animals may stem from disruptions in neuro-hormonal processes. Additionally, parity plays a role in temperament, influencing milk yield, and there is a trend of increasing calm temperament in cattle over successive years due to habituation. Considering the significance of dairy temperament, it's crucial to incorporate it into animal selection criteria for milk yield and milking performance.

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# Scientific Technique for Making Silage and its Quality Indicators

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## Abstract

*Silage is a way of conserving fodder to economize milk production costs, steady supply of fodder round the year and maintain a stable rumen environment. Silage is the end product of fermenting low dry matter forages (typically Maize, Sorghum, Oats, Ryegrass, and Bajra) in an anaerobic environment. To ensure high-quality silage, it is crucial to harvest the crop at the appropriate stage of maturity and moisture content. Silage undergoes four different phases in the pit: respiration, aerobic fermentation, anaerobic fermentation, and stabilization. The production of lactic acid by anaerobic fermentation helps to preserve the silage and prevent additional degradation, resulting in stabilization. This article describes the scientific basis of silage making technology, optimum stages of fodder harvesting, quality determinants of a good silage and method of its removal from pit to feed dairy animals.*

**Keywords**: *Dairy Animals, Feeding, Fermentation Process, Quality Indicators, Silage Making*

India has only 2.29% of the total land area of the world but hosts 17% of the humans as well as 11% of the total livestock population of the world. The area under fodder production in India is declining over the past few years, resulting in currently a net deficit of 35.6% green fodder for livestock. If this trend continues in the future, there will be a 67% deficit in the availability of green fodder for Indian livestock (Vision 2050). Moreover, under tropical climates, farmers are routinely faced with an acute shortage of green fodder twice a year, particularly during November–December and May–June, known as lean periods (Chaudhary et al., 2012). Forage availability and quality are major limiting factors that negatively affect animal productivity, health, and profitability (Sirohi & Michaelowa, 2007). To overcome this scenario, silage preparation is the best way to conserve the ample amount of good quality fodder for the feeding of animals throughout the year and particularly during the lean period. The importance of green fodder is well recognized, as feeding alone accounts for over 60% of the cost of milk production. Hence, by providing sufficient quantities of fodder instead of costly concentrates and feeds to the dairy animals, the cost of milk production can be considerably reduced (Vision, 2050).

Silage is a way of conserving fodder to economize production costs, meet demand for fodder during lean periods, and maintain a stable rumen environment. Silage is any forage, crop residues, agricultural or industrial byproducts preserved by acids, either

artificially added or produced by natural preservation in the absence of air. The main goal of silage production is to preserve maximum possible nutritional value of the original crop.

**Advantages of Silage Making**

- Silage can be used during scarcity of green fodder like in the months of May-June and November-December.
- Minimum loss of nutrients compared to other method of preservation.
- Succulent feed is made available round the year.
- It requires less area for storage as compared to hay.
- Helps to control weeds as the fodder is harvested from field at bloom stage.
- No danger of fire to silage.
- It is palatable and laxative feed.
- Compared to pasture there is less infestation of worms and parasites with silage feeding.
- Decreases the anti-nutritional factors present in various fodder crops

**Types of Silos:** Silo is a farm structure which stores and products the animal fodder providing an ideal condition. Silo may be of Trench, Bunker, Tower or Bag type (Fig 1)

- **Trench Silo**: It is often dug into an elevated location, bank or slope, sometimes lined with concrete and used mostly in the regions of the low rainfall for making and storing silage.
- **Bunker Silo**: For bunker silo, above-ground walls are constructed using concrete, soil or wood. Bunker silos require adequate drainage. Concrete floors are usually needed for easier loading and to minimize feed waste.
- **Tower Silo**: The tower silo is an above ground cylinder, with 6-9 m diameter and a 15-20 m height. Loading of tower silos is difficult. It heeds a mechanical loader or a large capacity blower for elevating the cut fodder.
- **Bag Silo:** Bag or tube may also be used by small or marginal farmers to prepare silage. Polythene used should be 0.1 mm thick and of low-density poly propylene grade.

The selection of silos for silage making depends on the number of animals, length of scarcity period, kind of soil and drainage, depth of water table, rainfall, and other weather conditions. One cubic meter silo pit has 5–6 quintals of storage capacity; accordingly, one can adjust the dimensions as per requirement. The silo size is determined by a

- Herd size
- Amount of daily feed


Fig. 1. Different types of silo structures

- Number of feeding days and
- Packed density of the raw materials

**An example of calculation for a herd of 10 dairy cows:**

- 10@ 20 kg/d for 180 days (number of feeding days)
- 10×20×180=36000 kg= 360 quintals
- 20% is added to account for packing/storage loss 360 × 10% = 36 Quintal
- The resulting calculated silo capacity= 360+36 = 396 Quintal
- The capacity of the silo: 1 $m^3$ = 6 Quintal fodder
- Size of silo = 396/6 = 66$m^3$
- Dimensions are 11m × 3m × 2 m = 66 $m^3$

**Fodders Used for silage Making**: The fodders used for silage making should have:

- High level of fermentable sugar (10-15% on DM basis or 2-3% on fresh basis)
- Low level of protein
- Low buffering capacity
- 30-35% DM at the time of ensiling

The most commonly used crops are Maize, Sorghum, Oats, Ryegrass, and Bajra. Generally, leguminous crops like Lucerne, Berseem, etc. are not considered suitable for silage making due to their high protein content and low carbohydrate and dry matter content. The time of harvest has a major impact on the nutritive value of the silage. Early harvesting reduces the dry matter content, and late harvesting decreases digestibility due to lignification of the stem. To have a good quality nutritive value in the silage, as per Tiwana (2015), the optimum stage of harvesting fodder is as follows:

| S. No | Fodder | Optimum Stage of Harvest and Time taken | Average Yield Quantity/Acre* |
|---|---|---|---|
| 1 | Maize | Dough stage; 55-60 days after sowing | 160-200 |
| 2 | Sorghum | From ear formation to grain formation; 60-85 days after sowing | 240 |
| 3 | Bajra | At the time of ear formation; 45-55 days after sowing | PCB 64: 210<br>FBC 16: 230 |
| 4 | Napier Bajra | At the height of 1 meter; 55-60 days after sowing | PBN 233: 1100<br>PBN 83: 960 |
| 5 | Oat | Full bloom to milking stage; 105-125 days after sowing | OL 9: 230<br>Kent : 210 |
| 6 | Rye grass | Full bloom to milking; First harvesting at 55-60 days after sowing and next after 30 days of harvest | 325 |

*May vary depending upon the variety of fodder

The silage from maize is prepared at two stages. One is as mentioned in the table, and the other is with kernels. For this, maize is harvested when the milk line is ½ to ¾ down the kernel (Fig. 2). This stage is mostly achieved 75–93 days after sowing, depending on the variety of maize to be sown.


Fig. 2. Milk line of maize kernels

Optimum dry matter in the fodder plays an important role in minimizing nutrient losses during silage. High moisture silage leads to effluent losses and it also promotes clostridial fermentation, which leads to excessive dry matter losses, high butyric acid

concentrations, and lower nutrient intake. High moisture (more than 75%) also promotes proteolysis by clostridia, which results in a loss of crude protein content. So, moisture judgement by grab test is of utmost importance before ensiling. Several methods are commonly used to estimate the moisture content of forages prior to being placed in the silo. Microwaves or ovens can be used to analyse a forage sample for moisture content; however, the dry matter content of fodder at farmer level can be easily estimated by using the hand or squeeze method (Grab method: squeeze a handful of green chops as tightly as possible for 90 seconds to make a forage ball) as follows:

| Condition of forage ball | Moisture (%) |
|---|---|
| Water easily squeezed out and material holds shape | More than 80 |
| Water can just be squeezed out and material holds shape | 75-80 |
| Little or no water can be squeezed out but material holds shape | 70-75 |
| Falls apart slowly and there is no free juice | 60-70 |
| No water can be squeezed out and material falls apart rapidly | less than 60 |

**Ensiling Technique:** Ensiling technique involves harvesting fodder at optimum stage, wilting (if required), chopping, filling of silo and airtight sealing with suitable material (Fig 3).

**Silo Pit**

- Silo pit must be at higher altitude/elevation to prevent seepage or rain water into silo pit.
- Location of silo is such that it is easy to distribute the silage to animals.
- It should be away from milking room/collection room to prevent its flavour creeping into milk.
- The bottom of silo must be free from moisture and usually should be above the water table.
- The walls of silo should be strong enough to withstand pressure of gases and there should be no entrance of fresh air.

**Feed Additive for Silage:** Generally, if fodder is harvested at the optimal stage, feed additives are seldom required, but presently, there are many feed additives available on the market, a few of which are as follows:-

| Commercial feed additive | Dose rate |
|---|---|
| Silage Savor | 1 pound/ton fodder |
| SilaTek | 10g/ ton fodder |
| Silage F400 | 1g/ ton fodder |

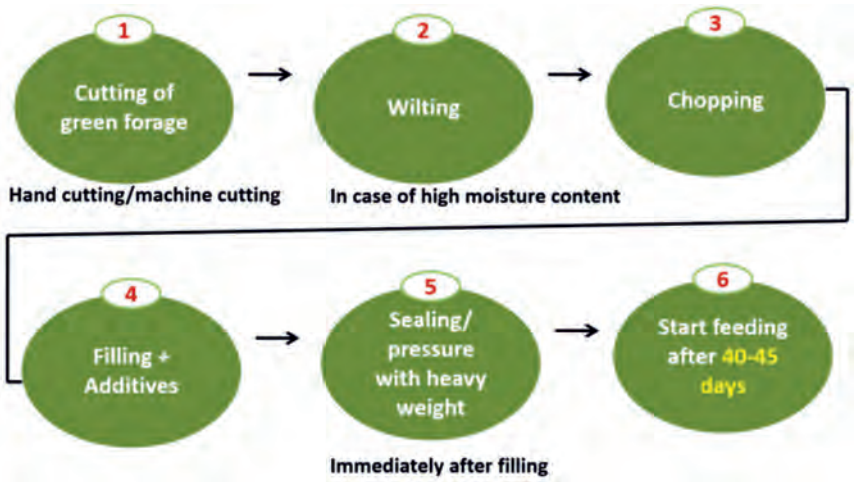


Fig. 3. Ensiling Process

**Harvesting of Fodder Crop**

- Crops at pre-flowering to flowering stage should be harvested.
- After harvesting, green fodder should be chaffed by using chaff cutter machine.
- 80% and 20% of crop must be chopped 1-3 cm and 3-5 cm, respectively for better packaging and quality silage preparation.

**Filling of Silo**

- After chaffing, start to fill green fodder in a pit.
- It is better to spread a layer of hay or straw at the base.
- Chaffed fodder should be evenly spread throughout the silo and pressed to expel the maximum air out and trap the minimum air in silo.
- After making 4" thick layer of ensiling mass, press it manually or with tractor to expel maximum air out of silo
- Follow the same procedure until filling of pit 1 to 1.5 feet above the ground level.
- Silo pit filling should be completed within 1-2 days.
- After thorough pressing, top should be covered with polythene followed by soil layer of 6 inches depth. Plug all possible areas of air or water entry.
- It will require 40-45 days to make good quality of silage.

**Fermentation Process:** Fermentation process can be divided into 4 phases

1. Phase I: Respiration: burning of carbohydrates in oxygen to $CO_2$ and water (1 to 2 days)
2. Phase II: 1 to 2 days

- Fermentation: natural conversion of carbohydrates to organic acids by bacteria
- Early fermentation, which produces acetic acid, formic acid and other organic acids as a result of the growth of facultative aerobic bacteria such as enterobacteria, which can live in the presence or absence of oxygen

3. Phase 3: Lactic acid fermentation by lactic acid bacteria that are strictly anaerobic, that is, they can only grow and multiply rapidly in the absence of oxygen (14 days) and pH drops to 3.7-4.8 during this phase.
4. Phase 4: Stabilization phase due to the presence of lactic acid, which inhibits further degradation (indefinite period). Aim for pH 3.8-4.2

**Characteristics of Good Quality Silage**

- **Smell**: Pleasant or vinegar type
- **Colour**: Bright and light green.
- **pH: 3.8-4.2.**
  - pH indicates how well preserved the silage is.
  - If pH < 3.8: means acidic silage: careful supplementation and/or buffering to avoid stomach upsets
  - Dry silages can have higher pH values and be well preserved such as 4.5 and above
  - If silage is prepared from legume, its pH range will be 4.2-4.8 which is due to high ash (> 15% of DM) and (or) protein content (> 18% CP)
  - Reasons for high pH: Dry silage, poor fermentation, silage with excess ammonia or urea, Clostridial silages, spoiled or moldy silages, silages containing manure
- Lactic Acid (LA) Content: >4%
  - Well-preserved stable silage: LA= 65-70% of total silage acids, sweet smelling
  - Standard of Fermentation: A good fermented silage has a per cent DM content of above 4-7%, while <4% indicates poorly fermented silage.
  - Low lactic acid
    a. Restricted fermentation due to high DM Content
    b. High in butyric acid content (Clostridial silages)
    c. Aerobic exposure of sample also degraded LA
- Acetic Acid (AA) Content: 1-3%
  - Higher AA: Extremely wet silages (<25% DM), prolonged fermentations (due to high buffering capacity), loose packing or slow silo filling

- Microbial inoculant *(Lactobacillus buchneri)* treated silages: higher level of AA-not mistaken as poor fermentation

- Butyric Acid (BA) Content: < 0.13%
  - Higher BA (>0.5%): indicates Clostridial fermentation
  - It has low in nutritive value and have higher ADF and NDF levels because many of the soluble nutrients are degraded
  - Foul smelling acid, lead to a lot of wastage in the pit
- Ammoniacal Nitrogen: <10% of total nitrogen
  - Best indicator of silage fermentation
  - Shows the proportion of N (including protein) that has been broken down during ensilage
  - Standard of Fermentation — Ammonia N (% of CP)

    | | |
    |---|---|
    | Excellent | <5% |
    | Good | 5-10% |
    | Moderate | 10-15% |
    | Poor | >15% |

  - High concentrations of ammonia are a result of excessive protein breakdown in the silo caused by a slow drop in pH or clostridial action.

**How to Remove the Silage**

- Silage quality can deteriorate rapidly during feed out
- If exposed silage surface open to air for longer periods of time, yeast and mold become active and result in silage heating which ultimately leads to DM and energy losses.
- So, feed out rate should be sufficient to avoid heating at the silage face.
- Feed at least 6 inches of silage per day from a bunker silo face; 4 inches per day in summer, 2 inches per day in winter, for upright silos
- Minimum disturbance of the feeding face, to minimize air penetration.
- Silage should not be removed prior to the time of feeding.
- There should be little to no silage left at the base of the face after feeding is done for the day

**Silage Testing**

- Prior to feeding, it is imperative to conduct a silage test when the silage pit is opened for the first time.

- If the moisture level of silage appears high after pulling it from the pit, it is advisable to promptly send a sample to the laboratory for analysis.
- If there is a presence of fungal infestation, it is necessary to conduct testing on the silage.
- Regular silage testing should be conducted every 1 to 2 months, regardless of the precautions taken during silage removal.

**Silage Feeding**

- After 40-45 days, silage is ready as feed for animals.
- Majority of silages are high in energy (70% TDN), moderate in protein (9%) and low in minerals Ca, P, Mg (0.2%) on DM basis.
- Feed silage @ 6% of body weight (if DM 30%)

  400 Kg: up to 24 Kg

  500 Kg: up to 30 Kg

- As silage is low in protein, so feed high yielders with concentrate having 20% crude protein.
- If prepare maize silage with grains: Feed 20% less concentrate i.e. 4 kg instead of 5 kg to the dairy animals.
  - Feeding of silage should start gradually i.e. 2-5 kg and after a week feed it at the rate of 20-30 kg/animal/day
  - Calves (< 6 m of age) should not be fed maize silage.
  - Unbred heifers could be fed maize silage only if they are fed high protein feeds, otherwise fat deposited in their developing udder.
  - Feed the silage at least 4 hours before milking or after milking.
  - Feeding of damaged or discoloured silage should be avoided.

**Bag Silage:** The basic steps in the preparation of bag/tube silage are the same as for bunker or trench silage. The bag is filled with chopped green fodder and pressed manually as shown in the picture (Fig. 1). After filling, the bag is tightly tied and left for 40–45 days. Plastic bags are an economical alternative to traditional silage storage systems for small farmers. It is an effective way to preserve feed with minimum nutrient loss. It has the additional advantage that all the acid is retained in the silage, unlike pit silage, where it seeps out as effluent loss. These bags of silage help in lowering the drudgery of women, especially from low landholding or landless families rearing animals that daily bring fodder from the field.

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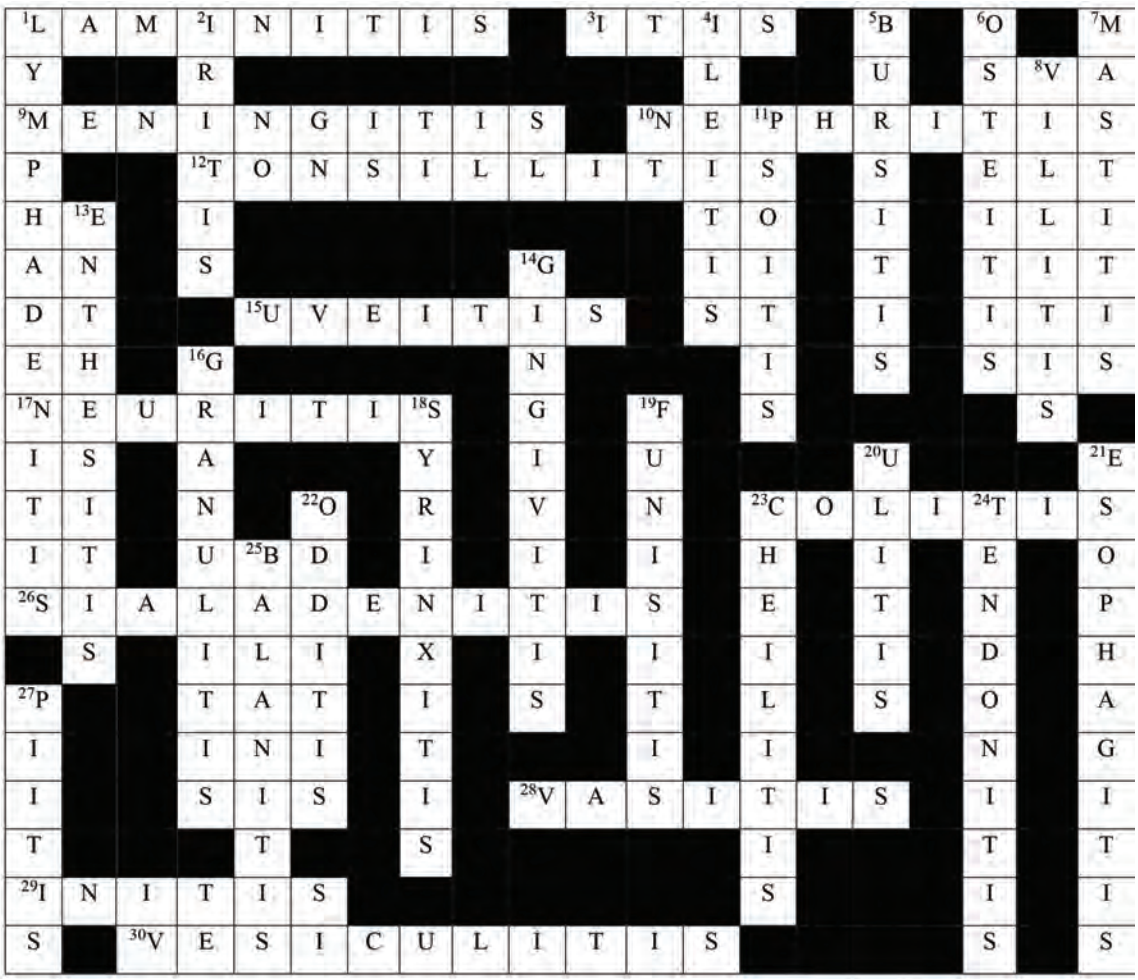
## Article Retraction Notice

All concerned please note that on the request of the corresponding author, the following article entitled "New and Alternative Approaches to Combat Antibiotic Resistance" by Tania Gupta and Deepti Narang which was published in the Vet Alumnus, June, 2023; 45(1): 47-50 has been retracted due to the duplicate publication elsewhere.

# Answers to the Crossword Quiz

## (Crossword Quiz on Page No. 56-57)



# Strategies to Bridle the Problem of Straw Burning in Northern India

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## Abstract

*Continuous rice and rotational wheat agriculture as well as intensive tillage for both crops, result in the adaptation of crop residue (CR) burning by the farmers of Northern India. The most severe consequences of residue burning are air and soil pollution along with a higher risk to public health. With time, the Indian government has taken several initiatives to provide support to farmers and also help in the development of various crop residue management technologies. These technologies can be adopted on the field (in-situ) and off-field (ex-situ). The residues, once thought to be waste, can be easily transformed into an essential natural resource with increased knowledge and further investigation.*

**Keywords:** *Burning, Crop residue, Straw, Strategies*

Since 2002, farmers primarily in Punjab, Haryana, and Uttar Pradesh have burned paddy straw from their paddy fields, known as stubble burning after harvesting the crop each year from April to May and October to November as a low-cost straw-disposal practice to reduce the turnaround time between harvesting of paddy and sowing of wheat. This practice has been identified as a major source of air pollution in Northern India. Every year about 13 million tonnes of stubble are burned and 19 million tonnes of carbon dioxide and other harmful gases are put into the atmosphere. It results in a four-fold increment of the ambient air quality standard for $PM_{2.5}$ (from 60µg/$m^3$ to 193-270 µg/$m^3$) (Central Pollution Control Board report, 2021). This further resulted in a two-threefold increase in respiratory symptoms like wheezing, breathlessness in exertion, etc., skin rashes, running nose, or itchiness of the eye posing a higher health risk to the public (Central Pollution Control Board report, 2021).

The Government has taken different initiatives to implement different schemes and to provide support to farmers for the adoption of various crop residue management (CRM) technologies. Such govt. initiatives and CRM technologies are narrated below.

## 1. Government Initiatives

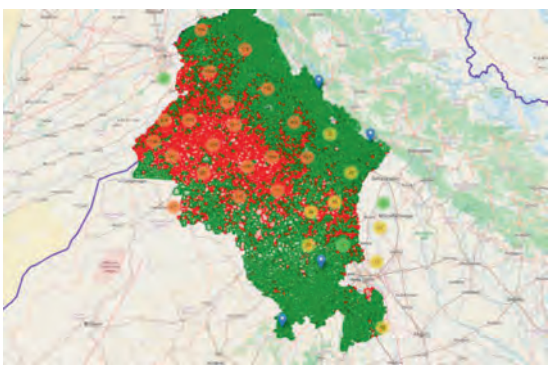
### ECOVISION - An AI-Driven Initiative for a Healthier North India

Eco Vision-an AI tool masterfully crafted to support NGO and government initiatives aimed at combating stubble burning in North India. Leveraging data from

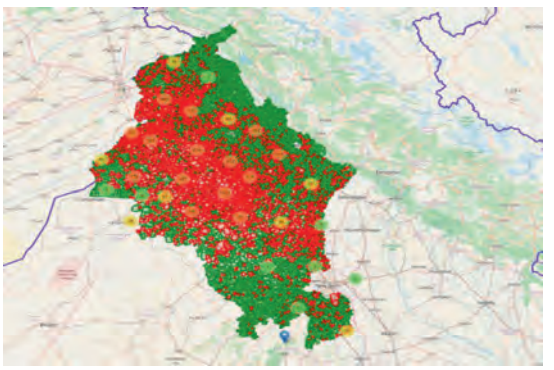
NASA's satellite imaging, Eco Vision applies artificial intelligence to accurately pinpoint villages severely affected by stubble burning. By effectively mapping areas requiring urgent attention and highlighting successful cases as "Good Green Villages", Eco Vision is instrumental in guiding strategic actions and channelling resources, contributing vitally to the battle against air pollution and the advancement of public health.

**Financial Support for Setting up of Pelletization and Torrefaction Plants**

Under Central Pollution Control Board (CPCB) guidelines, individuals/ entrepreneurs/ companies, interested in setting up pelletization and torrefaction plants, using only paddy straw can submit an application for obtaining a one-time grant under Environment Protection Charge (EPC) fund on capital investment (PSCST, 2022)



**ECOVISION 2022**



**ECOVISION 2023**

Using NASA satellite imagery, analysed with AI tools and Python, fire villages are identified as locations with prevalent stubble burning incidents in Northern India. These images depict villages affected by stubble burning, marked in red, across 2022 and 2023 (source: https://stopstubbleburning.in/).

**Emphasis on utilization as biofuel in Thermal Power Plants**

As per the revised biomass policy issued by the Ministry of Power on 16.06.2023, it mandates 5% biomass co-firing in TPPs from FY 2024-25. This obligation shall increase to 7% in FY 2025-26. Approximately 1,64,976 Metric Tonnes of agro residues-based biomass have been co-fired in 47 coal based thermal power plants till May 2023.

Crop residue management can be done in two ways (a) on the field (in-situ) and (b) off-field (ex-situ) management methods.

**1. In-Situ Crop Residue Management**

**a) Surface Retention and Mulching:** With the advent of farm machinery, farmers can now drill wheat directly into rice residue. The collection of leftovers on the surface helps protect the rich soil surface from wind and water erosion. Residues break down slowly on the surface, accumulating organic carbon and total nitrogen

in the top 5-15 cm of the soil, delaying erosion and leaving leftovers on the surface improving soil $NO_3^-$ content by 46 percent, N absorption by 29 percent, and yield by 37 percent when compared to burning. In comparison to no mulch, rice straw mulch enhanced wheat grain output, reduced crop water usage by 3-11 percent, and improved water use efficiency by 25%. Mulch retained soil moisture in deeper levels, resulting in 40 percent larger root length densities than no mulch.

**b)** **Farm Mechanization:** Advance technologies of zero-till seed-cum-fertilizer drill/ seed planters (happy seeder, spatial zero seed cum fertilizer drill) are available in the country for direct sowing of the consecutive crop in loose and anchored straw loads up to 10 t/ha**.** Whereas, Super Straw Management System (SMS) is a machine that attaches to the rear of a combine harvester to cut and spread loose residue across the field. It helps in retaining essential nitrogen, phosphorus and potassium in the soil (Nikam & Singh, 2020).

The Government has also been promoting and distributing other farm equipment including paddy straw choppers, cutters, mulchers, reversible mould board ploughs, zero till drills, shrub cutters, rotary slashers, rakers, balers, and rotavator (Nikam & Singh, 2020).

**2. Ex-Situ Crop Residue Management**

**a)** **Biogas Production:** Paddy residue biomass is an efficient source of energy generated by anaerobic digestion, gasification, and pyrolysis processes, providing an immediate reduction in $CO_2$ levels in the environment. About 300 $m^3$ of biogas containing 55-60% methane content may be produced by anaerobic digestion of one tonne of rice residue and the leftover slurry can be used as manure (Meena et al., 2022).

**b)** **Removal of Straw from Field by Use of Balers:** Straw baler machines are a promising technology commercially available for removing and collecting straw after combine harvesting and utilising the wastes for off-farm operations. After baling crop leftovers, it can also be used for paper and bioethanol processing, mushroom culture, bioconversion, and engineering uses.

**c)** **Livestock Feed:** Rice straw's nutritional value can be increased through a variety of methods. Physical, chemical (NaOH, $NH_3$ and Urea), and biological (white rot fungi and enzymes) treatments have been used on crop residues to weaken and break down lignocellulose connections, enhancing their nutritional value (Sarnklong et al., 2010). One animal is required to eat the straw from a one-hectare rice crop in a year; two animals are required if two crops are grown. Additionally, pre-treating rice straw improves both the amount of paddy straw consumed and the nutrients' ability to be absorbed. If given ad libitum, urea-ensiled straw can

be used as a full maintenance ration in conjunction with 3-5 kg of green fodder, 50g mineral mixture and salt to adult buffaloes. A scarcity feed can also be made by impregnating rice straw with 1% urea and 10% molasses. Nowadays, paddy straw based complete feed pellets could also be prepared by using guar gum as a binder in place of molasses having 14.35 to 17.4% crude protein for feeding of dairy animals (Bakshi and Wadhwa, 2023).

**d)** **Biochar Production:** The thermal decomposition of organic materials or biomass at temperatures between 500 and 700°C in a small amount of oxygen results in the production of biochar, which is a carbon-rich material and can be used as a soil amendment to improve soil fertility, carbon storage, and water filtration (Brewer, 2012).

**e)** **Usage as Manure/Compos**t: To solve the problem of stubble burning, scientists at ICAR, IARI Pusa and Harvesto came up with a new idea: Pusa Bio Decomposer. For about 15-20 days, this chemical fertiliser can make manure out of the straw that was left over (PIB, 2021). This manure can be further used to grow plants. To convert the straw into compost, scientists at IARI New Delhi have come up with a new technology to convert rice straw into compost using capsules, which cost about Rs. 5 to farmers.

**f)** **Agronomic and Cultural Practices:** Short duration varieties of rice would help in increasing the window between the harvesting of rice and the sowing of the next crop, giving more time to farmers for the management of straw. Transfer prices can be provided to the farmers when they are shifting from paddy or wheat crops to other crops.

**g)** **Use of Straw as Input to Industries/ Start-ups:** Rice bioparks are concepts propounded by Dr. M. S. Swaminathan, where industries will produce paper, board and animal feed from rice straw. Also, various start-ups have also been initiated to produce the product that can act as an input for the energy industry and can be used to develop disposable plates and other materials from it. These start-ups are also giving nominal money to farmers for taking the straw from the field.

**h)** **Straw for Other Uses:** It can be used as a raw material for mushroom cultivation by farmers. It can also be used as a packaging material for fruits, vegetables, sanitary items, etc., and also in nurseries for mulching, packing and protection of plants during extreme weather conditions. It can also be utilised in animal farms as a bedding material for animals, thatch roof material, wind barrier, for making wheat straw storage kupps, etc. Nowadays, various handicraft and home furnishing items are coming up in the market utilizing paddy straw as a major input material.

**Future Perspectives**

Technology-assisted in-situ crop residue management is advantageous to farmers and a workable way to prevent residue burning. It saves time by simultaneously shredding the harvested crop, strewing the stubble across the swath, and planting the wheat seeds. Studies have shown that it can save up to 10 lakh gallons of water on the first day of crop seeding and increase a farmer's annual profit by Rs 20,000 to Rs 25,000 per hectare. This causes farmers to gradually cut back on their use of nitrogen fertilizers.

Technology should be developed in a way that minimizes its impact on the environment and boosts farmers' income from growing rice. Even though these actions are short- to medium-term in nature, long-term crop diversification should be the main priority. Giving up the rice-wheat cropping system would help save water and restore ecological balance in addition to reducing the issue of burning straw.

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