

# THE SCIENCE WORLD



A Monthly e Magazine



**VETERINARY  
MICROBIOLOGY**



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UPCOMING FEB, 2022 ISSUE 2

# VETERINARY MEDICINE

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## VETERINARY MEDICINE



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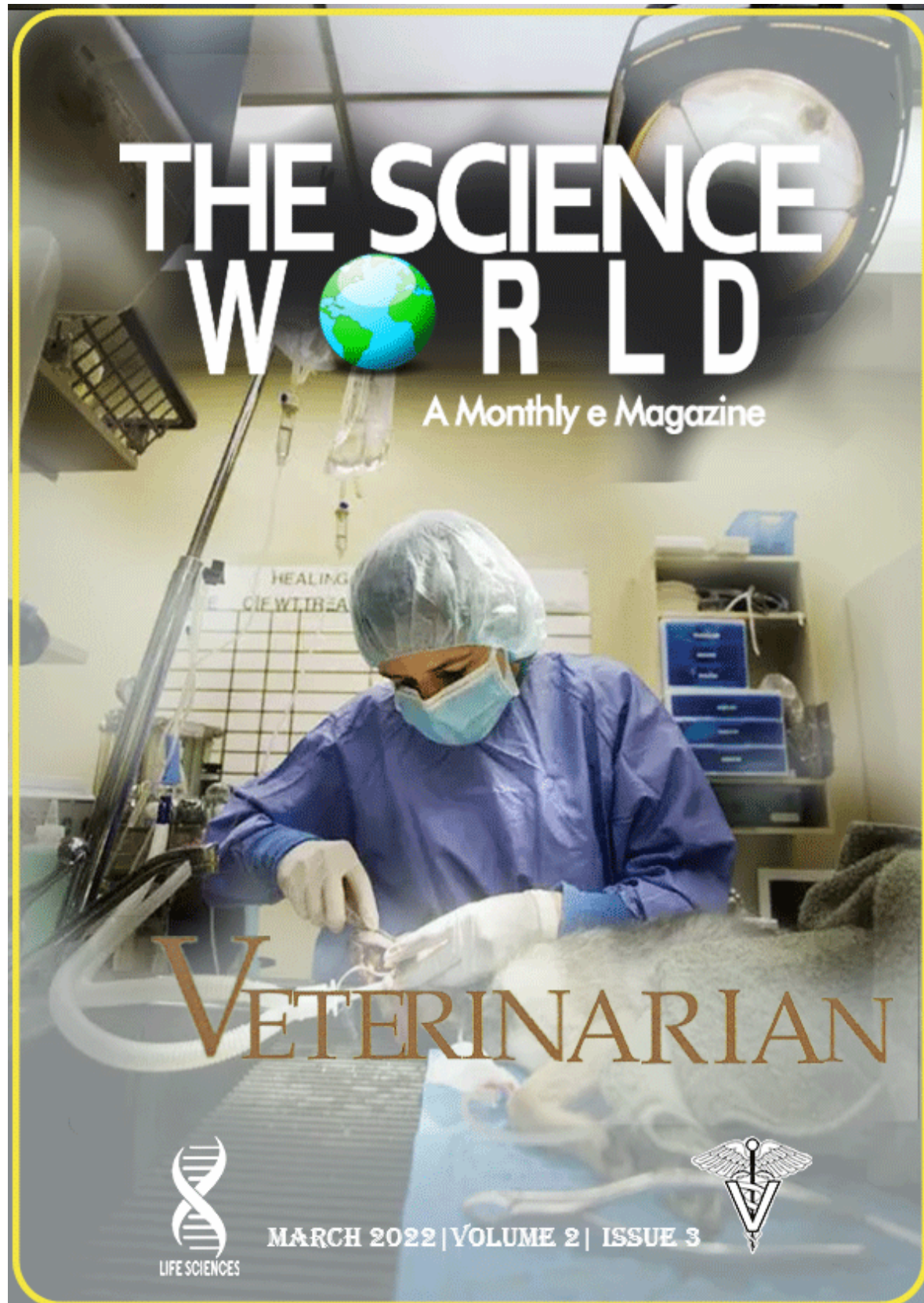
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# VETERINARIAN



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### **Application of Nanoparticles in Diagnostics**

Author: Dr Sudeep Solanki and Dr Durga Devi

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## Major Foodborne Viruses: An Emerging Public-Health Concern

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### Abstract

Foodborne pathogens are responsible for causing wide range of diseases which have significant impact on human health and economy. It is estimated that in the last 60 years, 30% of all emerging infections were caused by pathogens commonly transmitted through food. Over the last few decades, foodborne viruses raise great concern to the food industry and food safety monitoring authorities. Globally, they have been the leading cause of morbidity and mortality. according to food safety experts, different control measures should be incorporated throughout the food chain to control food-related hazards. This paper describes common foodborne viruses, which can be crucial to understand and control future food-related hazards.

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### Introduction

The World Health Organization (WHO) estimates that every year approximately 600 million people become ill due to consumption of contaminated food which results in about 4,20,000 deaths (Havelaar et al., 2015). It is estimated that between 1940-2004 about 30% of all emerging infections were caused by pathogens commonly transmitted through food (Jones et al., 2008). Among different diarrheal agents, norovirus and *Campylobacter* spp., are the common cause of foodborne illness (Havelaar et al., 2015). Between existing pathogens, viruses have been increasingly recognized as significant causes of foodborne diseases (Pal & Ayele 2020). On average, about 100 types of enteric viruses are responsible for causing different foodborne illnesses; the most common are Hepatitis A and Noroviruses. In addition to these, group B and C rotaviruses account for large foodborne outbreaks, while hepatitis E virus is linked with waterborne outbreaks. Other lesser common viruses like sapoviruses, astroviruses and aichi viruses were also occasionally associated with foodborne gastroenteritis outbreaks.

Though many foodborne illnesses of viral causation produce gastroenteritis (e.g., norovirus), some (e.g., hepatitis viruses) may lead to unusual symptoms that may not involve

díarrhea or vomítíng. Ínstead, these íllnesses may cause weakness, jaundíce and are sometímes attributed to long-lasting organ damage. Recently, advances ín víral detectíon and epídemiology have changed the way we detect, understand and prevent foodborne víruses. Thís has resulted ín the íntroductíon and widesread acceptance of preventíve públic health measures, thus better targetíng víral ínfectíons.

## Common foodborne víruses

Beíng obligate íntracellular pathogen, víruses requíre lívíg cells for propagatíon. Víruses have propertíes that make them quíte different from more commonly studíed foodborne bacteríal pathogens. Líke;

- Very few víruses are capable of producíng íllness
- Extremely high numbers of víruses shed ín stools of patíents sufferíng from gastroenterítís or hepatítís, up to  $10^{10}$  and  $10^{13}$  vírus partícles per gram of stool (Bosch et al., 2018).
- Víruses need specífíc lívíg cells ín order to replícate and therefore cannot do so ín food or water.
- Foodborne víruses typícally are quíte stable outsíde the host and are also acíd-resístant.

Enteríc víruses are responsíble for causíng: gastroenterítís, hepatítís and íllnesses that can affect other parts of the body such as the eye, the respíratory system and the central nervous system leadíng to conjunctívítís, políomyelítís, meníngítís and encephalítís.

1. **Norovíruses:** NoV belongs to Calícívírídae famíly, and ís non-enveloped, posítíve-sense, síngle-stranded RNA vírus. among the seven genogroups (G) of NoV víruses; GÍ, GÍÍ and GÍV ínfect humans (Nasherí et al., 2019). Ít causes ínfectíon to all age groups and the outbreaks are reported throughout year but peak reports usually obtáined duríng wínter months mostly ín temperate areas. Clínícal symptoms appear ín 24–48 h and ís characterízéd by acute onset of nausea, vomítíng, abdomínal cramps and non-bloody díarrhea. Ínfectíon ís transmíttered vía symptomatic and ínfected food handlers. Norovíruses bíd to Hístó Blood Group antígens (HBGa), and dífferences ín the expresíon of HBGas are essentíal determínants of susceptíbílíty to norovírus ínfectíon and dísease productíon (Gomara & Bríen 2016). Vírus can withstand varíyíng envíronmental condítíons, íncludíng wíde range of temperature (from freezíng to about 60 °C). Thís abílíty to persíst ín vast temperature range, food ítems and



artificial surfaces contributes to rapid dissemination, mainly via secondary spread (via food handlers or equipment's) (Glass et al., 2009).

2. **Hepatitis a:** Hepatitis a virus (HaV) belongs to the family Picornaviridae and is the sole member of the genus Hepatovirus. There is only a single serotype of HaV. Among the several genotypes: Ia, Ib, IIa, IIb, IIIa, IIIb are primarily found in humans, and IV–VI are primarily found in non-human primates. HaV differs from enteroviruses by specific biological characteristics such as marked tropism for liver cells, exceptional thermostability (it survives heating for 30 min to 56°C), acid-resistance (it tolerates pH 1) and slow replication without cytopathic effect on the host cell (Cromeans et al., 1994). The infection is primarily asymptomatic in children younger than six years while the severity increases thereafter. The virus is mostly transmitted via the fecal-oral route. This can be either by direct contact with an HaV-infected person or by ingesting HaV-contaminated food or water (Cromeans et al., 1994). Humans and vertebrates serve as natural hosts. The virus infects hepatocytes and Kupffer cells. Virus usually leads to mild symptoms without permanent consequences and is responsible for causing fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, clay-colored stools, joint pain and jaundice.
3. **Hepatitis E:** Hepatitis E Virus (HEV) is from the family Hepeviridae and genus Orthohepevirus and is a small non-enveloped icosahedral virus. It is usually 27–34 nm in size, with a single-stranded positive-sense RNA genome. The primary transmission for HEV is via the fecal-oral route. This happens via its shedding in the feces of infected individuals and the subsequent contamination of drinking water. It can also be transmitted by the consumption of raw or undercooked infected animals or shellfish. Other channels include blood transfusions or vertical transmission from pregnant women to the fetus (Shea et al., 2019). Although it primarily affects liver, HEV has also been associated with non-hepatic diseases. Primary examples of such are subacute and monophasic neurological disorders of the peripheral nervous system, acute pancreatitis, glomerulonephritis, mixed cryoglobulinemia, severe thrombocytopenia and haemolytic anaemia (Pischke et al., 2017).
4. **Rotavirus:** Rotaviruses are non-enveloped, segmented (11 segments) double-stranded RNA genome-containing viruses classified under the family of Reoviridae. among all age groups, young ones are primarily infected by Rotaviruses. The viral transmission is also by fecal-oral route, this can be due to processed and unprocessed food contaminated with fecal material at

the source or due to poor hygiene of food handlers. Among various human rotaviruses, group A is considered as the major cause of viral gastroenteritis in infants and young children (from six months to three years of age) (Vasickova et al., 2005). The usual signs are watery dehydrating diarrhea and vomiting, often accompanied by abdominal cramps and low-grade fever, lasting 6–10 days.

### Challenges of detecting foodborne viruses

Detecting viruses in foods is a more significant challenge than culturable bacteria. As mentioned before, viruses do not grow in food. Instead, they need living cells to replicate, and hence almost all food-borne viruses are strictly human pathogens. Most food-borne viruses are infectious, spreading rapidly from one individual to the next: also, for most food-borne viruses, only a few infectious particles (10–100) may result in a high probability of infection, whereas very high loads of viruses may be shed in stool samples of infected individuals ( $10^6$ – $10^7$  per gram of stool or more) (Newell et al., 2010). The situation is worrying as there is no systematic surveillance for food-borne viral disease detection. Also, the national surveys and statistics on food-borne viral disease are not readily available. In addition, if present, they are likely to reflect significant under-reporting.

### Conclusion

Although, NoV and HaV are recognized as priority concerns in foodborne viral transmission, the proper diagnosis of infection caused by these agents is often hindered. This can be due to sharing general symptoms with other diseases (fatigue, dehydration, nausea, vomiting, diarrhea, and some stomach cramping), failure of notification and relatively quick resolution of signs of illness. Lack of population-based estimates and the cost of pinpointing illness due to foodborne viruses form a significant hurdle in determining them. The most feasible solution to control foodborne viruses and reduce morbidity and mortality is by relying on food hygiene, good agricultural practices, appropriate post-harvest controls, and effective treatment of human sewage to prevent further transmission. We need to establish strategies to prevent viral foodborne disease and focus on primary prevention of food contamination early in the food chain, for example, by regulating the standards for irrigation water. More data on foodborne viruses as well as risk assessments incorporating data and information are needed to assist risk managers in controlling foodborne viral diseases.

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## Application of Nanoparticles in Diagnostics

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**N**anotechnology is recently employed as a tool to explore the darkest paths of the medical field in several ways like imaging, sensing, targeted drug delivery, gene delivery systems, and artificial implants. The new age drugs are nanoparticles of polymers, metals, or ceramics, which can combat conditions like cancer and fight human pathogens like bacteria. Applying nanotechnology in the treatment, diagnosis, monitoring, and control of diseases has been referred to as “Nano-medicine.”

Similarly, multi-parameter diagnostic systems are increasingly required to detect all the well-known and the more recently appeared biomarkers for different diseases. When the detection system requires a bio-molecular recognition event, antibody-based detection methodologies are still considered the standard assays in environmental, food, and clinical analysis. These assays are well established and they have been demonstrated to reach the desired sensitivity and selectivity. However, the use of antibodies in multi-analyte detection methods and the analysis of very complex samples could encounter some limitations mainly deriving from the nature and synthesis of these protein receptors. To circumvent some of these drawbacks, other recognition molecules are being explored as alternatives.

**Pathogen Recognition Elements-** A variety of recognition elements have been employed and include proteinaceous antibodies, nucleic acid aptamers, carbohydrates, and antimicrobial peptides. The recognition elements used in whole-cell biosensors are generally biomolecules that have an affinity for epitopes present on the pathogen surface. In the future, inorganic recognition elements such as molecularly imprinted polymers (MIPs) may also have utility for pathogen recognition.

**Antibodies-** antibody-based methods have been used extensively to detect bacteria, viruses, toxins, and spores, alike. Highly selective and sensitive antibodies are readily

available for many pathogens, and there are several well-established methods to conjugate antibodies to nanomaterials. For these reasons, immunological recognition by antibodies continues to be the most widely used tool for the selective capture and labeling of microorganisms. Three categories of antibodies are used in immunoassays: polyclonal, monoclonal, and engineered antibody fragments. Polyclonal antibodies (pabs) are produced in vivo and consist of a suite of antibodies that bind to several epitopes on the antigen. Monoclonal antibody (mabs) solutions are produced in vitro from hybridoma cell lines and consist of an identical, well-defined population of antibodies that bind to a single epitope.

**Carbohydrate-** Carbohydrates are a diverse class of biomolecules that play an important role in dictating pathogens, their toxin, recognition, and their attachment to human cells. For example, type I fimbriae on Enterobacteriaceae binds to mannose terminated glycoproteins, influenza virus binds to N-acetyl neuraminic acid, and rotavirus binds to galactose.

**Antimicrobial Peptides-** Similar to carbohydrates, AMPs exhibit a range of activity toward bacteria, viruses, and fungi and have been suggested for use in sensor arrays that incorporate multiple AMPs. AMPs targeting Gram-negative bacteria bind nonspecifically to the negatively charged lipopolysaccharide (LPS) of both pathogenic and nonpathogenic organisms, while AMPs targeting Gram-positive bacteria often target peptidoglycan precursors required for the synthesis of the bacterial cell wall.

**Aptamers:** Detection of biological threats, is a significant military and civilian challenge. Traditional analytical techniques for these targets are mainly based on immunological methods (Peruski and Peruski, 2003) such as conventional ELISA, immunomagnetic-electrochemiluminescence assays (Gatto-Menking *et al.*, 1995), or time-resolved fluorescence assays (Peruski *et al.*, 2002). However, in this particular field, aptamers can be of great advantage since all these methods are very dependent on the possibility of producing specific antisera for these toxic materials in animals. Aptamers due to their synthetic nature are independent of animals and they can be selected also for these toxic molecules. Aptamers specific for these particular targets, such as anthrax spores, cholera toxin, staphylococcal enterotoxin B, ricin, and abrin toxin, have been

selected in the past years (Bruno and Kiel, 1999, 2002; Kirby *et al.*, 2004; Tang *et al.*, 2007) and, by using these aptamers different detection systems have been developed.

Aptamers are single-stranded DNA or RNA ligands that can be selected for different targets starting from a huge library of molecules containing randomly created sequences (Tombelli *et al.*, 2005) or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. aptamers can be used for both basic research and clinical purposes as macromolecular drugs.

The selection process of aptamers is called systematic evolution of ligands by exponential enrichment (SELEX), first reported in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990). The SELEX process involves iterative cycles of selection and amplification starting from a large library of oligonucleotides with different sequences (generally 10<sup>15</sup> different structures). After the incubation with the specific target and the separation of the binding from the non-binding molecules, the oligonucleotides that are selected are amplified to create a new mixture enriched in those nucleic acid molecules having a higher affinity for the target. After several cycles of the selection process, the pool is enriched in the high-affinity sequences at the expense of the low-affinity binders.

The number of cycles required depends on the stringency conditions, but, once obtained and once the sequence is known, unlimited amounts of the aptamer can be easily achieved by chemical synthesis (Ngundi *et al.*, 2006). In addition to this aptamers can offer advantages over antibodies that make them very promising for analytical applications (O'Sullivan, 2002; Luzi *et al.*, 2003; You *et al.*, 2003).

## Recognition properties of aptamers

Conjugation of aptamers to either lipids or polymers such as polyethylene glycol improves their stability and distribution kinetics sufficient to produce therapeutic effects. The molecular recognition properties of aptamers are very similar to antibodies, which recognize a target with high affinity and specificity and in many cases effectively inhibit its function. Some of the best aptamers form complexes that have dissociation constants in the picomolar range, while many have dissociation constants that are similar to the antigen-binding fragment of antibodies. In terms of selectivity, aptamers can discriminate between very subtle structural differences, such as the presence or absence of a hydroxyl



group or structural enantiomers (mirror images that have an identical chemical composition) of the target. Due to their relatively small size compared with antibodies, aptamers can fit into clefts where bulky molecules such as antibodies would otherwise be excluded. Their flexibility allows them to fold and assume the shape of relatively small binding pockets, thereby maximizing surface contact with the target protein. These desirable properties of aptamers, combining the optimal characteristics of small molecules and antibodies, show great promise and have opened avenues for the development of therapeutic, antiviral, diagnostic, and targeted drug delivery tools in areas that have been hitherto refractory to other approaches.

### Advantages of aptamers

1. The main advantage is the overcoming of the use of animals or cell lines for the production of the molecules.
2. Antibodies against molecules that are not immunogenic are difficult to generate, toxins and molecules that do not elicit a good immune response and are not suitable targets for immunotherapy can be used as targets for the generation of high-affinity aptamers. Furthermore, aptamers are isolated by in vitro methods that are independent of animals: an in vitro combinatorial library can be generated against any target.
3. In addition, the generation of antibodies in vivo means; the animal immune system selects the sites on the target protein to which the antibodies bind. The in vivo parameters restrict the identification of antibodies that can recognize targets only under physiological conditions limiting the extension to which the antibodies can be functionalized and applied.
4. Moreover, the aptamer selection process can be manipulated to obtain aptamers that bind a specific region of the target and with specific binding properties in different binding conditions.
5. After selection, aptamers are produced by chemical synthesis and purified to a very high degree by eliminating the batch-to-batch variation found when using antibodies. By chemical synthesis, modifications in the aptamer can be introduced enhancing the stability, affinity, and specificity of the molecules. Often the kinetic parameters of aptamer–target complex can be changed for higher affinity or specificity.
6. Another advantage over antibodies can be seen in the higher temperature

stability of aptamers; in fact, antibodies are large proteins sensitive to temperature and can undergo irreversible denaturation. On the contrary, aptamers are very stable and can recover their native active conformation after denaturation.

### **Limitations of aptamers**

The primary limitation on the use of aptamers (mainly RNA aptamers) in bioanalytical methods has been their nuclease sensitivity which is very critical for their use in ex-vivo and in-vivo applications (Famulok et al., 2000). However, it has been shown that the stability of such molecules can be improved by chemical modification of the ribose ring at the 2'-position (Pieken et al., 1991). A different approach to stabilize aptamers comes from a selection of RNA aptamers binding to stereoisomers of an intended target molecule, followed by chemical synthesis of the mirror-image of the selected sequences. As a consequence of molecular symmetry, the mirror-image aptamer (L-ribose) binds to the natural target molecule. Because of the substitution of the natural D-ribose with L-ribose, the mirror-image aptamer is stable (Klussmann et al., 1996).

**Nanomaterials-** Three different groups of nanomaterials are raw materials, nanostructured materials, and materials such as nanotubes and fullerenes. The raw materials include nanoparticles and nanocrystalline materials which are more effective than bulk materials. Nanostructured materials are quantum dots and dendrimers. Nanotubes and fullerenes are 100 times stronger than steel, more conductive than copper, and have several medical applications. The most commonly used nanomaterials are fullerenes, nanotubes, buckyballs, quantum dots, dendrimers, nanoshells, etc., when nanomaterials compared to the macro materials are found stronger, lighter, more electrically conductive, more porous, and less corrosive than bulk materials. They can even change color viz., gold can appear red, blue, or gold depending on their size. Inorganic nonmaterial can detect electrical changes in biological molecules and help in detecting or treating a disease.

**Bucky balls-** They are pure carbon molecules composed of 60-80 atoms of carbon. Because a fullerene takes a shape similar to a soccer ball or a geodesic dome, it is sometimes referred to as Fullerene after the inventor of the geodesic dome, Buckminster Fuller. In the buckyball, each carbon atom is bonded to three of its neighbors. They are

mainly used in the delivery of medicine or radioactive material to a disease site.

**Nanotubes-** Nanotubes are essentially Buckey balls that have been opened on two sides with additional atom groups added in the characteristic hexagon shape to form a hollow carbon tube (cylinder). They are related to other forms of carbons such as graphite and diamonds. They are sheets of graphite rolled into a cylinder. Nanotubes are also called buckytubes.

**Quantum dots-** Among various nanomaterials, quantum dots (QDs) are nanoscale fluorescent semiconductor crystals with unique photochemical and photophysical properties. Their much greater brightness, rock-solid photostability, and unique capabilities for multiplexing, combined with their intrinsic symmetric and narrow emission bands, have made them far better substitutes for organic dyes in existing diagnostic assays. In biological applications, QDs have advantages over the traditional organic fluorophores due to their narrow, symmetric emission spectra while requiring only a single excitation wavelength to simultaneously resolve multiple photostable colors. The unique properties of QDs have enabled multiplexed imaging of cellular targets for studying cancer biology, multiphoton fluorescence studies for deep tissue imaging in live animals, and near-infrared imaging for sentinel lymph node (SLN) mapping at 1 cm tissue depth.

**Dendrimers (Polymers)-** These are synthetic three-dimensional man-made macromolecules formed using a nanoscale fabrication. The unique features of the dendritic architecture include a high degree of structural symmetry, intramolecular minimum value, and a well-defined number of terminal groups.

**Nanoshells-** Nanoshells are colloids that consist of a core of non-conducting material covered by a thin metallic shell. By varying the thickness of the metal shells, researchers can precisely tune the color of light to which the nanoshells respond. The infrared is suitable for whole blood immunoassay as it easily penetrates the whole blood well. When the antibody nanoshells particles are placed into a solution of whole blood containing the test molecules, it causes slight changes in the optical properties of the nanoshells. By monitoring the changes, it is possible to detect the slight concentration of antigens in the blood.



**Cantilevers-** Cantilevers are made of silicon nitride coated with gold on one surface are mechanical beams anchored at one end and free standing at the other, similar to a swimming pool diving board. The cantilever bends in response to the change in surface stress upon binding of the target molecule from body fluid such as serum. The bending can be measured both optically and electrically which can be scaled up to an array format with as many as hundreds of cells for simultaneous detection of multiple biomarkers requiring minimal clinical samples.

### **Applications-**

**1. Disease diagnosis:** Nanotechnology can be used in creating cheaper, faster, and more precise diagnostic tools. Nanotechnology can improve the quality of images produced by the ultrasound machine. Nanoparticles injected into the breast can help doctors to detect cancers at a very initial stage. Nanotechnology-based on gold nanoparticles and DNA can detect prostate-specific antigens (PSA) in the blood when present at extremely low levels. This method could be used to detect prostate cancer and to be used to monitor prostate cancer patients following surgery. In the conventional immunoassay, whole blood cannot be used as it is so viscous and gloomy that it interferes with the chemical reaction in the test. By adopting nanotechnology, researchers have made it possible to test whole blood by using optically active gold-coated glass particles commonly known as gold nanoshells. The nanoshell immunoassay can detect less than one billionth of a gram of IgG in 1 ml of whole blood in 30 minutes.

Future blood tests may use tiny bar codes to speed up disease diagnosis like scanning a bar code of a grocery item. Unique DNA tags called bio-bar-codes can be used to detect the disease markers. The tags can be scanned by an instrument to identify diseases starting from Alzheimer's disease to bio-terror agents such as anthrax, Ebola, Marburg, or smallpox. The test is easier, faster, more accurate, and less expensive than PCR. The new test called bio-bar code amplification (BCA) could be ready shortly and a drop of blood is enough to screen the patient against several diseases.

**2. Treatment-** The nanoshells with a targeted agent are injected into all animals and after a week animal's body are illuminated with infrared to raise the cell temperature to about 55°C to activate cancer-killing agents to destroy the tumor. The smart

superparamagnetic nanoparticles made up of iron oxides injected into the bloodstream target tumor receptor cells when subjected to a magnetic field by emitting an attached drug. Quantum dots may also be injected into the bloodstream of animals and upon stimulation with light capable enough to kill the cancerous cell. Nucleic acid engineered probes and methods offer powerful new ways to deliver therapeutics on preventive treatment for a particular disease. The major challenge is to develop a non-viral DNA delivery system that has low toxicity and cost but a high level of efficiency and specificity.

**3. Identity preservation-** The identity preservation (IP) system is a system that provides consumers with information about the practices and activities used to produce an agricultural product. Quality assurance of the safety and security of agricultural products could be significantly improved through IP at the nanoscale level. Nanoscale IP has the potential to continuously track and record the history of a particular agricultural product. The keys are biodegradable sensors for temperature and other stored data to track all stages in the life of the product including the birth of the animal, its medical history, the slaughterhouse, meatpacking plant, right through to the consumer's table.

**4. Animal breeding-** The management of breeding is an expensive and time-consuming problem in the dairy and swine industry. The nanotube implanted under the skin will provide information about the level of estradiol in the blood during estrous in animals by near-infrared fluorescence. The signal from this sensor will be incorporated in a central monitoring and control system to inseminate the animals for improving the conception rate/breeding performance.

**5. Drug delivery-** Nanomaterials such as bucky balls and dendrimers can be used in drug delivery systems. Bucky balls are inert, non-toxic perfectly smooth and can interact easily with cells, proteins and viruses. additionally, they are hollow inside where drugs can be put so that they can release the drugs inside the cells. Dendrimers are synthetic polymers in various predetermined sizes and can be used as a delivery vehicle as they can hold a drug inside. They can enter cells very easily and release drugs right on target. They do not trigger an immune response and execute a five-step task while dealing with the treatment of tumors (1) dendrimers may be able to find tumor cells in the body by looking for tumor receptors (2) bind and pass through the cell membrane (3) perform chemical

analysis to know the type of tumor (4) release chemotherapy or radioactive agents inside the cells (5) confirmation of the death of tumor cells by chemical analysis. Besides, targeting tumor cells the drug delivery systems, dendrimers showed promising results as tools in MRI and gene transfer techniques. Dendrimer-based nanocomposites are being studied as possible anti-microbial agents against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli*. Nanotechnology has also entered the field of vaccinology. Synthetic oligonucleotides and antigens in bio-degradable nanospheres can be used as an alternative approach for immunization. a better immune response seems to be obtained with biodegradable nanospheres than with vaccines produced by conventional methods. Nowadays antibiotics, probiotics, and pharmaceuticals are delivered to animals primarily through feed or injection. The medicine is delivered as a preventive measure or as a treatment once the disease organism has multiplied and symptoms are evident. Nanoscale devices can detect and treat an infection, nutrient deficiency, or other health problems long before symptoms are evident at the macroscale. This type of treatment could be targeted to the affected area and have multifunctional characteristics viz., time-controlled, spatially targeted, self-regulated, remotely regulated, and pre-programmed. Smart delivery systems can also have the capacity to monitor the effects of the delivery of pharmaceuticals, nutraceuticals, nutrients, food supplements, bioactive compounds, probiotics, chemicals, and vaccines.

**Toxicity-** The application of nanotechnology in different fields is not free from drawbacks. The particle size and surface are important characteristics when considering the toxicity of a material. as the size of the particle decreases, the surface area increases exponentially which allows for more potentially reactive groups to interact with the environment on the surface. It has been well established fine particles in the air can increase morbidity and mortality from pulmonary and cardiovascular diseases with long and short-term effects. For example, exposure of human keratinocytes to carbon nanotubes was associated with oxidative stress and apoptosis. However, not all nanotubes are composed of the same functional groups, and nanomaterials with the appropriate coating will have minimal toxicity. On the other hand, when injecting nanomaterials into humans as contrast agents, therapeutic carriers, or sensors, one has to consider the rate of clearance. Iron oxide nanoparticles, for example, have been used as contrast agents and can be ingested by

living cells, and the biodegradation of the particles results in free iron that can be incorporated into Hb and the body is free of residues of iron oxide nanoparticles after months. Quantum dots encapsulated with the best protective shells will slowly break down in the body and eventually expose the core and release toxic iron.



## Successful treatment of a Wild Makhna Elephant in Kaziranga National Park

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**I**t was reported to me on 30-09-12 that a wild Makhna elephant was lying down in a pool of water in a rivulet of Brahmaputra River in 6<sup>th</sup> addition of Kaziranga National park opposite Silghat, by the forest officials of Northern range. A team comprising of range officer, me and animal keeper named Bikh Bahadur along with other forest staffs went on to check the Elephant. To reach the site we had to come to Bhomuraguri in Tezpur and from there had to take a mechanised boat in the main stream of Brahmaputra river and a country boat to reach the exact location in the rivulet. We checked the elephant on that day itself, it was a makhna around 40 years of age and 9 feet in height. It was lying in lateral recumbency position and unable to get up. According to the fisherman who had first reported the case to the forest staffs it was lying there for two days. They were providing it with banana stems and bananas.



**Injured Makhna elephant in a pool of Water**  
**Photo: Dr. Jahan ahmed**

after checking the elephant, I decided to administer it with pain relievers (Melonex 30 ml), multivitamins (Tribivet, 30ml), antibiotics (Fortiver, 30 ml) and antihistaminic (Chloril, 30 ml) intra muscularly. It was decided that me and my animal keeper would be camping near the elephant in a ship named Unicorn, which was a patrolling ship of Kaziranga National Park involved in anti poaching activities in the Brahmaputra river and its banks. It was a double decker ship with 4 rooms having a bunker bed where two persons can sleep. It also had a deck with railings where people can sit having thatched roof on top. From 1<sup>st</sup> of October 2012, treatment to the elephant was provided twice daily, once in the morning and once in the evening. as I had carried limited stock of medicines, some more additional supplies along with few new drugs were brought from Tezpur, which was the nearest town by local villagers who were constantly providing feeds like banana stems, banana, elephant apple etc to the elephant.



**Unicorn the ship where we stayed during the treatment of the elephant**  
**Photo : Birkhe Bahadur**

The elephant was lying down in a pool of water, adjoining a rivulet in the Tintikia chapori, of Panpur Reserve Forest, Northern range, Kaziranga National Park, it had strength in its three legs but the left hind leg was completely immobile, and was buried in the sand.

The elephant could not be tranquilized for treatment because half of the elephant was submerged in water in lateral recumbency position.

Ideally in such situation we lift the elephant with the help of JCB or hydra vehicle and provide treatment but in this case, it was not applicable due to two reasons, firstly as the area was inaccessible by road there was no possibility to bring a JCB or Hydra vehicle and secondly as the elephant was wild it would be difficult to restrain it.



**Injured elephant being administered medicines**  
**Photo: Birkhe Bahadur**



**The elephant being continuously administered medicines**  
**Photo : Birkhe Bahadur**

After relentless effort on 2nd oct, 12 night, after administering the timely dose of the medicines namely antibiotics, pain killers, multivitamins, steroids, at 10.00 pm, we noticed

the elephant getting up on its legs, the injured left hind leg still didn't had the strength to support its body weight so it fell down again with tremendous force, we could also notice that the left hind leg was bent outward an abnormal position, it seemed a case slipping out of hand, so we returned back to the ship where we had been staying for the past three days. I decided to administer medicines at 8 hourly interval beginning at 6 aM in the morning, 2 PM and 10 PM at night. Though it was risky to approach the elephant at night in the watery location, there was no alternative. Intra venous fluid couldn't be administered as approaching it from front was difficult.



**The elephant was administered with required medicines at night also**  
**Photo : Birkhe Bahadur**

After continuing the treatment for two days, it was observed that the elephant was gaining strength and was able to lift its body but couldn't stand up. Hind limbs didn't had strength to support the weight. It had no strength to get up completely and would fall down into the water





**Approaching the elephant at night for administering the injectable medicines**  
**Photo :Bírkhe Bahadur**



**Improvement was observed in the condition of the elephant as it was able to lift its anterior body region.**  
**Photo : Dr. Jahan ahmed**

On 4<sup>th</sup> October night after administering the regular dose of medicines to the elephant, it got up on its legs and looked at us in a charging motion but couldn't move and fell down again making a huge splash of water. We run towards the ship in darkness. Had it charged we were left with no option than to jump in the Brahmaputra river which still was flooded.

On 5<sup>th</sup>, at 3 aM there was an earth quake and there was loud splashing of water on the shores and our ship was moving with the waves created due to the earthquake. I got up from my bed and was terrified thinking poachers may have attacked us poaching of rhinos for the horn was at peak during those days. Lal Das, a contractual boatman who had been continuously working and helping me in the treatment of the elephant asked me not worry and told me that it was an earthquake. I had never experienced an earthquake in water or a ship before and was terribly shocked.



**The elephant was able to put pressure on t fore legs but couldn't get up as hind legs were not able to bear weight.**

**Photo: Dr. Jahan ahmed**

At 5 aM when we went to check the elephant and administer the medicines we saw foot marks of a small herd of elephant not more than 7-10 individuals in the nearby sandy areas. We were surprised to see that the sick elephant was not there in the area. We were

perplexed at not finding the elephant. Local Member of assembly (MLa) was to visit the area in the morning, what would we say. Few fishermen had come to check their fishing nets in country boats. We borrowed a country boat and set sail into the rivulet following the foot marks of the wild herd of elephants and after moving for a distance of 700 meters we could see a lone elephant moving slowly in the water towards the forest. We had doubted that it may be the same elephant that we were treating. We moved cautiously towards the elephant without making any noise as it might disturb it and we may be charged upon by the elephant. We didn't have a binocular to see from a distance, so had to approach it from a close distance. We anchored our boat at an approximate distance of 200 meters after confirming that it was the same elephant that we were treating. We could confirm that it was the same elephant from the pinkish white triangular spot at base of the tail on the left side of body. We observed the elephant walk slowly in the stream for 20 minutes, the stream was almost straight so we could watch the elephant easily after which there was horse shaped curve so we had to sail our boat again to observe it. We observed for 2 hours till 7.30 aM for a distance of more than 2 kms approximately when the elephant got up from the water into the forest and walked into the woods.



**The elephant walking slowly in the stream into the forest. It was recognized from the triangular mark in the sacro coccygeal region on the left side.  
Photo: Dr. Jahan ahmed**

It was moving normally without limping or abnormal movement. We also saw a huge lone tusker in musth nearby which made us decide not to delve further in pursuit of the

elephant. For some reason, I forgot to carry my camera so had to take some blurry photos with my Nokia phone.

We came back to the location where the elephant was treated to observe for any signs that might indicate that the wild herd might have helped it get up on its feet. The herd came exactly to the location where the elephant was lying down. We felt that while the sick elephant tried to get up, it was helped by the wild herd members to stand up. as the lone elephant walked slowly, the wild herd moved ahead of it.



**Foot marks of wild elephants near the area where the lone elephant was found and treated**

**Photo: Dr. Jahan ahmed**

Forest staffs had monitored the elephant for more than a month in the Panpur area of 6<sup>th</sup> addition of Kaziranga National Park. After six days of relentless work we were happy and satisfied that the elephant was able to get up and walk back to the forest on its own.

I would like to acknowledge the help and support provided by Kaziranga National park authorities, Wildlife Trust of India, Tierpark Hagenbeck Zoo, Boatman Lal, animal keeper Birkhe Bahadur for providing support and logistics during the treatment of the elephant. My seniors Help and support provided by Mr. Sunil Kyarong, Dr. Rathin Barman and Dr. Bhaskar Choudhury of Wildlife Trsut of India in attending the case is duly acknowledged.



## Milk to Man

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### Introduction

Milk is the mammary secretion obtained from various farm animals for direct consumption or as processed form by human to get nutrients through it. Raw milk is defined as temperature of milk when less than 40°C without any heat treatment which can harbor zoonotic pathogens and toxins even though it is having some beneficial bacteria, prevention of lactose intolerance. India with the world's largest milk producer with 198.7 million tons of milk produced in 2019-2020 with the growth rate of 5.68% and per capita availability 406 grams per day against ICMR recommendations of 280 grams per day. According to EFSA, the major milk transmitting pathogens in milk especially during mastitis were *E. coli*, *Campylobacter* spp., *Salmonella* spp., *Bacillus cereus*, *Brucella abortus*, *Brucella melitensis*, *Listeria*, *Mycobacterium*, *Staphylococcus*, *streptococcus* (EFSA, 2015) and parasites *Toxoplasma gondii*, *Cryptosporidium parvum*. Young children, the elderly, pregnant women, immunocompromised (YOPI) people, and those who are ill should be aware of the dangers of drinking raw milk and raw milk products (Brom *et al.*, 2020)

### Common milk organisms and diseases

Biggest concern with the dairy farms and storage facilities developed in India lies with the milk safety and milk quality where the contamination occurs at any stage of milk handling from animal to human consumption. Major aspects lie with milk safety is milker's hygiene, animal hygiene, environmental hygiene, milk handling and chemical residue in milk.

Singh *et al.* (2020) conducted a study in Punjab with about 300 farmers and found out that 24% of milk samples have contamination with *E. coli* samples, 5% samples with water adulteration and 3% samples with urea adulteration. Bhatt *et al.* (2011) in his study in Gujarat by milk metagenomic profiling by pyrosequencing of Kankrej, Gir and crossbred cattle found that mastitis milk have high *enterobacteriales* predominance followed by *pseudomonadales*, *bacillales*, *lactobacillales*. The most common bacteria detected in Kankrej and Gir cattle were

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*Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas medica*, *Shigella flexneri* and *Bacillus cereus*. In crossbred cattle, *Staphylococcus aureus* was found first, followed by *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and *E. coli*. Joseph and Kalyanikutty (2021) examined 42 samples of raw milk from household vendors and cooperative milk marketing agencies and found prevalence of Shiga-toxigenic *E. coli* (STEC) with multidrug resistance genes against penicillin, cefalexine, rifampicin, methicillin and novobiocin with potential public health threats like hemorrhagic colitis, hemolytic uremic syndrome.

*E. coli* also a gram-negative bacterium mostly by fecal contamination due to poor hygiene causes diarrhea mostly young ones, hemorrhagic colitis, hemolytic uremic syndrome and in extreme cases seems fatal. These are usually shed in ruminant faeces, which the animal defaecating while milking is major concern of pathogen transmission through milk (Martín and Beutín, 2011). Coliform are more susceptible at high temperature short time pasteurization (72°C for 15 seconds)

*Staphylococcus aureus* an important opportunistic pathogen which is a most common pathogen for mastitis, causing major economic loss to farmers. *Staphylococcus* are most common in skin commensals cause endotoxin release that affect humans by causing food poison with symptoms of vomiting, diarrhea within 2-6 hours and some staphylococci are becoming resistance to antibiotics called MSRa that pose risk to consumers due to over usage of antimicrobials.

*Campylobacter*, gram-negative bacteria mostly affect intestinal pathway leads to abdominal cramps, discomfort, diarrhea, fever and bloody stools with severe cases leads to nervous damage. They are environmental pathogen contaminated by faeces or direct excretion into milk. Effective pasteurization can control these microbes

*Bacillus* and *Clostridium* spp are environmental pathogens contaminate the milk by spore release, silage, feces, poor floor hygiene cause diarrhea by toxin release. *Clostridium* grown under anaerobic condition release two major type of toxin called neurotoxin, enterotoxin. Most famous zoonotic pathogen of milk transmission that can withstand high temperature and nowadays using as an indicator organism for milk pasteurization is *Coxiella burnetii* which cause Q-fever can infect wide range of species like ruminants, human leads to abortion. Some other zoonotic pathogens that excreted through milk is *Mycobacterium* spp. and Brucellosis which should be

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vaccinated at early ages of life to prevent them infection to humans through milk and screening for tuberculosis, brucellosis to be done for atleast once in year and the animal should be culled if presented positive. Tuberculosis can be screened by intradermal test and brucellosis by plate or tube agglutination test.

Brockmann *et al.*, 2016 in Germany encountered two human cases of tick born encephalitis transmitted by unpasteurized goat milk and cheese which was confirmed by serology analysis. TBE is caused by RNA virus of flavivirus genus which is transmitted by tick bite, shed through the milk and taken by oral route by humans.

Arena *et al.*, 2021 developed a bovine coronavirus immune milk (BIM) which confers passive immunity against SARS-CoV-2 infection as an immunostimulant therapy, that activate the intestinal immune system. This is possible due to the similar phylogenetic relationship between COVID-19 and BCoV viruses.

## Conclusion

Major ill effects that transfer through milk comes from consumption of raw milk that too under unhygienic and under maintenance of environment, transport, processing and dispatch of milk. Hence, good farming and clean milk production, cold chain maintenance helps to reduce the environmental bacterial contamination. Recommendation of non-intake of raw milk especially by infants, pregnant women, elderly patients, immunocompromised persons.

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*Review article*

## Staphylococcal Food Intoxication: A Review

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### *Abstract*

Food poisoning is a serious problem all around the world. Many types of food poisoning are caused by bacterial infections or sometimes food intoxication. Among the predominant bacteria involved in the diseases, *Staphylococcus aureus* food poisoning is an intoxication caused by ingestion of food containing staphylococcal enterotoxin (SE). It is characterized by an acute onset of nausea, vomiting, abdominal cramps and diarrhea, and is one of the most common food-borne diseases in the world. Because of their stability of enterotoxins at high temperatures (100°C for 1 h) in *Staphylococcus aureus*, have been identified as a possible biological risk and ability to incapacitate individuals for several days to two weeks. Here, a brief review of Staphylococcal food intoxication is given.

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**Keywords:** *Staphylococcus aureus*, food intoxication, enterotoxins.

### **Introduction**

*Staphylococcus aureus* is a facultative anaerobic gram-positive coccus, non-motile, catalase, and coagulase-positive of the micrococcaceae family. It is not only a commensal colonizer but can also cause serious infections, toxinoses, and life-threatening diseases, likewise skin and tissue infections, toxic shock syndrome, septicemia, and food intoxication (1). Staphylococcal food poisoning is among the most common types of food poisoning globally, and it is caused by the ingestion of staphylococcal enterotoxins, which produces constant vomiting, diarrhea, and stomach pains. due to the hot and humid climate of India, infection rates are higher. (2). As staphylococcal colonization and infection are widely spread, contamination of foodstuff by food handlers may represent a major source of staphylococcal food intoxication. as Staphylococcal food intoxication isolates are difficult to obtain, to date, there is only very limited

information on the source of enterotoxigenic *S. aureus* strains that lead to cases of food poisoning (3).

### Morphology of staphylococcus aureus

*Staphylococcus aureus* is unique with many typical phenotypic characteristics as rigid cell due to high content of teichoic acid helping in tolerance to drying, high salt, and sucrose concentration enabling it to grow at a temperature range of 15 to 45 °C and sodium chloride concentrations as high as 15%. They develop in grape bunches, groups, pairs, and sometimes short chains that grow by aerobic respiration or fermentation, producing lactic acid in the process. However, the organism *S. aureus* was named so because of the production of golden-colored colonies but the production of variable pigments viz. pale yellow, mustard, and white by this organism has also been reported.



**Figure 1:** Mannitol salt agar used for the isolation of *Staphylococcus aureus*.

### Pathogenesis of *S. aureus* infections

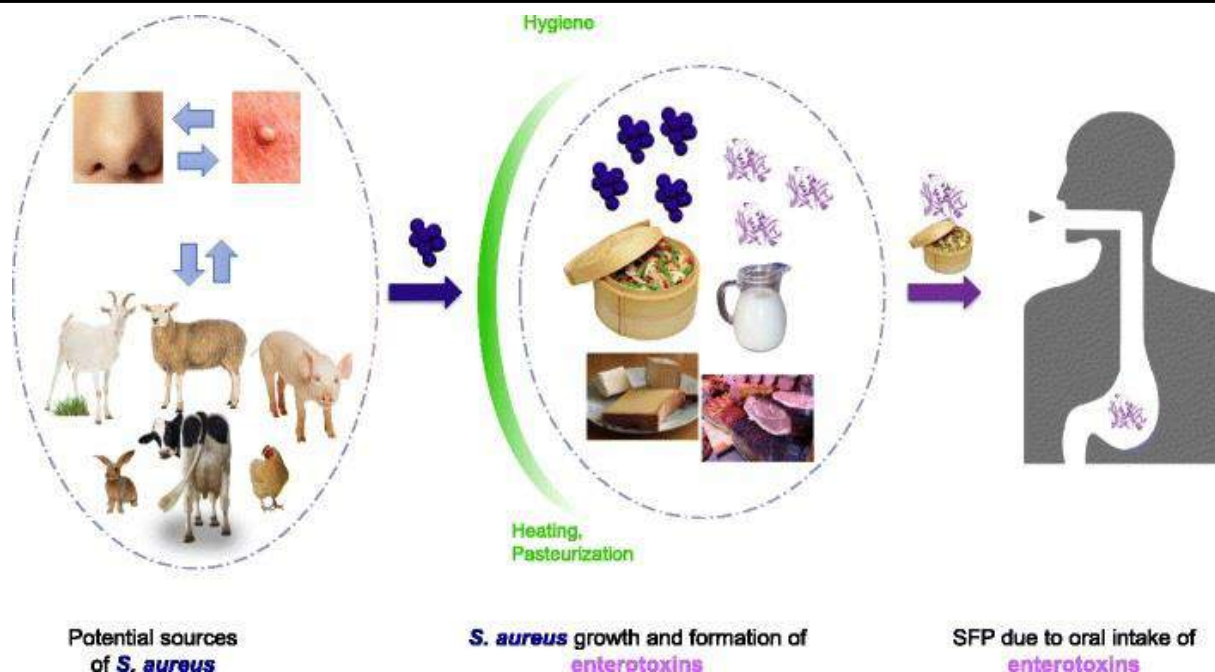
Toxins, adhesion, biofilm development, and antibiotic resistance are all factors that contribute to *S. aureus* pathogenicity. *S. aureus* is commonly found in hospital-acquired and community-acquired infections. It is found in the normal flora of humans and has the potential to become harmful. as a result, life-threatening disorders such as pneumonia, meningitis, endocarditis, septicemia, mastitis, phlebitis, urinary tract infections, osteomyelitis, and endocarditis have been reported (4,5).

**Physical symptoms:** The appearance of symptoms of food poisoning depends upon the quantity, type, and toxicity of the toxin. The gastrointestinal symptoms typically appear after 1-6 hours whereas the other effects may appear after a long time like neurological and

hematological effects. Symptoms can range from mild, moderate to severe and include abdominal cramps, nausea, vomiting, diarrhea, fever, and dehydration and in severe cases, death may be the result. Like any other organism, *S.aureus* needs temperature, moisture, nutrients, and time to grow. The presence or absence of oxygen, salt, sugar, acidity, and microflora in the environment are other important factors for the growth of bacteria in the gastrointestinal tract. In the appropriate conditions, one bacterium may multiply by binary fission to become four million in eight hours. Because bacteria and toxins cannot be smelled or seen with the naked eye, adopting food hygiene recommendations is the best approach to assure wholesome food. (6).

**Biological symptoms:** Staphylococcal food poisoning is defined by a characteristic set of histological abnormalities in the gastrointestinal tract, chief among them being neutrophil infiltration and blood accumulation in the epitheliums and basement membranes of the stomach, the upper part of the small intestine, and jejunum. In the lumen of the duodenum, oozing of mucus and pus is also noted (6).

**Incriminate food:** Incriminating Food is frequently causing staphylococcal food poisoning mainly including meat and meat products; poultry and egg products; chicken; bakery products such as cream-filled pastries and chocolate; sandwich fillings; and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning. Staphylococci bacteria can be found in a variety of places, including the air, dust, sewage, water, milk, milk products, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. They're found in 50 percent or more of healthy people's nasal passages and throats, as well as on their hair and skin. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment, and environmental surfaces can also be sources of contamination with *S. aureus*. Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough 7.2°C, 45°F or below (7).



**Figure 2:** Human and other food-producing animals colonized or infected with *S. aureus* represent sources that can introduce enterotoxigenic *S. aureus* to the food matrix.

### Clinical prevention of staphylococcal infection

Staphylococcus infection is mainly spread as a nosocomial infection through nasal secretions etc, consumption of contaminated foods, and through healthy carriers. Though there is no 100% prevention for a Staphylococcus infection several easy steps can be taken to lower the risk of a “minor infection” or “minor cut” from becoming a “major infection.”

This include:

- Wash hands and wounds with soap and water after treating a “minor wound.” The proper washing technique is to scrub aggressively for 30 seconds or more. anti-bacterial soaps offer no more cleaning power than other soaps. The time spent washing is more important. Wash hands after treating another person’s wound.
- After coming into contact with your wound or damage, you must dry your hands with towels or napkins.
- Disposable gloves should be worn when treating another individual to prevent colonization from spreading from the caregiver to the patient and vice versa.
- Bed linens and clothing should be changed and washed if wounds are oozing through protective bandages.



- Treatment areas, such as sinks and countertops should be cleaned immediately after use.
- The patient's environment should be cleaned routinely and when soiled with body fluids.
- Notify physicians and other healthcare personnel who may care for the affected individual that they may be infected with antimicrobial-resistant bacteria.
- Avoid contact with another person's wounds or any material that may have been contaminated from the wound.
- Hospital kitchens should be regularly checked for the presence of *Staphylococcus aureus*.

## Conclusion

Foodborne illness caused by *S. aureus* is a major problem across the world. Predictions of *S. aureus* growth and SE production might be useful tools for assessing microbial risk in the food manufacturing industries. Several factors influence *S. aureus* growth and enterotoxin formation in foods, and further research is required to integrate such prediction tools. Proper food preservation can reduce the likelihood of food illness. The temperature of the bacteria on our food that might cause illness is normally managed by heating (cooking) and/or cooling (refrigerating) it. While reusing the refrigerated food, one should heat it properly it needs to be restored or eaten after some time. Not only this, but the quantity of bacteria also rises due to bacterial multiplication. They may cross-contaminate other foods – especially cooked and ready-to-eat foods. Good kitchen and personal hygiene practices as well-cooked foods are important to help control the consumption of contaminated foods and hence food poisoning. a considerable research effort is still required for a better understanding of the interactions between *S. aureus* and the toxin production and of the mechanisms of SE production in a variety of food. Research is also necessary for the examiner of new SEs and of new enterotoxigenic staphylococci. Much effort is being put into developing better and more sensitive ways for detecting SE in foods. Taken together, these studies should lead to better control and a subsequent reduction of staphylococcal food poisoning outbreaks.

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## Urea molasses mineral blocks as a feed supplement

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### Abstract

The principal forages used in India for livestock feeding are low in nitrogen, minerals and vitamins resulting in low productivity. To overcome this strategic nutrient supplementation is essential in this scenario to improve the use of poor quality roughage. The Urea Molasses Mineral Block (UMMB) was developed at NDDDB and supplementation of UMMB can show promising effects on productivity of animals. UMMB supplementation significantly increases feed intake, milk yield and growth rate and is therefore a cost-effective. UMMB provides fermentable nitrogen, energy and minerals necessary for optimum microbial growth. Molasses is noted for its sugar content and urea is a non-protein nitrogen compound. Most urea contains about 46% nitrogen and protein contains 16% nitrogen: crude protein equivalent value of urea is about 281%. Feed UMMB to ruminants only and do not feed to monogastric and pre-gastric animals. The number of blocks fed to sheep and goats should be limited to 100 grams/day while for cattle it should be limited to 500-700 grams/day.

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### Introduction

Most people in India live in rural areas and their livelihoods are based on the production of crops and livestock. The production of livestock is an integral part of farming systems. The principal forages used in India for feeding livestock are crop residues and dry grasses. These crop residues are low in nitrogen, minerals and vitamins but high in fibre and lignin, limiting animal intake and digestibility: hence low productivity. The main limiting factors for animal productivity in the nation are poor nutrition and poor feed availability. as a result, animal performance is often sub-optimal, which is reflected in sluggish growth, delayed maturation, longer inter-calving periods and poor milk yield. Strategic nutrient supplementation is essential in this scenario to improve the use of poor quality roughage. Considering the availability and price of concentrate mixture, poor farmers can hardly afford them. To overcome this limitation, animal nutritionists across the world have shown that the nutritional value of crop residues can be enhanced if it is complemented by deficient nutrients (Makkar, 2002).

The Urea Molasses Mineral Block (UMMB) was developed at NDDDB (1983) and has been further enhanced at IVRI (Garg *et al.*, 1998). Supplementation of Urea Molasses Mineral Block (UMMB) can show promising effects on improving the use of nutrients and also the productivity of animals (Prasad *et al.*, 2001). Urea Molasses Mineral Block (UMMB) supplementation can increase fibrous feed digestibility by up to 20 percent, increase the nutrients the animal receives, and increase feed intake by 25 to 30 percent (ESGPIP, 2007). UMMB can be fed throughout the year, but during the dry season or when the animals graze low-quality fodder, they are more beneficial. Liquid supplements based on molasses with added nitrogen, minerals and vitamins have recently been shown to improve growth rate and reproductive performance in cattle as another approach to increasing nutrient utilisation. The benefits for feeding UMMB are:

- a) Safe and uniform micronutrient delivery vehicle
- b) Safe delivery Vehicle for NPN
- c) Increase NPN utilisation
- d) Vehicle for delivery of molasses or fermentable rumen carbohydrates to rumen microbes
- e) Reduce dustiness of concentrate feed
- f) Reduce feed sorting
- g) Increase the feed intake as a whole and enhanced palatability.

## Composition

UMMB is made from various ingredients, each of which in the mixture has its own contribution. In general, it consists of Molasses, Urea, Mineral mixture, Salt, Calcite powder, Bentonite, Cottonseed meal, de-oiled mahua seed cake, Wheat bran and crushed maize. Composition of Urea Molasses Mineral Block is given below. The composition may vary according to availability of feed ingredients.

Ingredients	Example I	Example II
<b>Molasses</b>	45 %	40 %
<b>Urea</b>	15 %	5 %
<b>Mineral mixture</b>	15 %	3 %
<b>Salt</b>	8 %	2 %
<b>Calcite powder</b>	4 %	-

<b>Bentonite</b>	3 %	-
<b>Cotton seed meal</b>	10 %	-
<b>De-oiled mahua seed cake</b>	-	10 %
<b>Wheat bran</b>	-	20 %
<b>Crushed maize</b>	-	20 %

## Benefits

Urea-molasses-mineral block (UMMB) is a strategic feed supplement for ruminants that promotes the growth of rumen microbes throughout the day with a constant source of fermentable nitrogen. UMMB supplementation significantly increases feed intake, milk yield and growth rate and is therefore a cost-effective approach to maximising the use of locally available feed resources for increased productivity. UMMB provides fermentable nitrogen, energy and minerals intermittently through licking, which is necessary for optimum microbial growth. Microbial protein can add 30-40 % of an animal's crude protein requirement. UMMB supplementation in the ration is quite beneficial, especially when fed crop-residue-based diets, as ruminants can produce microbial protein from non-protein nitrogen.

Molasses is noted for its sugar content and sugars usually contribute 60-65% of the solids in sugarcane molasses. Increasing the rate of carbohydrate fermentation could result in more effective capture of rumen degradable protein and improved supply of metabolizable protein to the dairy animals. Urea is a non-protein nitrogen compound. That is, the nitrogen portion of urea is used as the building block for the production of protein by rumen microbes. Most urea contains about 46% nitrogen, and protein contains 16% nitrogen. Therefore, when urea is converted to protein, the crude protein equivalent value of urea is about 281%. Urea contains no other useful feed components such as energy, minerals, or vitamins. Ruminants convert urea to protein via ammonia and carbon dioxide production. The ammonia released from urea is either used for microbial protein production or is detoxified and excreted in the urine.

## Precautions while supplementing UMMB

- Feed to ruminants only (sheep, goats and cattle).
- Do not feed to monogastric animals i.e. horses, donkeys, or pigs.
- Do not feed to young ruminants less than six months of age (kids, lambs).



- Blocks should be used as a supplement and not as the basic ration.
- A minimum of coarse forage in the rumen is essential.
- Never give blocks to an emaciated animal with an empty stomach. There is the risk of poisoning due to excessive consumption.
- The amount of blocks fed to sheep and goats should be limited to 100 grams/day while for cattle it should be limited to 500-700 grams/day.
- The blocks should never be supplied in ground form or dissolved in water as this can result in over consumption
- Supply sufficient amount of water ad lib.

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## Biopesticides Usage in India

Popular article

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### Abstract

Synthetic pesticides are the central pillar of insect pest control. The major threats such resistance, resurgence, environmental issues caused by these synthetic pesticides laid avenue for shifting towards usage of biopesticides as integral part of pest management strategies. Biopesticides mainly microbial biopesticides include entomopathogenic fungi, viruses, bacteria and nematodes. The effectiveness of these biopesticides is tested under laboratory conditions and also in field conditions with great success in combatting many insect pests causing economic losses. Registered biopesticides products are commercially available for the management of insect pest and diseases.

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### Introduction

In India, agriculture and its allied sectors are not only imperative for country's Gross Domestic Product but also serve as principal livelihood for an estimated 58.00% of its population. On farm yield losses from pests has been estimated to a magnitude of 10-30%. Currently, India ranks fourth in production of pesticides and the industry is expected to reach Rs.316 billion by 2024, with a compound annual growth rate of 8.10%. There are 293 pesticides registered in India of which 104 pesticides are still being produced or used in the country despite being prohibited in two or more nations elsewhere. In the interim, pesticides are withdrawn or restricted or banned due to variety of environmental, health concerns and insecticide resistance thus shifting towards development and promotion of non-chemical based pest management strategies, precisely the biopesticides. In India, only 12 different kinds of biopesticides under the Insecticide act of 1968 have been recorded. The main microbial biopesticides manufactured and used in India are entomopathogenic fungi, *Bacillus thuringiensis*, NPV and *Trichoderma*. Biopesticides occupy 4.20% of the Indian pesticide market. as of 2017, at least 15 microbial control agents have been developed as biopesticides in India with 970 commercial formulations registered. as of 2017, a total of 188 mycoinsecticides, 39 myconematicides, 51 bacterial insecticides and 27 nucleopolyhedrovirus products were registered through the CIBRC.

## Different biopesticides used in India

### 1. Entomopathogenic Fungi

These Entomopathogenic fungi belong to the divisions, deuteromycota, ascomycota, and Zygomycota. These entomopathogenic fungi being eco-friendly and bio-persistent, make all distinct stages of insect life cycle vulnerable. The most widely studied species for pest management include as follows.

#### i. *Beauveria* Genus

It causes White Muscardine disease. In India, at least 87 products based on *Beauveria bassiana* have been registered for use against a range of lepidopteran, coleopteran, and hemipteran pests on crops. The cadavers covered with white colour conidia. Commercial formulations are typically applied in the range  $10^7$ - $10^8$  conidia/ml and 400-750 L/ha, depending on the crop. It produces toxins namely beauvericin, bassianocide etc. *Beauveria* products have been used to reduce the menace of white grubs in Indian sugarcane. The related species, *B. brongniartii*, has emerged as a promising biocontrol agent in sugarcane.

#### ii. *Lecanicillium lecanii*

*Lecanicillium lecanii* is broadly distributed, which can cause huge epizootics. Over 60 products based on *L. lecanii* are manufactured in liquid and dry formulations in India for the management of aphids, scales and other soft-bodied sucking pests on a range of crops in both field and green house conditions.

#### iii. *Metarhizium* Genus

It causes Green Muscardine disease. The genus *Metarhizium* includes, *Metarhizium anisopliae*, *M. album*, and *M. flavoviride*. *M. anisopliae* is a potential entomopathogenic fungus. a total of more than 30 products based on *M. anisopliae* are used for control of both foliar and soil pests in India, especially in plantation crops and vegetable crops.

#### iv. *Nomuraea* Genus

*Nomuraea rileyi* is a potential insect-infecting fungus, is a dimorphic hyphomycete that can cause epizootic death in various insects. In India, natural epizootics of *N. rileyi* has been observed on lepidopteran pests including castor semilooper, *achaea janata*, cut worm *agrotis ipsilon*, gram podborer, *Helicoverpa armigera* and tobacco caterpillar *Spodoptera litura*.

#### v. *Paecilomyces* Genus

*Paecilomyces* is important biocontrol agent against whiteflies and causes “yellow muscardine” It is effective against *Bemisia* and *Trialeurodes* spp. both in field and greenhouse

conditions. The cadavers covered with grey or yellow conidia are by *Paecilomyces* spp. or in some cases, pink or pink-grey conidium is usually by *P. fumosoroseus*.

#### vi. *Hirsutella* Genus

*Hirsutella* includes three important species, *H. thompsonii*, *H. gigantea*, and *H. citriformis*. Mycohit, product based on isolate of *H. thompsonii* is used to reduce the menace of coconut mite, *aceria guerreronis*.

#### vii. *Isaria* Genus

Three *Isaria fumosorosea* products are sold for control of soft-bodied insect pest's viz., whiteflies, aphids, thrips, mites, leaf miners, plant bugs and several soil pests in food and ornamental crops.

### 2. Bacteria

Many species of bacteria are known to infect different varieties of insects, but those bacteria belonging to the genus *Bacillus* are most widely used as biopesticides. *Bacillus thuringiensis* is among the most successful and widely produced microbial control agents, against Lepidoptera, diptera, Coleoptera and Hymenoptera. The first formulation based on *B. thuringiensis* was developed under the name “*Sporéine*” in France in 1938. During sporulation, *Bt* synthesizes a parasporal crystalline inclusion containing proteins known as  $\delta$ -endotoxins or Cry proteins, which have insecticidal properties. Genes that express the delta-endotoxins are called “*cry genes*”. The  $\delta$ -endotoxins form two classes of toxins, Cry proteins and Cyt. proteins. after ingestion, the crystals are solubilized in the alkaline (pH 9 to 12) releasing protoxins which are converted to active toxin by midgut proteases and bind to specific receptors in the microvilli of the apical membranes of the columnar cells thus causes opening or pore formation followed by osmotic imbalance between the intracellular and extracellular environments and cell disruption. This destroys the microvilli, causing the insect to stop feeding, leading to its death. There are many reports of such *Btk* formulations for use against bollworms, loopers, and other lepidopteran pests in India.

### 3. Viruses

Baculoviruses comprise of nucleopolyhedro viruses (NPVs) and granulo viruses (GVs). NPVs produce occlusion bodies (OBs) referred as polyhedra, while that of GV's are called granules. during the course of infection two forms of virions are produced: Budded Virions (BVs) and OB-derived virions (OdVs). BVs consist of only a single nucleocapsid surrounded by an envelope; whereas OdVs embedded within OBs contain either single nucleocapsids known as single NPV's (SNPVs) or multiple nucleocapsids known as multiple NPVs (MNPVs). NPV

has a biphasic infection cycle. When susceptible larvae feed the leaves infected with baculoviruses, the occluded bodies get dissolved in the alkaline pH of insect midgut and the virus start multiplying within the host cell nuclei after binding to the brush border membrane vesicles of the mid-gut columnar cells thus giving rise to progeny viruses which later form budded viruses and cause secondary infection. In later phase of secondary infection OdVs are formed infecting other insect tissues, which are then embedded within OBs and are released into the environment following the death of infected larvae by liquefaction. Infected host may die within 3-4 days when environmental conditions are favourable. In India, two nucleopolyhedro viruses (NPV) are commercially produced or imported to manage bollworms *Helicoverpa armigera* and *Spodoptera litura* which are polyphagous pests on economically important crops including castor, citrus, cocoa, cotton, groundnut, legumes, maize, potato, rice, rubber, tobacco, tomato, sorghum and many other vegetables.

#### 4. Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) are soil-inhabiting, lethal insect parasites that belong to the Phylum Nematoda and belonging to the families Steinernematidae and Heterorhabditidae. EPNs are mutually associated with bacteria carried by Steinernematidae is *Xenorhabdus*, and by Heterorhabditidae is *Photorhabdus*. The third juvenile stage of EPNs is referred to as the “infective juvenile” (IJ). IJs of both genera when locate a susceptible host larvae enters the insect host through the mouth, anus, spiracles or thin parts of the host cuticle and release their corresponding bacterial symbionts in the insect host body and develop into fourth-stage juveniles and adults. Mortality of the infected insect occurs mainly due to “septicemia”. Normally EPNs take 1-3 weeks of duration starting from infecting the insect host, development and reproduction within the cadaver. The insect cadaver becomes red if the insects are killed by *Heterorhabditids* and brown or tan if killed by Steinernematids.

## Conclusion

Utilization of viable microbial entomopathogens is important tool in present day pest management program and also as a vital component of integrated pest management strategy. Need for identifying and isolating native virulent strains of microbial pathogens and their commercial production, genetic modification for enhancing the virulence and exploitation of the unexplored microorganisms are to be enunciated for the effective management of insect pests in varied agro-ecosystems in a sustainable way.



## An Overview of Rumen Microbial Ecosystem

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### *abstract*

The rumen microbial ecosystem is diverse and complex and the complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses. These microorganisms work symbiotically to break down feedstuffs consumed by ruminant animals. The microbiome controls the production efficiency of the animal, with certain pathways (such as those associated with methane production) resulting in energy loss in the animal. The microbiome also affects end-product quality (milk and meat) but also contributes to environmental pollution. Understanding the rumen microbiome and its connection to the ruminant itself; is important for producing quality products, increasing profitability and reducing environmental impacts.

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### Introduction

The rumen can be viewed as an anaerobic and methanogenic fermentation vat that contains microorganisms that have the ability to utilize, and increase the productivity of, cellulolytic feeds (i.e. straw, hay, silage and grass). The rumen dynamics are almost solely responsible for providing nutrients to the host animal. The rumen and its microbiota play a particularly important role in the degradation of feedstuffs. As a result of fermenting feedstuffs, carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>), which are the main electron acceptors and donors of the ecosystem, are produced in the rumen. The rumen microbiome, i.e., the community of microorganisms that inhabits the rumen, is characterized by its high population density, extensive multiplicity (encompassing bacteria, archaea, protozoa and fungi) and complexity of interactions. The continuous fermentation carried out by these microorganisms leads to broken down of ingested fibrous feed into their subcomponents.

There are 3 intersecting micro-environments found in the rumen that contain these microbes; the **liquid phase** making-up 25% of the microbial mass, the **solid phase** making-up 70% of the microbial mass, and the **rumen epithelial cells and protozoa**, containing 5% of the

microbial mass. a nutritionally balanced diet is important as it provides an atmosphere that maximizes the growth and activity of these microbes.

A rumen microorganism is anaerobic or facultatively anaerobic, and produces end products that are either directly utilized by the host or by other microorganisms as energy. The ruminal pH is kept relatively constant (Generally 6 to 7), but may vary depending on diet. Such variations can result in a change in the microbial populations, and the levels of volatile fatty acids (VFAs) produced. These fatty acids such as propionate and butyrate, can be absorbed across the gut wall to serve as an energy source for the ruminant.

Buffering of the rumen to maintain a relatively constant pH is facilitated by the large quantity of saliva produced by the ruminant, which is high in sodium and potassium bicarbonate and urea. The saliva is swallowed into the rumen and then absorbed through the rumen walls. Further buffering is provided by ammonia produced during fermentation, which can then be used for microbial growth in the rumen. Ruminants, through the action of their microbiota, can utilize indigestible components of feed i.e. lignocellulose. The interplay between the host and microbes in the rumen is synergistic, i.e. the host provides heat, moisture and food, while the microbiomes produce protein and by-products of digestion, such as VFAs, for use by the host. The complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses.

### 1. Rumen bacteria –

The most abundant microbes in the rumen liquor with concentration  $10^{10} - 10^{11}$  cells/ml and are of over 200 species. The bacterial composition of rumen liquor depends upon a number of factors including precedence of certain diets, energy requirements, and resistance to some toxic metabolic end-products. Rumen bacteria are mainly Gram negative (when fed on high forage diet), but on high grain diets more Gram positive bacteria are seen in rumen liquor. The use of molecular techniques for the analysis of rumen microbiology for example, the conserved 16S rRNA gene to determine bacterial composition, predict their functionalities or enumerate targeted microbes within a complex ecosystem without the need for culturing. This is important because only 20% of the rumen microbiota can be cultured using standard techniques. Sequencing (using 16S rRNA) also reveals that *Prevotella*, *Butyrivibrio* and *Ruminococcus* are the most dominant bacteria in the rumen, and that community structure is affected by changes in the diet of the host.

It has been also discovered that diet complexity favors increased microbial diversity. Due to the high forage diets of ruminants, particularly grass-based diets, cellulose digesters are more in number. These **cellulolytic bacteria** degrade the main components of plant fiber i.e. cellulose and hemicellulose. The ability to degrade cellulose is strongly dependent on the type of forage, crop maturity and the accessibility of the cellulolytic bacterial communities. The plant fiber matrix is complex in nature and is composed of  $\beta$ -1, 4 linked glucose residues for cellulose and  $\beta$ -1, 4 linked xyloses for hemicellulose, and thereby requires the coordination of a number of hydrolytic enzymes in order to break it down. Although there are many cellulose degrading bacteria, *Fibrobacter succinogenes* and *Ruminococcus albus* are the most desirable cellulose degraders. Their ability to digest cellulose is much higher than that of other cellulolytic bacteria, which is due to the fact that they possess a number of genes encoding enzymes which are involved in fibre degradation. Fermentation end products of cellulolytic bacteria include acetate, butyrate, propionate and  $\text{CO}_2$ . Formic acid and lactic acid are also formed but are quickly used by other bacteria.

Starch is also an important constituent of the ruminant diet, in particular for highly productive dairy cows. High grain diets result in an increase in the amount of starch in the rumen. *Streptococcus bovis* (an **amylolytic** bacterium) is normally present in low numbers in cows fed high forage diets and in high abundance in cows that consume high grain diets. *S. bovis* requires low pH for their optimum growth and its high abundance following consumption of high grain diets is attributed to a sudden increase in glucose levels in the rumen and the loss of protozoa due to the more acidic environment created by high grain diets. More specifically, lactic acid is produced from starch and, as lactic acid is not metabolized by the animal, therefore gets absorbed through the rumen wall; causing an increase in lactic acid in the blood and reduced blood pH.

Sudden change in animal diet, results in an accumulation of VFAs in the rumen liquor, causing a detrimental effect on the microbiota and the host animal. These severe and sudden changes lead to a decrease in ruminal pH and an increase in *S. bovis* and *Lactobacillus* species. Some anaerobic bacteria acquire energy from the pectin degradation, with the most important **pectinolytic** species, *Lachnospira multiparus*, *Prevotella ruminicola* and *Butyrivibrio fibrisolvens*, being capable of reducing pectin to oligo-galacturonides, yielding large quantities of acetate. Citrus by-products like citrus pulp are widely used in ruminant feeding systems and contain a high percentage of pectin substances. These by-products can be used as an alternative to highly

fermentable grains, preventing the excessive growth of *S. bovis*, and associated ruminal acidosis, and some researchers suggest that they may even improve the feed utilization efficiency for milk production.

Microorganisms associated with milk fat yield are **Firmicutes** and **Bacteroidetes**. A decreased abundance of Bacteroidetes in comparison to Firmicutes results in increased milk fat %.

## 2. Methanogenic archaea –

Archaea, due to their broad spectrum of unusual and distinctive metabolisms, can survive in a variety of different environments. Rumen archaea are strictly anaerobic and are the only known rumen microorganisms to produce methane (CH<sub>4</sub>). Such archaea are referred to as methanogens. Archaea are found in the rumen in the range of 10<sup>6</sup> to 10<sup>8</sup> cells/ml, accounting for less than 4% of the microbial community. Archaea are present at the bottom of trophic chain due to their need to use the end products of fermentation as their substrates.

The domain archaea is broken into two different kingdoms-

A) Euryarchaeota, consisting of methanogens and extreme halophiles, and

B) Crenarchaeota, consisting of hyperthermophiles and non-thermophiles.

Methanogens of kingdom Euryarchaeota; requires very low redox potential and are strictest anaerobes known. According to meta-analysis of global data, 90% of rumen methanogens belong to the following genera; *Methanobrevibacter* (63.2% of methanogen population), *Methanomicrobium* (7.7% of methanogen population), *Methanosphaera* (9.8%) "Rumen Cluster C", now referred to as *Thermoplasma* (7.4%) and *Methanobacterium* (1.2%).

Most methanogens remove hydrogen gas by reducing CO<sub>2</sub> with H<sub>2</sub> gas to form CH<sub>4</sub>. In contrast, *Methanosphaera stadtmanae* only produces methane through the reduction of methanol with H<sub>2</sub>, having one of the strictest energy metabolisms of all methanogenic archaea. Producing methane; keeps low ruminal hydrogen concentrations, allowing methanogens to promote the growth of other species, and thereby enabling a more efficient fermentation.

Eruktion of methane; which is also a green-house gas (GHG), leads to atmospheric pollution. Therefore, following efforts to mitigate emissions of rumen methane includes -

- I. Vaccines (targeting rumen methanogens through the generation of antibodies to selected methanogen antigens that enter via saliva, binding to targets on the methanogens)

- II. Small-molecule inhibitors (targets enzymes essential for the growth of methanogens)
- III. Additives and Breeding approaches.

In a study carried out by *Goopy et al.* (2014), it was found that sheep that emitted low methane levels had a smaller rumen in comparison to high methane-emitting sheep. There was no difference in dry matter intake or digestibility between the two groups. The study also found that low methane-emitting animals had a shorter mean retention time for both solid and liquid phase. Hence, this may be the basis for breeding animals with a smaller rumen size to reduce methane emissions. However, most effective and straightforward method of lowering rumen methane emissions is dietary manipulation because, as selective breeding is slow and selection of specific traits may affect favorable variants.

Methane production by archaea represents an energy loss of about 2–12% of gross energy intake (GE<sub>i</sub>), means this energy is no longer available for animal growth, lactation, maintenance or pregnancy. Manipulating the ruminant's diet; to reduce the number of methanogens would therefore be helpful to reduce the negative impact on the environment, and will also improve the efficiency of livestock production.

Methanogenesis is the only mechanism of ATP synthesis for methanogenic archaea. The methyl coenzyme M reductase (McrA, encoded by *mcrA* gene) catalyzes the final step in methanogenesis. Thus, use of anti-methanogens, such as bromochloromethane, can be used to inhibit methane production. Because, Bromochloromethane reacts with reduced vitamin B<sub>12</sub> and inhibits the cobamide-dependant methyl transferase step, which is responsible for McrA synthesis. Therefore, targeting this step may break the pathway and inhibit production.

A recent study, investigated the use of an invasive species of macroalgae *asparagopsis taxiformis* as a means of reducing methane emissions in vitro by its secondary metabolite-bromoform, which is similar to bromochloromethane in its ability to decrease methanogenic activity.

### 3. Ciliate protozoa –

Ciliate protozoa are found in the range of  $10^4$  –  $10^6$  cells/ml in rumen liquor and are amenable for 30 to 40% of overall fiber digestion. They are also relatively active in lipid hydrolysis and can produce hydrogen via their hydrosomes. The *Entodinium* genus is the most dominant protozoan in high grain diet and it rapidly degrades starch, engulfs it and converts it to an



iodophilic storage polymer. Degradation occurs through a combination of debranching, amylase and glucosidase enzymes.

Defaunation is a mechanism used to prevent the growth and/or remove, protozoal population from the rumen. This can be done by several different means such as through the isolation of calves after birth, chemical defaunation through the use of CuSO<sub>4</sub>, calcium peroxide, alcohol ethoxylate, coconut oil, linseed oil or soya oil hydrolysate. Another method of defaunation, involves an invasive method to remove the rumen contents from the host, followed by carefully washing of rumen mucosa and then the rumen contents are treated by either heating or freezing to eliminate protozoa. The rumen contents are then returned back to the rumen. This method also removes protozoa-associated methanogens (methanogens sequestered within rumen protozoa) which account for approximately 37% of methanogenesis in ruminants, and as defaunation would also eliminate them, leading to decrease in methane production.

Some studies revealed that methane emissions increased with protozoal abundance and stated that protozoa showed the strongest link with methane emissions (in comparison to other domains) after qPCR using DNA extracted from rumen contents. The presence of protozoa can probably have both beneficial and negative effects on the rumen microbiome, as protozoa engulf bacteria and other smaller microbes and particles in the rumen, but also larger molecules including proteins and carbohydrates. They actively ingest the bacteria as a source of protein and also act as a stabilizing factor for end products of fermentation.

#### 4. Amoeba –

Amoebae can represent an important reservoir for bacteria in the environment, but their role in the rumen is unclear. In the vegetative cycle (multiplication by binary fission), amoeba, similar to ciliate protozoa, survive by ingesting bacteria through phagocytosis. It is known that some bacteria can survive phagocytosis by protozoa and live as endosymbionts. For instance, *Campylobacter jejuni* has been shown to invade *Acanthamoeba polyphaga* and can replicate in vacuoles. *C. jejuni* and *C. fetus* can have large effects on cow fertility, immunity and overall health. Amoeba therefore may be important in rumen and general bovine health.

#### 5. Fungi -

Rumen fungi ( $10^3 - 10^6$  zoospores/ml) are anaerobic, falling into the class Neocallimastigomycetes, consisting of 6 genera (*anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces* and *Piromyces*) with 21 known species and, using

molecular techniques, 2 genera are recently discovered *Oontomyces* and *Buwchfawromyces*. anaerobic fungi may influence the rest of the microbial community since they produce  $H_2$  during initial degradation of plant tissue and this  $H_2$  can be used as fuel for the degradation mechanisms of other communities.

Fungi colonizes plant cell walls and accounts for approx. 8–12% of total ruminal microbial biomass, but this figure varies widely depending on the animal diet. While most plant fiber-associated fungi are retained in the solid phase, the liquid phase may contain smaller particulate matter that fungi may have attached to. Fungi are the best degraders and they produce high levels of celluloses hemicelluloses, as well as also possess the ability to break down xylan due to the production of xylanases enzyme. Fungi appear to initiate the feed breakdown process, indicating that anaerobic fungi may be pivotal for feed utilization efficiency and animal growth and production in pasture-fed ruminants.

By breaking down the carbohydrates, fungi produce metabolites which can be used for nutritional purpose by the host. They have many features which make them unique in comparison to fungi found outside the rumen, including polyflagellate zoospores (the reason why rumen fungi were originally mistaken for ciliate protozoa), cellulosomes, hydrogenosomes and a wide enzymatic spectrum.

## 6. Bacteriophage –

Bacteriophage are obligate pathogens of bacteria and they occur in dense populations of approximately  $10^7 - 10^9$  particles/gram of digesta in the rumen. Like others; bacteriophage population are also influenced by external sources, meaning they may also be controlled through different strategies.

The bacteriophage and virus population found in a rumen sample is referred to as the **virome**. The high number of rumen bacteriophage suggests that they may have an important function in the balance of the ruminal ecosystem, but there is little known about the effect of the rumen virome on the system it inhabits. Some studies showed that this was important in the transfer of glycoside hydrolase between bacteria and rumen fungi. Also some research propose that this may also be important in the case of rumen ciliate protozoa, whereby horizontal gene transfer occurs from rumen bacteria in order to aid in protozoa adaptation to the carbohydrate-rich environment of the rumen, transferring genes encoding plant cell wall degradation. This information indicates that bacteriophage may represent a shared gene pool for the rumen system.

Metagenomics can also be used to determine the origin of these genes. In a study carried out by it, it was found that most viruses found in the rumen were associated with the most dominant phyla, namely Firmicutes, Bacteroidetes and Proteobacteria. Bacteriophage interaction can be identified by the presence of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins in the microbial population. These genes are fundamental to provide adaptive immunity in some bacteria and archaea, which enable the organism to respond and eliminate invading genetic material. Once infected by these invading genetic materials, the new DNA is integrated into the host CRISPR locus as new spacers, ultimately encoding a unique spacer sequence.

## CONCLUSION

The rumen microbiomes play an exigent role in shaping the host physiological parameters. The complex rumen ecosystem consists of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses. Although bacteria are the most prominent microorganisms in the rumen, but fungi are the best degraders. Identification of certain metabolic pathways of these microbiota and then further investigation of those pathways; may be helpful to determine the best diet for ruminants; in order to minimize the energy losses, to reduce the methane production and to increase the nitrogen utilization efficiency. Examination of the rumen microbiome can even identify the effects of diet on the microbiome and in turn, the effects on milk yield, milk fat %, protein percentages, urea percentage (used as an NPN indicator) and milk protein yield.

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## Bio-preservation: A Natural Approach for Food Safety

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**D**espite modern advances in technology, the preservation of foods is still a debated issue, not only for developing countries but also for the industrialized world. Need for development of new technologies for better processing, preservation, and storage of food. To achieve improved food safety, the food industry makes use of chemical preservatives or physical treatments e.g., high temperatures. These preservation techniques have many drawbacks which include the proven toxicity of the chemical preservatives e.g., nitrites, the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems.

Bio-preservation refers to extended storage life and enhanced safety of foods using the natural antimicrobial compounds that are of plant, animal, and microbial origin and have been used in human food for a long time, without any adverse effect on human health.

### Natural antimicrobials for Food Biopreservation

1. **Antimicrobial Substances derived from Bacterial Cell Metabolism-** Bacteria produce many compounds such as organic acids (Lactic acid, acetic acid, Propionic acid) CO<sub>2</sub>, Hydrogen peroxide, Bacteriocins, diacetyl, etc that are active against other bacteria, which can be harnessed to inhibit the growth of potential spoilage or pathogenic microorganisms. These include fermentation end products such as organic acids, hydrogen peroxide, and diacetyl, in addition to bacteriocins and other antagonistic compounds. Bacteriocins are ribosomally synthesized single polypeptides that are usually inhibitory only to closely related bacterial species. Bacteriocins become one of the weapons against microorganisms due to the specific characteristics of a large diversity of structure and function, natural



resource, and is stable to heat Ex- Nisin (*Lactococcus lactis* subsp. *lactis*) Pediocin a, Pediocin ACH, etc.

Bacteriocin-based biopreservation strategies include a purified/semi-purified bacteriocin preparation as an additive in food, incorporation of an ingredient previously fermented with a bacteriocin-producing strain, by using a bacteriocin-producing culture to replace all or part of starter culture in fermented foods to produce the bacteriocin in situ.

The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives, such as:

- They are generally recognized as safe substances, they are not active and nontoxic.
- They become inactivated by digestive proteases, having little influence on the gut microbiota.
- They are usually pH and heat-tolerant.
- They have a relatively broad antimicrobial spectrum against many food-borne pathogenic and spoilage bacteria
- They show a bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane: no cross-resistance with an antibiotic.

Moving towards the better tomorrow we can identify new bacteriocins for application in food safety, alter the specificity of existing bacteriocins, Increase the level of bacteriocin production, development of bacteriocin-producing lactic starters through the gene transfer system.

## 2. Antimicrobials from animal Sources-

**Lactoperoxidase** is a protein or glycoprotein enzyme present in raw milk, colostrum, saliva, and other secretions (Taylor *et. al.*, 2014, Seifu *et. al.*, 2005). Lactoperoxidase reacts with thiocyanate and hydrogen peroxide forming the termed lactoperoxidase system (LPS) with antimicrobial capacities.

**Lysozyme** is an enzyme naturally present in avian eggs and mammalian milk and is generally recognized as safe (GRaS) for direct addition to foods (FDA, 1998). The white lysozyme of hen eggs is a bacteriolytic enzyme widely reported for its antimicrobial application in food products (Tiwari *et al.*, 2009) and commonly used as a preservative for meat, meat products, fish, fish products, milk, and dairy products, and fruits and vegetables (Cegielska-Radziejewska *et al.*, 2009). The ability to hydrolyze the  $\beta$ -1, 4 linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of the microbial cell

wall accounts for lysozymes antimicrobial activity (Juneja *et al.*, 2012). Lysozyme has been used primarily to prevent late blowing defects in cheeses, caused by *Clostridium tyrobutyricum*.

**Lactoferrin (Lf)**, an iron-binding compound of milk, has been shown to possess antimicrobial activity against a wide range of bacteria and viruses (L  onnerdal, 2011). Recently, Lactoferrin Lf has been approved for application on beef in the United States and has been applied as an antimicrobial in a variety of meat products (USda-FSIS, 2010; Juneja *et al.*, 2012). Lactoferrin shows antimicrobial effect against foodborne microorganisms including *Carnobacterium*, *L. monocytogenes*, *E. coli*, and *Klebsiella* (al-Nabulsi and Holley, 2005; Murdock *et al.*, 2007).

3. **Antimicrobials derived from Plants-** Herbs and spices have been recognized to contain a broad spectrum of active compounds that are having antibacterial, antifungal, antiparasitic, and/or antiviral properties. Essential oils have been used for a long time as part of natural traditional medicine. They are plant material's aromatic oily liquids (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). The principal components responsible for essential oil's effective antimicrobial activity include saponins, flavonoids, carvacrol, thymol, citral, eugenol, linalool, terpenes, and their precursors (Burt, 2004). The antimicrobial activity of plant essential oils is due to their chemical structure, in particular to the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components (Jorman and Deans, 2000). Usually, the compounds with phenolic groups as oils of clove, oregano, rosemary, thyme, sage, and vanillin are the most effective (Skandamis *et al.*, 2002). They are more inhibitory against Gram-positive than Gram-negative bacteria (Mangena and Muyima, 1999; Marino *et al.*, 2001).

**Bacteriophages-** Lytic bacteriophages offer great potential as natural bio preservative agents, due to their capacity to selectively control the bacterial population. Phages can be safely used in the food industries for bio-control at various levels thus representing a new era of novel food safety agents that are potential replacements for chemical preservatives and antibiotics. The advantages encourage their use in the food chain system for several reasons.

- (i) High specificity to target their host determined by bacterial cell wall receptors, leaving untouched the remaining microbiota, a property that favours phages over other antimicrobials that can cause microbiota collateral damage.

- (ii) Self-replication and self-limiting, meaning that low or single dosages will multiply as long as there is still a host threshold present, multiplying their overall antimicrobial impact.
- (iii) As bacteria develop phage defense mechanisms for their survival, phages continuously adapt to these altered host systems.
- (iv) Low inherent toxicity, since they consist mostly of nucleic acids and proteins;
- (v) Phages are relatively cheap and easy to isolate and propagate but may become time-consuming when considering the development of a highly virulent, broad-spectrum, and no transducing phage;
- (vi) They can generally withstand food processing environmental stresses (including food physiochemical conditions);
- (vii) They have proved to have a prolonged shelf life.

## General control measures for management of parasitic diseases

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### Abstract

Parasites are present from thousands of years and infect both human as well as animal. They are responsible for reducing the health and productive status of animal and in turn can hamper the country development. Some time they also cause mortality in human also. Control of parasitic disease is little bit difficult because they are multicellular having complex life cycle, many intermediate stages and more chance of developing resistant against chemical based controlling agents. So, for effective control of parasite complex interaction of events in the fields of public health, education, political will and medicinal science are needed. Current manuscript deal with different managerial practices which can be used for effective control of parasitic infection.

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**Keywords:** Parasitism; control; management

### Introduction

Diary animals are very sensitive to the effects of internal parasitism. Parasitism can cause decreased fertility, abortion, un-thriftiness, increased susceptibility to other disease and death. Delay in puberty age due to parasitism is well documented in dairy animals. Besides these, parasitic diseases are known to cause decrease in fertility and reduced body weight gains especially in meat animals. The quality of hair coat and wool is affected by parasitism as rough hair is known to be associated with many internal parasites. Besides, abortion in small ruminants due to toxoplasmosis is a very well-known problem. Alongside, coccidiosis is known to significantly alter the health and production of dairy animals. Moreover, quite a few of parasitic infections are zoonotic in nature thereby, posing

public health significance.

The control of parasitic diseases of livestock has been undertaken since the deleterious effects of parasitism on animal health and well-being are very much significant. Subclinical parasitism in grazing animals is a major cause of economic problem, severely limiting the productivity of animals throughout the world, but in actual sense, the effect of subclinical parasitism is more severe in countries like India. An exhaustive review commissioned to prioritize animal health research for poverty reduction in the developing World, by a consortium consisting of the WHO, OIE and FAO, concluded that gastrointestinal (GI) parasitism had the highest global index as an animal health constraint. It is a common observation that the number of infective stages present in the environment at any given period is directly related to the number of worm eggs passed by the host and this ultimately determines the number of parasites that are potentially capable of being established in a susceptible host. Characteristics such as breed, age and nutritional status of the host have a marked influence on the damage to the host. Subclinical parasitism poses enormous threats to global food security by causing up to 20% reduction in milk yield of dairy animals and 52% loss in body weight. This is in addition to the losses incurred from clinical parasitism resulting in death of newly born or young animals or even adult animals during productive age. The different approaches to control from diagnosis to treatment and cure of the clinically infected animal to control the transmission within the herd by preventative chemotherapy and vector control are outlined in this article. The various strategies that should be strictly and stringently followed for management and control of parasitic infections in a farm include-

1. **Integrated Parasite Management (IPM)**: IPM encourages a multidisease control approach, integration with other disease control measures and systematically considered application of a range of interventions, often in combination and synergistically (WHO 2004). The IPM is cost-effectiveness, ecological sound and sustainable. IPM strategies are very much similar to those of integrated pest management systems that are used in agriculture. Such integrated approaches help to preserve the ecosystem alongside encouraging the biological control measures. IPM favors the combined application of four approaches: (a) environmental management that includes environmental manipulation for parasite control; (b) biological control methods using natural predators; (c) chemical control methods with the use of chemical compounds/ drugs (d) social and behavioural measures that includes public awareness. The



details of these measures are summed up below:

**a. Environmental Management:** aim of environmental management is to alter the maintenance of vectors by long-term physical changes in abiotic factors, often incriminated as environmental modification (WHO 1982). Environmental manipulations include flushing or streaming of canals, providing intermittent irrigation to agricultural fields, temporarily flooding or draining of wetlands, or removing specific types of vegetation that provides larval habitats for mosquitoes. Remarkable success is achieved by draining swamps to remove larval breeding sites of mosquitoes alongside using oil to kill larvae of mosquito by asphyxia. *Glossina* (tsetse flies) fly has been effectively controlled in savanna and riverine forest habitats by the selective destruction of breeding lands. The general effectiveness of environmental management ultimately depends on how well the particular intervention is matched with the ecology of the particular disease caused by a specific vector population. These also include managerial practices like-

- i. MacLean county system for control of ascarid infections by proper treatment of sows before and after farrowing. The sows and the piglets are treated with piperazine and are reared in ascarid free environment upto weaning.
- ii. Gilt only policy for control of kidney worms of pigs. The gilts are sold and slaughtered before the prepatent period of the parasite thereby suppressing the environmental contamination.
- iii. destruction of marshy areas for snail population control for trematode infections using Molluscicides like copper sulphate and N-tritylomorpholine.

**b. Use of Natural Predators/Biological Controls:** Biological methods involve the use of toxins of biological origin and/or using natural enemies of a particular pest or parasite so as to achieve effective vector control. an additional advantage of using biological control over traditional chemical control is the reduction in ecological disturbance affecting the other animal and plant species. Larvivorous fish (*Gambusia* for mosquito control) and copepods as well as the toxic products of bacterial agents have been successfully used to control vectors. Microbial larvicides are safely added to drinking water and in environmentally sensitive areas and are being used regularly in western countries. They do not accumulate in the environment or in body tissues and hence, are not toxic to vertebrates and other animal and plant fauna through food chain (WHO 1999). Research on the potential use of naturally occurring pathogens, parasitoids and predators for the biological control of animal pests is far

behind that for plant pests as much more studies have been conducted in agriculture sector in comparison to veterinary sector. among the biological agents used, entomopathogenic fungi played a uniquely important role in the history of microbial control of insects and helminths. Use of spore of predacious fungi such as nematode-trapping fungi (*arthrobotrys oligospora* and *Duddingtonia flagrans*) and egg parasitic fungi (*Paecilomyces lilacinus* and *Verticillium chlamydosporium*) can also reduce the parasitic burden in the pasture (Sanyal et al 2007; Singh et al 2010). Haemipterans and ants have been used to control ticks (Soulsby 2005). Certain plants like stylosanthes are used for control ticks while smoking with neem and Eucalyptus have been traditionally been used to control flies and mosquitoes (Soulsby 2005). Tannin rich plants are often used for control of gastrointestinal parasitism particularly against nematodes. duck rearing alongside the pond is very much recommended for snail control. Traditional animal health care practices involve the use of medicinal plants to treat livestock diseases. This Ethno veterinary knowledge (EVK) and practices play a major role in complementing modern approaches in the control of diseases. Certain plants have repellent activities against ticks, mosquitoes and flies and hence are used as 'push strategy' to keep these insects away. Tobacco leaves are used by people of certain states of India especially Bihar, as an indigenous measure of controlling ticks. Methanolic and other extracts of certain plants like neem (*azadirachta indica*) leaves and bark, nochi (*Vitex negundo*) leaves, etc are known to have acaricidal activities.

**c. Chemical Control:** Chemical control methods are definitely fast in results, highly effective, if used efficiently. They may be used as a non-residual application (effective over a short time-scale, killing only those which are currently exposed) or a residual (persistent) application that is effective over a period of weeks to months. Chemicals remain the backbone of IPM. However, there are concerns over the impacts of chemicals, especially those which are persistent in nature as they are organic pollutants and undergo bioaccumulation in food chain. a wide range on commercial anthelmintics and acaricides are available in the market for application. Some of them are:

anthelmínthics and antíprotozoals		Effective against
Píperazíne salts @200-300 mg/ kg b wt orally, Pyrantal palmoate @5-10 mg/ kg b wt orally		Nematodes especially ascaríds
Albendazole, Fenbendazole @5-7.5 mg/ kg b wt orally		Parasític gastro enterítis including haemonchosis, Hookworms
Prazíquantal @10-15 mg/ kg b wt orally		Tapeworms
Oxyclozaníde @15 mg/ kg b wt orally		Flukes
Tríclabendazole @10-12 mg/ kg b wt orally		acute fasciolosis
Buparvaquínone@2.5mg/kg b wt Í/M		Theíleriosis
Ímídocarb @ 2-4 mg/ kg b wt Í/V		Babesiosis
Tetracyclínes		Rícketsial infections
Ívermectín		as end-ectocíde
amprolíum @10 mg/ kg b wt orally		Coccídiosis
acarícídes		Concentration and route of application
Coumaphos	1% dust, 0.34-0.46% spray, 10 mg/kg as pour on	
Díchlorvos	8.9% as dog collar, 30-60 ml 1% soln. as spray, 20% as tag	
Malathión	4-5% dust, 0.5-0.6% spray	
Carbaryl	5% dust, 0.5% spray	
Líndane	1% dust, 0.03% spray, 0.04-0.05% díp	
Amítraz	250 ppm in water	
Deltamethrín	1% soln. as spray	
Permethrín	0.25% dust, 0.05% spray, 10% tag	
Ívermectín	0.2 mg/ kg as inícción subcutaneously or líquid or paste or tablet	

**General strategies for the use of chemical anthelmintic:**

- i. Prophylactics:** Here the treatments are either done at regular intervals or the drugs with residual effect are used. Treatment is done irrespective of the fact that the animal is infected or uninfected. a broad spectrum anthelmintic like albendazole or a drug combination containing flukicide, anti-nematodal and anti-cestodal preparation can be used for common dosing against trematode, nematode and cestode to prevent multiple drug resistance.
  - ii. Curatives:** These treatments are based solely on clinical diagnosis. Only the infected animal is treated. There reduce expenses for anthelmintics as few animals are treated and selection for resistance is significantly reduced. disadvantage includes the fact that it requires regular monitoring which increases labour costs.
  - iii. Measures against intermediate hosts:** Molluscicides like copper sulphate and N-tritylmorpholine are commonly used for snail control. Molluscicides are usually applied in spring or mid-summer. The spring application is easy to apply and highly effective, killing off wintered infected snails and parent snails which would supply the majority of the next year's breeding population. Mid-summer applications kill off infected snails prior to emergence of summer infection of trematodes on to the pasture in late summer season.
2. **Health awareness and Behaviour:** Vector-borne diseases are much more serious especially in cases when the affected animal or human host is malnourished, or in ill health, or suffering from existing disease. Thus, there is a strong link between overall social well-being and actual impact of parasitic disease. Improved awareness of hygiene and sanitation is very much important in reducing the disease amongst the susceptible hosts. awareness like the early identification of disease based on symptoms; the need to maintain attendance for drug delivery; and refraining from interfering with traps for vectors such as Tsetse fly should be promoted in endemic areas. These steps have provided enormous success in african countries. Indoor rearing of animals at night should be practiced in endemic areas and is known to give very significant results in vector control.
  3. **Vaccines:** although a very few vaccines are available commercially in markets for parasitic control. These include dictol/ difil/ Huskvac for lung worms, Tick Guard and Gavac for ticks alongside a few for protozoan parasites like Raksha Vac T for theileriosis, Pirodog for babesiosis and Coccivac/ Livacox/ Pracox for poultry coccidiosis.

## General guidelines to farmers for controlling parasitic infections at grass root level:

Following measures are recommended against free living and parasitic stages of ticks and other vectors:

- (a) Elimination of the tick/ fly shelters (cracks in the wall and perches, stagnant water source) for getting rid of larvae, nymphs and adults of insects.
- (b) Pre-monsoon burning and/or rotation of pastures in the endemic areas of helminths.
- (c) Natural predators (birds, rodents, ants and *Hymenoptera* spp. wasps) feed upon engorged and free-living vectors and reduce their population (especially for tick control).
- (d) In indigenous and cross bred cattle, exposure with tick and fly infestations, confer strong resistance amongst the herd.
- (e) Periodic use of acaricides and insecticides against free living stages of vectors in recreational parks, dog kennels, perches, barns and human dwelling is very much advised.
- (f) Destruction of breeding grounds of mosquitoes and flies like stagnant water resources, etc significantly helps in vector control.
- (g) Duck rearing for snail control alongside use of chemicals like copper sulphate, N trityl morpholine.
- (h) Use of tannin rich plants for control of Gastrointestinal parasites as a part of herbal approach for control of parasitism.
- (i) Use of vaccines particularly in endemic areas especially for poultry coccidiosis.
- (j) Regular deworming of infected animals throughout the year on regular basis.

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## Brucellosis: an Important Re-Emerging Zoonotic Disease

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### abstract

Brucellosis is a zoonotic disease with high rate of morbidity and lifetime of sterility. It has been eliminated in many industrialized nations, but the disease has a consequential impact on both animal and human health. abortion in the last trimester is a predominant sign in cattle, while in humans it is characterized by undulant fever, malaise and arthritis. It is the need of the hour for rapid, more sensitive and more specific test methods. Public awareness regarding health education and safe livestock practices should be given importance. active co-operation between human medicine and veterinary services should be promoted.

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### Introduction

Brucellosis is a zoonotic bacterial disease which is associated with animal husbandry with worldwide distribution. It is one of the most common zoonosis according to Food and Agriculture Organization and World Health Organization, which has serious public health consequences (WHO, 2012). It is caused by various *Brucella* species, and it primarily affects cattle, swine, goats, sheep and dogs. The existence of brucellosis as a public health issue is due in part to the expansion of animal industries and urbanization, as well as a lack of hygienic measures in animal husbandry and food handling.

### Etiology

The etiological agent of brucellosis are Gram-negative coccobacillus, *Brucella* spp. which are non-spore-forming, non-motile, aerobic pathogens. Twelve species are currently described in the genus that infect various domestic as well as wildlife animal species. *Brucella abortus* (cattle), *B. ovís* (rams), *B. melitensis* (goats and sheep), *B. suis* (pigs), *B. canis* (dogs) and *B. neotomae* (common voles) are the six *Brucella* species classified according to preferred hosts and pathogenicity. *B. melitensis*, *B. suis* and *B. abortus* are the most important pathogenic species in humans (Whatmore *et al.*, 2016).

## Transmisión

Íngestión, inhalación, contact with the conjunctiva and skin abrasions/wounds are all possible modes of transmission. The main sources of infection include aborted fetus, placenta and uterine secretions. Infected animals milk also contains microorganisms. The virus is spread to the animals through contaminated feed and water, as well as contact with aborted fetus, foetal membranes and uterine discharges. Infected bulls can potentially disseminate infection from one herd to another through spontaneous or artificial insemination. Humans become infected after consuming Brucella-infected raw or unpasteurized milk and milk products. Veterinarians and animal handlers are at a very high risk of contracting the disease (Khurana *et al.*, 2021).

## Pathogenesis

*Brucella* spp. multiplies intracellularly in phagocytic cells like macrophages and dendritic cells once it has entered the host body. When a female conceives, the bacteria travels through the bloodstream to the placenta, then to the trophoblasts and mammary gland, where it multiplies to cause abortion. Abortion happens as a result of bacteria causing harm to the placenta, as well as stress-induced hormonal changes. The presence of erythritol in the placenta and foetal fluid from the fifth month of pregnancy is regarded to be a significant factor in animal abortion. Females allantoic fluid components promote Brucella growth, making the uterus and reproductive tract of pregnant female the bacterial predilection location (de Figueiredo *et al.*, 2015). Bacteria continue to proliferate and shed in the environment in non-pregnant animals through various body fluids and excretions. Because *Brucella* spp. are intracellular pathogens that can persist within phagocytic cells by employing numerous escape mechanisms to resist the host's immune system, they can develop from acute to chronic and carrier forms in the host. Humans, typically, do not produce clinical abortions due to brucellosis infections, thus constituting a dead-end host (amjad *et al.* 2019).

## Clínical Signs

The incubation period lasts from 5 days to several months. Chills and fever, severe headache, joint and low back pain and occasionally diarrhoea are among the common symptoms,

which are followed by an intermittent fever that lasts 1 to 5 weeks. Over months or years, the febrile phase recurs in waves (undulations) and remissions, and it can present as a fever of unclear etiology. anorexia, weight loss, stomach and joint discomfort, headaches, backaches, weakness, irritability, sleeplessness, depression and other symptoms may also be present. Lymph nodes may be slightly or moderately swollen, and splenomegaly may develop. Hepatomegaly affects up to 50% of individuals. Endocarditis or severe central nervous system problems are the most common causes of death in people with brucellosis (Hull and Schumaker, 2018). In animals it mainly causes reproductive problems like abortions, infertility, still birth, etc. Other signs include arthritis, lameness, mastitis and fistulous withers.

### Diagnosis

Serological assays like Complement Fixation Test, Mercaptoethanol, Standard Tube agglutination Test (STaT), Rose Bengal Plate agglutination Test (RBPT), Milk Ring Test (MRT) and Coombs Test whereas in molecular assay Polymerase Chain Reaction is used for the diagnosis of brucellosis.

### Treatment

If antibiotics are given, combination therapy of two or three drugs are more effective because relapse rates with monotherapy are high. Either doxycycline plus rifampicin / streptomycin or trimethoprim-sulphamethoxazole plus rifampicin / streptomycin are effective in doses for 21 days. The gold standard treatment is streptomycin 1g IM for 14 days and oral doxycycline 100 mg b.i.d. for 45 days (concurrently). Gentamicin 5mg/kg BW IM o.d. for 5 days can also be substituted for streptomycin.

### Prevention & Control

- Test and slaughter method will be the most rational approach
- Hygienic disposal of aborted foetus, uterine discharges
- Calfhood vaccination: Vaccination of all calves between 4-8 months of age with strain-19 vaccine
- avoid consumption of unpasteurized milk, cheese, ice cream or raw meat
- When handling animals or animal tissues wear glasses and gloves
- While handling livestock always cover open wounds on your skin

- While handling female during parturition wear protective clothing and gloves

## Conclusions

Brucellosis is a global public health issue as well as a threat to livestock. Because of the zoonotic significance of Brucella infection, it is critical to properly manage the disease, and specific tests should be utilized to screen for brucellosis in both humans and animals. To control and cure brucellosis, an accurate and prompt diagnosis is required. Farmers education and requisite training would also aid in the control of the illness.

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## Leptospirosis in dogs

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**L**eptospirosis is an acute chronic or clinically inapparent contagious disease of domesticated and wild animals as well as human. The characteristic manifestation of the disease being fever, anemia, icterus and abortion. Leptospirosis is a bacterial zoonosis that is common worldwide, especially in developing countries.

Synonyms of Leptospirosis are Weil's disease, Stuttgart disease, rice field workers disease, sugarcane workers disease, canine typhus. The organism affects kidneys, liver, lungs, brain and uterine tissues of the animal and causes multiple organ failure. Pathogenic *Leptospira* species have been found worldwide, on all continents except Antarctica. Leptospirosis is most common in warm and humid environment.

In India, Canine *Leptospira* has been recorded in Madras in 1932 by Ayyar and which is the earliest report in India. Leptospirosis is endemic in 5 states & 1 union territory of India. The endemic states are Gujarat, Maharashtra, Kerala, Tamil Nadu, Karnataka & the union territory of Andaman & Nicobar Islands.

The disease is seasonal in temperate climate and year-round in tropical climate and it is directly associated with the amount of rainfall. All the available evidence from endemic states suggests that leptospirosis is now emerging in India as an important public health problem.

In dogs leptospirosis is caused by *Leptospira interrogans* serovars i.e. *L. icterohaemorrhagica*, *L. canicola* and *L. Pomona*. Morphologically *Leptospira* is a thin, flexible, filamentous bacteria made up of fine spirals with hook-shaped ends.

Leptospirosis is mostly transmitted by the urine of an infected animal and is contagious as long as the urine is still moist as the bacteria can't survive dryness for a longer period. The transmission is possible through drinking water contaminated by the urine of rodents or wild animals. Dogs acquired the infection through ingestion of infected carcasses. The dogs which are vaccinated for the *Leptospira* vaccine may acquire the infection and shed the organism through urine while dogs remain asymptomatic.

Humans can contract the disease through contact with the urine or body fluids, excluding saliva, from infected animals, or contact with contaminated water, soil or food. The initial clinical signs are usually nonspecific and may include fever, depression, anorexia, stiffness, myalgia, shivering and weakness. The mucous membranes are often injected. Later, there may be signs of kidney involvement, with polydipsia, polyuria,

oliguria, or anuria, and haematuria or dehydration in some cases. Fever may or may not be apparent in dogs that present with kidney signs. Vomiting, diarrhea, abdominal pain, grey stools, weight loss, jaundice, conjunctivitis, and abortions can also be seen. Hemorrhagic syndromes occur in some dogs: the mucus membranes may have petechial and ecchymotic hemorrhages, and in later stages of the disease, there may be other signs such as hemorrhagic gastroenteritis and epistaxis. Leptospiral pulmonary hemorrhage syndrome has been seen in dogs and can cause coughing, tachypnoea or dyspnoea. Vasculitis may result in peripheral edema and mild pleural or peritoneal effusion. Evidence of pancreatitis or myocardial involvement has also been reported. Deaths can occur at any time, including the acute stage. Chronic kidney disease can be a sequela.

Microscopic agglutination test (MAT) is the internationally recognized test for the diagnosis of *Leptospira*. For treatment, intramuscular injection of penicillin, streptomycin or tetracycline, is recommended. The treatment should be continued for 5-7 days. Supportive treatment should be given to monitoring kidney function. Corticosteroids may be given to speed up the elimination of urea.

The first line of leptospirosis prevention is to avoid exposure. avoid contact with floodwater, and do not eat food contaminated with floodwater. Treat unsafe or potentially contaminated drinking water by boiling or chemically treating it. Keep rodent populations (rats and mice) or other animal pests under control. Do not eat food that may have been exposed to rodents and possibly contaminated with their urine. Infected animals must be segregated from the rest of the animals

Four serovars are used for regular vaccination in India. They are *Ícterohaemorrhagiae*, *Canicola*, *Pomona*, and *Gríppotyphosa*. a killed mixed vaccine of above mention leptospira is used against leptospirosis in dogs.

## Canine Hypothyroidism

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### Introduction

Hypothyroidism is the most frequently diagnosed endocrinopathy in dogs and is characterized by diminished production of the thyroid hormones thyroxine (T4) and thyroxine (T3). Thyroid hormones influence a large number of metabolic processes in the body and in the event of disorders in hormone production, symptoms can arise from a number of organ systems. In addition to the most commonly occurring symptoms such as dermatological changes and signs of general metabolic disturbances, a number of neurological manifestations have been reported to occur in hypothyroidism in dogs. Neurological symptoms of hypothyroidism can originate from the central and peripheral nervous systems as well as from the muscles.

### Thyroid Hormone

Thyroxine (T4) and 3, 5, 3'-triiodothyronine (T3) are iodine-containing amino acids. Thyroid hormone synthesis requires iodine and is dependent upon ingestion of adequate iodine from the diet. Iodide is actively transported from the extracellular fluid into the thyroid follicular cell by the sodium-iodine symporter (NIS), where it is rapidly oxidized by thyroid peroxidase (TPO) into a reactive intermediate. At the apical membrane, iodine is incorporated into the tyrosine residues of Tg (Salvatore *et al.*, 2011). TPO also catalyzes the coupling of the non-biologically active iodinated tyrosine residues (monoiodotyrosine [MIT] and diiodotyrosine [DIT]) to form the biologically active iodothyronines-T4 and T3. These iodination reactions are referred to as organification.

### Hypothyroidism Classification

Hypothyroidism is the most common thyroid disorder in dogs and may be acquired or congenital. Hypothyroidism is classified as primary if it is due to an abnormality at

the level of the thyroid gland, secondary if it is due to decreased TSH secretion, and tertiary if it is due to TRH deficiency.

#### (a) Acquired Hypothyroidism

##### (1) Primary Hypothyroidism

It is the most common cause of naturally occurring thyroid failure in the adult dog, accounting for more than 95% of cases. Two histologic forms of primary hypothyroidism are recognized in dogs- lymphocytic thyroiditis and idiopathic atrophy. Other much rarer causes of primary hypothyroidism include iodine deficiency, goitrogen ingestion, congenital hypothyroidism, thyroid gland destruction by neoplasia, drug therapy, surgical thyroidectomy and treatment with radioactive iodine.

##### (a) Lymphocytic Thyroiditis

Lymphocytic thyroiditis is an immune-mediated disorder, where both humoral and cell mediated immunity play a role in pathogenesis. The major thyroid antigens that initiate an immune response in the thyroid gland are Tg and TPO. Lymphocytic thyroiditis is characterized histologically by diffuse infiltration of lymphocytes, plasma cells, and macrophages into the thyroid gland, resulting in progressive destruction of follicles and secondary fibrosis. Graham *et al.*, (2007) proposed four stages in the development of lymphocytic thyroiditis in dogs.

Stage 1	Stage 2	Stage 3	Stage 4
Subclinical Thyroiditis	Antibody Positive Subclinical Hypothyroidism	Antibody Positive Overt Hypothyroidism	Non-Inflammatory Atrophic Hypothyroidism
Focal lymphocytic thyroid gland infiltration.	Loss of greater than 60% to 70% of thyroid mass.	Most functional thyroid tissue is destroyed.	Replacement of thyroid tissue by fibrous and adipose tissue.
Positive Tg and Thyroid hormone autoantibody test.	Compensatory increase in TSH, which stimulates the thyroid gland to maintain normal T4 concentrations.	Decreased serum thyroid hormone concentrations and Increased TSH concentration.	Disappearance of inflammatory cell and circulating antibodies.

### **(b) Idiopathic atrophy**

It is characterized microscopically by a progressive reduction in the size of the thyroid follicles and replacement of the degenerating follicles with adipose tissue. The parathyroid glands are not affected, and variable numbers of parafollicular cells remain. Idiopathic thyroid atrophy may be either a primary degenerative disorder (Gosselin, *et al.*, 1981) or an end-stage of lymphocytic thyroiditis as evident by initial degenerative thyroidal parenchymal changes, which progressed to progressively worsening inflammation, subsequent fibrosis, and thyroid gland destruction.

### **(c) Neoplastic Destruction**

Clinical signs of hypothyroidism may develop following the destruction of more than 80% of the normal thyroid gland by an infiltrative tumour. Tumours may arise from the thyroid gland or unlike for may metastasize to or invade the thyroid gland from adjacent tissues. Interpretation of thyroid hormone concentrations in dogs with thyroid tumours is complicated by the effects of concurrent illness on serum thyroid hormone concentration (Benjamín *et al.*, 1996).

### **(d) Miscellaneous Causes**

Acquired primary hypothyroidism may rarely result from ingestion of goitrogens, administration of anti-thyroid medications (e.g. propylthiouracil and methimazole), and chronic use of high doses of potentiated sulfonamides. a palpable goiter may develop in dogs treated chronically with potentiated sulfonamides (Taeymans and O'Marra, 2009).

## **(2) Secondary Hypothyroidism**

Secondary hypothyroidism results from failure of pituitary thyrotrophs to develop due to pituitary malformation or acquired dysfunction of the pituitary thyrotrophs causing impaired secretion of TSH. Deficiency of TSH leads to decreased thyroid hormone synthesis and secretion and thyroid gland hypoplasia (Gal *et al.*, 2012).

Potential causes of secondary hypothyroidism include congenital malformations of the pituitary gland, pituitary destruction, and pituitary suppression. In the dog, secondary hypothyroidism caused by naturally acquired defects in pituitary thyrotroph function or destruction of pituitary thyrotrophs (e.g. pituitary neoplasia) is uncommon.

## **(3) Tertiary Hypothyroidism**

Tertiary hypothyroidism is defined as a deficiency in the secretion of TRH by peptidergic neurons in the supraoptic and paraventricular nuclei of the hypothalamus. Lack of TRH secretion causes deficiency of TSH secretion and follicular atrophy of the



thyroid gland. Neurologic signs and additional pituitary dysfunction may be present, depending on the cause. Tertiary hypothyroidism is assumed to be rare in dogs.

### **(B) Congenital Hypothyroidism**

Congenital hypothyroidism is rare in dogs. Unfortunately, congenital hypothyroidism frequently results in early puppy death, and the cause of death is rarely documented. A defect anywhere in the hypothalamic-pituitary-thyroid axis or of the thyroid hormone receptor can result in congenital hypothyroidism. Congenital hypothyroidism with goiter (CHG) develops if the hypothalamic-pituitary-thyroid gland axis is intact; TSH binds appropriately with its receptor, but there is an intra-thyroidal defect in thyroid hormone synthesis (dyshormonogenesis). Increased serum TSH concentrations result in the development of thyroid hyperplasia and a goiter.

## **Clinical Features of Hypothyroidism in the adult Dog**

### **A. General Metabolic Signs**

Most adult dogs with acquired hypothyroidism have clinical signs that result from a generalized decrease in metabolic rate. Clinical signs due to the decreased metabolic rate include mental dullness, lethargy, exercise intolerance or unwillingness to exercise, cold intolerance, and a propensity to gain weight without a corresponding increase in appetite or food intake.

### **B. Dermatologic Signs**

alterations in the skin and hair coat occur in 60% to 80% of hypothyroid dogs and are the most commonly observed abnormalities in dogs with hypothyroidism. Thyroid hormone is necessary to initiate and maintain the anagen or growing phase of the hair cycle. With thyroid hormone deficiency, hair follicles prematurely enter the telogen phase of the hair cycle. Excessive shedding with a lack of hair regrowth leads to alopecia. Decreased concentrations of cutaneous fatty acids and prostaglandin E<sub>2</sub> in canine hypothyroidism may lead to sebaceous gland atrophy, hyperkeratosis, scale formation, seborrhea sicca, and a dry and lusterless hair coat. In the early stages of hypothyroidism, hair loss is often asymmetric and develops over areas of excessive wear or pressure, such as the caudal thighs, ventral thorax, tail base, and tail (i.e. development of a "rat tail;". as hypothyroidism becomes more severe or chronic, alopecia becomes more symmetric and truncal, eventually developing into the classic cutaneous finding of bilaterally symmetric, nonpruritic truncal alopecia.

Hyperpigmentation is common in hypothyroidism, especially in regions of alopecia and areas of wear, such as the axilla and inguinal regions. In severe cases of hypothyroidism, the hygroscopic glycosaminoglycan, hyaluronic acid may accumulate in the dermis, bind water and result in increased thickness and non-pitting edema of the skin referred to as myxedema or cutaneous mucinosis. Myxedema predominantly affects the forehead, eyelids, and lips that contribute to the development of the classic “tragic facial expression” described in hypothyroid dogs.

### **C. Reproductive Signs**

Reproductive malfunctions include lack of libido, testicular atrophy, and oligospermia or azospermia so hypothyroidism appears to be a cause of infertility in male dogs.

additional reproductive abnormalities have been reported as weak or silent estrus cycles, prolonged estrual bleeding, and inappropriate galactorrhea and gynecomastia which may develop following a thyroid hormone deficiency-induced increase in TRH secretion, which in turn stimulates prolactin secretion.

### **D. Ocular Signs**

Corneal ulceration, uveitis, lipid effusion into the aqueous humor, secondary glaucoma, lipemia retinalis, retinal detachment, keratoconjunctivitis sicca (KCS), and Horner’s syndrome have been reported in hypothyroid dogs.

### **E. Gastrointestinal Signs**

Constipation may occur, presumably as a result of alterations in electrical control activity and smooth muscle contractile responses in the gastrointestinal tract. Diarrhea has also been reported with hypothyroidism.

### **F. Neurologic Signs**

Both the peripheral nervous system and CNS may be affected by hypothyroidism. Diffuse peripheral neuropathy characterized by exercise intolerance, weakness, ataxia, quadriplegia or paralysis, deficits of conscious proprioception, and decreased spinal reflexes have been reported to occur in dogs with hypothyroidism.

### **➤ Other Neurologic Disorders**

- Laryngeal Paralysis and megaesophagus may both occur in association with hypothyroidism.
- Myasthenia gravis
- Myxedema Coma

## **Díagnósis and clínico-pathologic abnormalities of Hypothyroidism**

- Complete Blood Count
- Serum Biochemistry Panel
- Urinalysis
- Conventional Radiography
- Ultrasonography
- Nuclear Imaging
- Baseline Serum Total Thyroxine Concentration
- Baseline Serum Total Triiodothyronine Concentration
- Baseline Serum Free Thyroxine Concentration
- Thyrotropin Stimulation Test
- Thyrotropin-Releasing Hormone Stimulation Test
- Tests for Lymphocytic Thyroiditis

## **Treatment**

The initial treatment of choice is synthetic L-T4 sodium. The same treatment protocol is used for both a therapeutic trial and definitive therapy. Treatment with L-T4 sodium preserves normal regulation of T4 to T3 de-iodination, which allows physiologic regulation of individual tissue T3 concentrations. The recommended initial dose for otherwise healthy hypothyroid dogs is 0.02 mg/kg by mouth every 12 hours. The dose for treatment of hypothyroid dogs is 10 times higher than the dose used in hypothyroid humans because of poorer gastrointestinal absorption and a shorter serum half-life of T4 in dogs compared to humans.

## **Response to Levothyroxine Sodium Therapy**

Thyroid hormone supplementation should be continued for a minimum of 6 to 8 weeks. So that all of the clinical signs and clinic-pathologic abnormalities associated with hypothyroidism should resolve.

- 1) As evident by an increase in mental alertness and activity usually occurs within the first week of treatment this is an important early indicator that the diagnosis of hypothyroidism was correct.
- 2) Some hair regrowth may be observed during the first month in dogs with endocrine alopecia, it may take several months for complete regrowth and a marked reduction

in hyperpigmentation of the skin to occur. Initially, the hair coat may appear to worsen as hairs in the telogen stage of the hair cycle are shed.

- 3) If obesity is caused by hypothyroidism, it should also begin to improve within 2 months after initiating L-T4 sodium therapy along with adjustments in diet and exercise.
- 4) Improvement in myocardial function is usually evident within 1 to 2 months, but it may take as long as 12 months for complete recovery.
- 5) Neurologic deficits improve rapidly after treatment, but complete resolution may take 2 to 3 months.

## Conclusion

The thyroid gland is an essential gland in the body, producing a number of hormones, including T3 and T4 both of which are required for normal metabolism in the body. So it is the most frequently diagnosed and one of the most over-diagnosed endocrinopathies in dogs. Don't rely on T4 alone to diagnose hypothyroidism. a normal or low TSH does not rule out hypothyroidism. fT4ED testing is an ideal test to help confirm hypothyroidism – Low Total T4 combined with low fT4ED has a diagnostic accuracy > 95% in hypothyroidism. If the non-thyroidal illness is involved, postpone additional thyroid diagnostics until NTI is resolved. Replacement therapy with synthetic L-thyroxine is the most appropriate treatment

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## *Aspergillus fumigatus* Infections in Cattle: A review

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### *Abstract*

The importance of aspergillosis in cattle has increased over the last decades. *aspergillus* species are found worldwide in almost all domestic animals, causing a wide range of diseases from localized infections to fatal disseminated diseases. Some prevalent forms of animal aspergillosis are mycotic abortion, mastitis, pneumonia, gastrointestinal aspergillosis, and Hemorrhagic bowel syndrome (HBS) in cattle. The aspergillosis causes heavy economic losses to the farmer. Hence proper management practices are implemented to reduce these infections in animals. This article represents a comprehensive overview of the most common infections reported by *aspergillus* species.

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**Keywords-** *Aspergillus fumigatus*, Mycotic abortion, Mycotic mastitis, Mycotoxins

### **Introduction**

**A** *aspergillus fumigatus* is an infectious, cosmopolitan opportunistic saprophytic fungus. It causes severe clinical conditions such as mycotic abortion, mastitis, pneumonia, gastrointestinal aspergillosis, and Hemorrhagic bowel syndrome (HBS) in cattle (Puntenney et al., 2003). These usually occur during the winter and spring months since cows are often kept in total confinement and are exposed to moldy hay or silage. It drives economic loss to the farmer. The financial losses are due to the loss of young ones, reduced milk production, expenditure on treatment. The development of many complications such as retention of the placenta, endometritis, infertility, sterility, pyometra, and delayed conception is seen in such animals (Radostits et al, 2007). It is also responsible for the contamination of milk with several mycotoxins, which renders it unfit for human consumption and consequently affects the milk industry.

The respiratory tract is considered the primary portal of airborne conidia of *A. fumigatus*. Primarily lungs are affected due to the inhalation of the organisms from the environment, and the

The infection spreads to other vital organs. The microorganisms also enter by ingestion of fungal spores in mouldy fodder. Cattle consuming spoiled silage demonstrated generalized deterioration typical of protein deficiency, malnutrition, diarrhoea, irritability, abnormal behavior, and occasional death (Whitlow et al 2019).

Mycotoxins are produced by these fungi cause mycotoxicosis in animals after their exposure. Exposure is usually by consuming contaminated feeds but may also be by contact or inhalation. The nutritional diet of domestic cattle consists of forages, concentrates, preserved feeds, by-product feeds, and wet feeds. They are exposed to a broader range of mycotoxins at concentrations that are perhaps higher than are found in dry grain mixtures. The rumen flora depresses the activity of several mycotoxins while others pass through intact or transformed into metabolites with biological activity. as a result, the rumen's barrier function significantly controls the vulnerability of dairy cows to specific mycotoxins. These mycotoxins affect dairy cows by reducing feed consumption and nutrient utilization, altering rumen fermentation, suppressing immunity, and altering reproduction. The mycotoxins produced by *aspergillus fumigatus* are one of the most significant concerns to cattle welfare. The present paper highlights the most important clinical infections of these fungi in cattle.

### **Mycotic abortion:**

The chief fungus associated with mycotic abortion is *aspergillus fumigatus*, which has been recorded from over 60% of cases (Pal M, 2015). This form of abortion occurs sporadically, and its prevalence is influenced by poor quality contaminated fodder harvested in wet seasons. *aspergillus fumigatus* can proliferate in damp hay, in poor quality silage, and brewer's grains. Infection, which reaches the uterus hematogenous, causes placentitis leading to abortion late in gestation. There are no specific clinical signs observed in animals aborting due to fungal infections. However, the affected animal may exhibit vaginal discharge, hyperaemia of cervical mucosa, reduced appetite, fever, and retention of the placenta. The placentas were thickened, necrotic, haemorrhagic, and oedematous in most cases (Quinn et al, 2002). The aborted foetus may show discrete, raised lesions on the skin of the head and neck. The fungi can be recovered from the placenta, amniotic fluid, fetal abomasal contents.

### **Mycotic pneumonia**

Pulmonary mycoses are essential from public health and economic point of view as they lead to high mortality, especially in immune-compromised animals and can be of great zoonotic importance. although mycotic pneumonia is rare in cattle, it can be caused by *aspergillus* species and other opportunistic fungal pathogens. The inhalation of spores causes



infection. Mycotic pneumonia is associated with lesions such as epithelial metaplasia of the airways, bronchitis, bronchiectasis, and interstitial fibroplasia. Respiratory signs like coughing, nasal discharge, dyspnea, generalized weakness are visible in affected animals (Cordes et al, 1964).

### **Mycotic Mastitis:**

Mycotic Mastitis occasionally results from the accidental introduction of *a. fumigatus* spores into the mammary gland on an intramammary tube. The cattle with immunocompromised status are susceptible to mycotoxins produced by these invading fungi resulting from poor animal hygiene. *aspergillus fumigatus* is isolated from the milk and mammary tissue of the cow. The affected udder tissue showed many nodules granulomata, with caseous centers containing fungal hyphae. Infection of the mammary gland leads to abnormalities such as a watery appearance of milk, flakes, clots, blood or pus in milk (Thompson et al, 1978). Mycotic Mastitis leads to a decline in potassium, lactoferrin, and casein content in milk and milk yield.

### **Gastrointestinal aspergillosis:**

This ailment is observed in cattle that ingest heavy loads of spores of *aspergillus fumigatus* from moldy feedstuffs. Clinically, all animals were generally poor and showed inappetence, lack of rumen contractions, diarrhea and melena. The target organ for fungal infection was the omasum, followed by the rumen, reticulum, and abomasum. The relatively short interval between onset of gastrointestinal symptoms and death, the predominance of acute and sub-acute lesions, and the occurrence of multiple lesions indicate that mycotic infections of the bovine gastrointestinal canal are established quickly, simultaneously, and terminally (Jensen et al, 1994).

### **Hemorrhagic bowel syndrome (HBS):**

It is also known as a jejunal haemorrhagic syndrome or jejunal hematoma. It is a relatively new and increasing disorder reported as sporadic, acute and necrohemorrhagic enteritis with high fatality rate in dairy and beef cattle. Clinical signs of the disease are decreased feed intake, depression, reduced milk production, dehydration, abdominal distension and dark clotted blood in the feces. *aspergillus fumigatus* have been suggested as the potential cause, because these organisms have been isolated from the lesions of clinical cases (Owakí et al, 2015). However, apparent causes of the disease are still not known.

### Prevention and control of *aspergillus fumigatus* infections in Cattle:

- The addition of mycotoxin binders to contaminated diets has decontaminated mycotoxins in the feed by binding them strongly enough to prevent toxic interactions with the consuming animal and prevent mycotoxin absorption across the digestive tract. Potential absorbent materials include activated carbon, aluminosilicates, etc.
- Drought and insect damage are most important in instigating mould growth and mycotoxin formation in the field. Therefore, varieties resistant to fungal disease or insect damage should be encouraged as feed for cattle.
- After harvest, grains should not remain at moisture levels greater than 15 to 18%. To reduce the moisture level, sufficient storage, aeration and cooling of grains should be done.
- The feed should be protected from rain or water by a vapor barrier and it should be checked regularly.
- All the storage sites are cleaned to reduce contamination.
- Preservatives can be used for high moisture levels for proper storage.
- Dry cows, springing heifers, and calves should receive the cleanest feed possible.

### Conclusion:

Ingestion of *aspergillus fumigatus* contaminated feed in large amounts can be the primary agent causing acute clinical conditions in cattle and significant factors contributing to chronic problems such as higher incidence of diseases, poor reproductive performance, or

suboptimal milk production. It also affects human health as toxin residues may be present in milk. Thus, it is imperative to adopt better and cost-effective management practices to decrease the occurrence of clinical conditions in cattle caused by *aspergillus fumigatus*

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# Clinical Application of Liver Function Test A Brief Review

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## INTRODUCTION

The liver has a tremendous capacity for regeneration and its functional mass in health greatly exceeds the body's needs. The liver creates bile to aid digestion and help rid the body of waste. The liver also helps metabolize fat, protein, carbohydrates, vitamins, minerals. additionally, the liver filters out the toxins and waste found in foods and medications.

Liver function tests are the biochemical investigation to assess the capacity of the liver to carry out the functions it performs. a liver function test will help to detect the abnormalities and the extent of liver damage.

### **FUNCTIONS OF LIVER:**

#### **1. Metabolic Function:**

- a) **Carbohydrate metabolism:** Glycolysis, Glycogenesis, Glycogenolysis, Gluconeogenesis, conversion of Galactose and Fructose, blood glucose regulation.
- b) **protein metabolism:** protein catabolism, urea cycle, amino acid metabolism, hormone synthesis.
- c) **fat metabolism:** fatty acid breakdown/ oxidation, cholesterol synthesis.
- d) **citric acid cycle (TCA)& ATP synthesis**

#### **2. Synthetic Function:**

- a) **protein metabolism:** synthesis of plasma protein, coagulation factors, enzyme synthesis.
- b) **fat metabolism:** synthesis of cholesterol, triacylglycerol, Fatty acids and lipoproteins, and digestion of lipids: with the help of bile salts.
- c) **excretory function:** bile pigments, bile salts, and cholesterol are excreted in the bile into the intestine.

#### **Protective function & detoxification**

- ammonia is detoxified to urea.
- Kupffer cell of liver perform phagocytosis to eliminate foreign compounds.
- Liver is responsible for the metabolism of xenobiotics.

**Storage function:** Glycogen, trace elements - iron, vitamin A, D & B<sub>12</sub>

## CLASSIFICATION BASED ON CLINICAL ASPECT

### Marker for Liver Dysfunction

- Serum bilirubin: van der Bergh reaction, icteric index.
- Serum: total protein, albumin, globulin, serum a: G ratio.
- Prothrombin time determination
- Urine: bilirubin, urobilinogen, urobilin.
- Fecal: stercobilinogen, stercobilin.
- Blood ammonia.

### Markers for Hepatocellular Injury

- Serum glutamate pyruvate transaminase (SGPT)
- Serum glutamate oxalate transaminase (SGOT)
- MaRKERS FOR CHOLESTaSIS
- alkaline phosphatase (aLP)
- Gama glutamyl transferase (GGT)

### Abnormal Protein Electrophoretic Patterns in Liver Diseases

- The rise in gamma globulins will have wide base, suggestive of Polyclonal gammopathy.

LÍVER DÍSEaSES	aBNORMaL PROTEÍÑ ELECTROPHORETÍC PaTTERNS	LEVEL
acute hepatitis	Per albumin	REDUCED.
Cirrrosis	albumin	REDUCED
	Gamma Globulins	ÍÑCREASED.
Hepatocellular Diseases	alpha-1 Globulins.	REDUCED
Biliary obstruction	alpha-2 Globulins, Beta Globulins	ÍÑCREASED.

### Liver Diseases and Transaminases

In viral/toxic hepatitis plasma aLT increases more than the aST where the cytoplasm sustains major damage. In cirrhosis increase in plasma aST more than the aLT where the cytoplasm and mitochondrial membrane are damaged. Plasma aLT, aST, and GGT are highly elevated in Cholestasis. aLT: aST ratio reversed in alcoholic hepatitis.

LÍVER DÍSEaSES	TRaNSaMÍÑaSES	LEVEL
Parenchymal liver cell damage	Plasma aLT and aST	Íñcreased
Viral / toxic hepatitis	Plasma aLT and aST	Íñcreased
Cirrrosis	Plasma aST>aLT	Íñcreased
Chronic active hepatitis	aLT and aST	Normal or Slight Íñcreased

Cholestasis	Plasma aLT and aST	Slight Increased
alcoholic hepatitis	Plasma aLT : aST ratio	Reversed

### Van Der Bergh Reaction

1. This is a reaction between **bilirubin** and **Ehrlich diazo reagent** giving a **reddish-purple** compound (Hepatic Jaundice).
2. **Conjugated bilirubin** reacts directly with the reagent. This is called: **direct bilirubin** (Obstructive Jaundice).
3. **Unconjugated bilirubin** does not react with the reagent directly except after the addition of methyl alcohol. Thus, it may be called: **indirect bilirubin** (Haemolytic Jaundice).

### HAEMOLYTIC (Pre-Hepatic) JAUNDICE

In serum, unconjugated bilirubin will increase. aST, aLP, and aLT would be normal. In urine, bilirubin was not excreted and urobilinogen excretion would be more.

### OBSTRUCTIVE (Post-hepatic) JAUNDICE

In serum, conjugated bilirubin will increase. aLP would be increased and also aLT and aST would be marginally increased. In urine, bilirubin was excreted and urobilinogen excretion would be normal or decreased.

### HEPATIC (Intra-hepatic) JAUNDICE

In serum, both conjugated and unconjugated bilirubin will increase. aLT and aST would be increased and also aLP would be marginally increased. In urine, bilirubin was excreted and urobilinogen excretion would be normal or decreased.

### OTHER DIAGNOSTIC PROCEDURES

#### Radiology

Plain radiographs may be helpful in confirming hepatomegaly. The liver is the largest solid organ in the body. so, in plain radiography examination of the liver is unreliable. Contrast radiography is primarily indicated in diagnosing portal caval shunts.

#### Ultrasound

Ultrasound examination of the liver may assist in differentiating homogeneous enlargement from cellular infiltration and in differentiation hepatic from post-hepatic-cholestasis.

#### Biopsy

The hepatic biopsy is usually the only method by which the type of hepatic pathology can be characterized. Dogs with obstructive jaundice and chronic hepatocellular disease could be mainly deduced by hepatic biopsy done at the site on the Right side (9<sup>th</sup> to 11<sup>th</sup> intercostals space).

### CONCLUSION

Increased liver enzyme activities are common results in small animal practice and can suggest patterns of liver diseases. To evaluate the liver function, the laboratory test plays an important role in diagnosing along with the combination of history, clinical signs, physical examination,



diagnostic imaging, cytologic or histologic assessment of liver tissue, and other liver function test results must be considered.

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# Preventive and Health Management of Elephants

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## INTRODUCTION

**W**ildlife conservation, Veterinary, public health, and other organization allocated human, physical, and economic resources according to perceived needs and available resources. Asian elephants are an endangered species that illustrate the challenges of optimizing and integrating resources allocation for animal health and conservation. So, there is a need to identify strategic investment in Asian elephants' health that will yield maximal benefits for overall elephants' health and conservation.

## PREVENTIVE CARES

Elephants are susceptible to several diseases. many of which can be minimized by regular vaccination. these include tetanus, anthrax, elephant-pox, pasteurellosis, rabies, and clostridial infection. anaphylaxis is a potential complication associated with potent vaccination, so elephants should monitor closely 1 hour after vaccination. One can administer an intradermal test dose of 0.1 ml of a vaccine to look for the harmful effect of i/m or s/c full dose in sensitive elephants. Dosages for adult elephants are two to three times the suggested dosages for adult horses and cattle.

## COMMON DISEASES AND DISORDERS

### NUTRITIONAL DISEASES

General malnutrition was common in calves when they were imported or orphan

#### 1. Rickets

It occurs in young growing elephants. Occur mainly due to improper calcium: phosphorus ratio in feeding diet. Clinically manifested by lameness. inward buckling of the tibiotarsal joint one or both rear legs, this problem probably results from abnormally rapid growth that is due to a high-protein diet can be seen with or without rickets. By correcting lameness 8- 9 % protein in the diet may help to treat any associated rickets. During treatment, an orthotic brace may support the limb.

## **2. Hypokalemic Tetany**

It can be seen mainly in captive elephants. Which housed indoors without any exposure to sunlight for longer periods. The effect of vitamin D deficiency leads to improper uptake or utilization of calcium. Tetany has also been reported as a result of stress (e.g. Rail transport) imposed on elephants with marginal calcium reserves.

## **3. Long – term Iron Deficiency**

It can cause iron-deficiency anemia in elephants. to avoid or prevent this problem this condition includes the addition of iron supplements (e.g. ferrous sulfate) to the diet or regular exposure to the clean earth.

## **4. White Muscle Disease**

It has occurred in newborn elephants. the calf was never able to stand and even a born live calf died after two weeks. One can prevent this by feeding pregnant cows includes 0.1 to 0.4 mg selenium/kg feed or injection of selenium with vitamin E to the cow 5mg / 70 kg body weight. The dose should be administered 6 to 8 months before parturition and repeated one month before parturition and administration to newborns.

## **VIRAL DISEASES**

### **Herpes Virus**

#### **Elephant Endotheliotropic Virus (EEHV)**

Elephant endotheliotropic virus is an infectious type of herpes virus found only in elephants. it is a complex disease and the symptoms include swelling of the head and trunk, ulceration of the mouth, and internal hemorrhaging. it seems that young elephants between 2 and 8 years old are particularly susceptible to death from equal endotheliotropic herpes virus. it is a very significant risk to young elephants in zoos and there is no vaccine or reliable care.

#### **Encephalomyocarditis Virus (ECMV)**

acute or per-acute deaths are seen in ECMV. Postmortem lesions include pulmonary edema or congestive and cardiomyopathy. It's a zoonosis, but infections in humans are mild inapparent.

### **Elephant pox**

It's a vesicular skin disease. lesions are mostly seen in the head and trunk region, conjunctivitis and swollen, draining temporal gland, erosion, ulceration of mucous membrane, hoof sloughing, and lameness.

## **BACTERIAL DISEASES**

### **Tuberculosis**

elephant are susceptible to both human and bovine types of *Mycobacterium tuberculosis* and therefore attended should be TB free this disease which causes weakness and weight loss is provided by air-born infected droplets and appeared to be an increasing problem in captivity

### **Anthrax**

anthrax is an acute infection with a high mortality rate. it occurs in enzootic and epidemic form in tropical countries which anthrax is epidemic, it is best to immunize regularly. Sign of anthrax in elephant includes death and sudden collapse, high fever and hemorrhagic mucous membrane, colic, subcutaneous swelling, trembling, bloody diarrhea, severe pain, paralysis.

### **Salmonellosis**

It is often fatal to elephants and has been reported frequently. signs associated with elephants are diarrhea (usually diffuse and watery sometimes with blood and mucous), weakness, fever, and anorexia. always Salmonellosis should consider as part of differential diagnosis for diarrhea. diagnosis made by stool culture.

### **Tetanus**

This is caused by anaerobic spores forming bacillus *clostridium tetani*. the clinical signs include cleared gait, the muscle of mastication, or very badly affected especially the lower Jaw. signs similar to that of the horse. Proper vaccination should be given to maintain immunity. Chloral hydrate is used to control muscle tetany.

### **Hemorrhagic Septicemia (Pasteurellosis)**

HS is a common infectious disease, especially amongst wild elephants. symptoms or and frequent yawning, swelling of various parts of the body, respiratory disorders, and conversion received death by suffocation when domesticated elephants are in contact with the diseased cattle or buffaloes. it is strongly advised that the elephants be immunized.

### **Colibacillosis**

Colibacillosis is common in newborn calves that do not receive colostrums from cows. In poor sanitary conditions, Young elephants are exposed to a high concentration of *Escherichia coli*. Signs that are similar to the bovine calves.

## PARASITIC DISEASES

In elephants, all protozoa, trematodes, cestode, and nematodes infections are reported. protozoan like Trypanosoma, Babesia, and Piroplasma. Trypanosoma is commonly reported among elephants. Trematodes that are capable to cause infection are *Protofasciola robusta* (live in the bile duct or duodenum), *Fasciola hepatica*, and *F. Jackson* (causes liver diseases). Nematode parasites that cause diseases include ascarids (muscle, large arteries, fascia, bile duct, intestine), oxyurids, strongylids (similar to that of a horse), paramphistomes (large intestine), cyclostomes, syngamids (gapeworm), and filarids. Drugs used to treat internal parasites of elephants include thiabendazole, dichlorvos, piperazine, tetramisole, bisphenol, etc.

### Foot Disorders

Foot disorder is common in captive elephants compared to wild space. disorder of foot includes overgrown sole, cracked sole, cracked heel, overgrown sole and nails, split nails, ingrown nails, wounds, abscessation, and laminitis. foot rot is common in elephants where the sole gets worn and bacterial infection set in. care should be taken for keeping the elephants under clean condition by using 5 %  $\text{CuSO}_4$ , 10 % Formalin solution was applied on the affected area. this is reinvested by a broad-based antibiotic administrator.

### Disorders Of Digestive System

gastrointestinal disorders include colic, diarrhea, dental abscess, dental caries, constipation, foreign body injection, intussusceptions, hepatitis, cholelithiasis.

### Dermatological Condition

an Elephant's skin is very sensitive to the sun and in the wild individual will use mud or a dust bath to protect their skin. this also helps keep the skin in good condition. which is important in regulating body temperature. a prolonged period in an Indoor enclosure creates the opportunity for pathological skin conditions not seen in wild standing or laying in the same place where an animal urinates and defecating me also causes skin irritations.

### Work-Related Injuries

common accidental injuries occur in the elephant when where keep kept especially for working may suffer from trunk injuries and tusk injuries.

**Trunk Injuries:** such as crusting laceration damage to motor nerves trunk paralysis may occur in some cases due to nerve injuries.

**Tusk Injuries:** If the tusk breaks over the root canal there is hemorrhage and pain and with the danger of regulator infection leading to loss of the entire tusk

### MISCELLANEOUS

some elephants such as juveniles that had been rescued and brought to the elephant orphanage have died of either a broken heart or sudden death syndrome. The broken heart reaction may be avoided by providing other elephants as traveling companions. Thunder-storm also causes sudden death syndrome.

### CONCLUSION

a focus on one or a few diseases that are not strategically selected risks wasting limited resources by investing in programs that have little or no impact on overall elephant conservation efforts. There is a need to clarify the tradeoffs that exist and establish the outcomes, that will likely result from investment in elephant health. Consequently, elephant health programs should be well-supported as a part of overall conservation efforts. Elephant is important to save. Not just so for generation can grow with them, but because they are keystone species. That means they have an important role to play in the lives of another animal within their habits.

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# Neonatal coccidiosis in goat kids- A threat to goat husbandry

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## Abstract

Coccidiosis in goats is a protozoan infection caused by *Eimeria* coccidia parasites that develop in the small and large intestine and primarily affect goat kids. Several species of *Eimeria* are known to involve in causing the disease, however, only a few are highly pathogenic and manifest clinical diseases. Coccidiosis is of major economic concern due to losses caused by clinical disease (diarrhoea) and subclinical infections (low weight gain in particular). Clinical diarrhoea and the presence of *Eimeria* species oocysts in faeces are used to diagnose coccidiosis. The use of anticoccidial drugs and hygienic measures between kidding and weaning seasons are the mainstays of control of the disease.

**Keywords:** Coccidia, Diarrhoea, *Eimeria*, Kids,

## Introduction

The global goat population has increased dramatically in the recent years, reaching 875.5 million heads, with Asia having the highest goat population (FaO, 2013) mostly in developing countries including India and Pakistan (Aziz, 2010). However, gastrointestinal parasites are a serious concern for livestock production in tropical countries like India and coccidian parasites of the genus *Eimeria* are the most common cause. They continue to be one of the most significant restrictions to ruminant production, particularly in goats, because they can produce a variety of subclinical consequences such as growth depression, milk supply reduction, appetite loss, hypoproteinaemia and digestive inefficiency. Coccidiosis in goat is a serious disease caused by complicated parasite-host interactions, with a variety of factors impacting disease severity. Under specific management situations, coccidiosis is a substantial contributor to enteric disease, especially in young goats, and is also a great economically important disease (Smith and Sherman, 1994) because of the losses due to clinical symptoms (diarrhoea) and subclinical infections, particularly poor weight gain. The disease is more severe in 3-6 months old kids and also when animals of any age are kept

in unhygienic and overcrowded houses. Coccidian parasites frequently destroy intestinal epithelial cells and disrupt intestinal microbiota (Mohammed et al., 2010).

### Aetiology

It is caused by *Eimeria* coccidian parasites, which contribute to gastrointestinal disease, particularly in young or stressed goats under inadequate farm management, resulting in high goat kid mortality. Seventeen species of *Eimeria* spp are known to infect goats throughout the world. However, *E. arloingi*, *E. ninakohlyakimovae*, *E. hirci* and *E. christensenii* are particularly pathogenic to goat kids. Poor farm management, intake of contaminated food and water, nutritional deficiencies, age of kids, and climate conditions are all predisposing factors that contribute to the disease's spread.

### Symptoms

Clinical signs include

1. diarrhoea with or without mucus or blood (Fig. 1)
2. dehydration
3. emaciation
4. loss of body weight
5. rough and dull body coat (Fig: 2)
6. anorexia, and even
7. Some goats are actually constipated and die acutely without diarrhoea.



Fíg. 1. Díarrhoea in goat kids

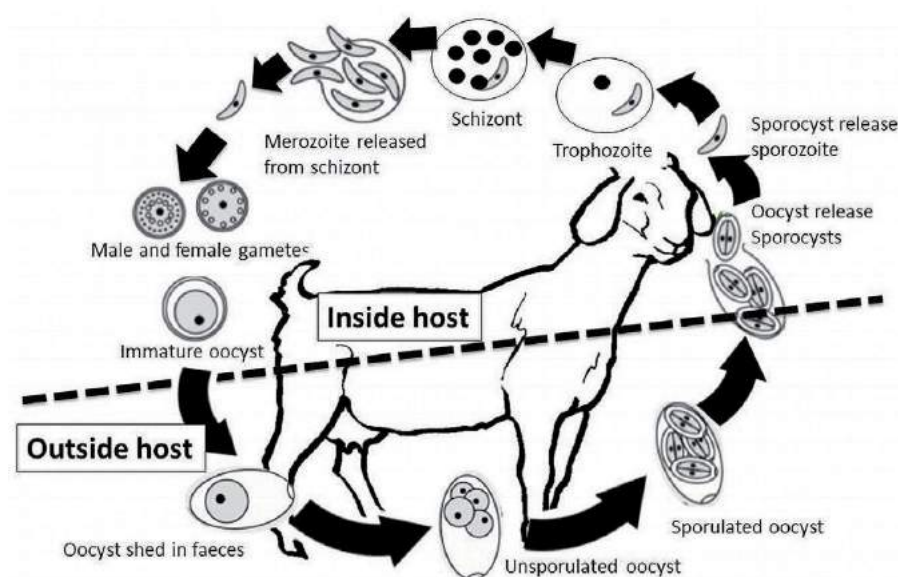


Fíg. 2. Rough body coat

## Pathogenesis

A variety of factors determine pathological and clinical results of coccidiosis, including the presence of *Eimeria* species, infection dosage, replication capacity, inflammatory response and concurrent infections by other pathogens, as well as treatment and associated stress. *Eimeria* species generally requires only one host to complete their entire life cycle. Two phases, schizogony/merogony and gamogony, develop within a host's intestinal cells. On the other hand, Sporogony/sporulation takes place outside of the host within an oocyst protecting infectious sporozoites. The life cycle includes an extracellular oocyst maturation stage (sporogony) and a parasitic intracellular stage inside the host with sexual and asexual reproduction (Foreyt, 1990). After passing through the faeces, unsporulated oocysts become infected after 2–7 days, depending on the *Eimeria* species and environment condition. The single cell is divided into four sporoblasts, each of which develops into a single sporocyst containing two sporozoites.

After being consumed by the host, the oocyst's walls degrade, releasing sporozoites from the sporocysts. Sporozoites penetrate the small intestine via an epithelial cell and develop into first-generation schizonts. Schizonts generate motile merozoites, which could either generate a second generation of schizont, gametes, and then non-sporulated oocysts, which are excreted with the faeces. The second generation schizogony in the large intestines generally



**Fig:** Life cycle of *Eimeria* species in goat (Taylor *et al.*, 2016)

occurs when another generation of merozoites invades epithelial cells and develops the sexual phases, male (microgametocytes) and female (macrogametocytes) gametocytes. Second-generation schizogony and gametocyte fertilisation (gametogony) results in functional and systemic abnormalities of the large intestine. In goats, the prepatent time for *Eimeria* species is around 19 days (Jones *et al.*, 1996); however, the prepatent period is 10 to 23 days depending upon the *Eimeria* species (Kusiluka and Kambarage., 1996).

### Diagnosis

Coccidiosis may be suspected when there are digestive problems in young goats due to poor hygienic conditions in the stall or intensive housing system. The most effective and reliable method for the diagnosis of *Eimeria* species is coproscopical examination. In an appropriate epidemiological situation, a low growth rate should lead to a diagnosis of subclinical coccidiosis.

### Prevention and control

1. In small ruminants, coccidiosis is normally controlled through a combination of appropriate management and anticoccidial drugs or prophylactics.
2. Monensin, amprolium, Sulphonamides, Decoquinate and Diclazuril are the drugs generally used for the prevention and treatment of these infections.
3. As there is no vaccine available for coccidiosis, prevention is mostly focused on herd management, which includes hygienic measures of the farm.
4. Coccidiosis outbreaks are an issue that affects whole herds and is triggered by stress. To prevent major infection, infected animals require a responsive immune system. as a consequence, reducing or eliminating stresses such as feed changes, severe weather conditions, crowding, frequent transportation, animal grouping, and exposure to other disease pathogens is critical to disease prevention.
5. Colostrum feeding is crucial for newborn ruminants since it protects them from infections. although *Eimeria* prevention is ineffective, protection from other infections helps to promote and avoid immune system problems in animals.
6. Coccidiosis control strategies need appropriate hygienic measures of the farm and the elimination of predisposing elements in the environment. Kidding pens should be maintained dry and clean, as well as bedding should be discarded when it becomes old or contaminated with oocysts. Buildings must be cleaned and disinfected using clean water or with surface disinfectants.

7. Feed and water troughs should be high enough to avoid faeces contamination. Feeding animals on the ground should be avoided, particularly when overcrowding. The regular rotation of pastures for parasite control will also assist in the controls of coccidial infection.

## Conclusion

Coccidiosis is a managerial disease of small ruminants particularly affecting young goats. It has great economic concern due to poor growth rate of the affected animal and mostly kids are diarrhoeic. Several factors are associated with the causation of the disease in kids where hygienic and overcrowding of the pen or farm determines the intensity of the disease. The presence of *Eimeria* species, infection dosage, replication capacity, inflammatory response, and concurrent infections by other pathogens, as well as treatment and related stress, are all factors that influence the pathological and clinical outcomes of coccidiosis. Maintaining sanitary measures and periodical use of anticoccidial drugs are the practices need to be followed in the farm for healthy and productive kids.

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## Etíopathogenesis, diagnóstico and therapeutic management of canine pyoderma

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### Abstract

Staphylococcal skin infection also known as staphylococcal pyoderma is the most frequently encountered dermatological affection in pet animal practice. *Staphylococcus pseudintermedius* is the commonly isolated bacteria from these lesions. Canine pyoderma can be superficial or deep and may be primary or secondary in origin characterized by pruritus, erythema, papule, pustule, and epidermal collarettes. Immune dysfunction is believed to be the predisposing factor. The emergence of antibiotic resistance has been a topic of zoonotic importance posing a hindrance to the effective management of the disease. The antibiotic resistance of the staphylococcal species to penicillins and cephalosporins is attributed to the presence of the *mecA* gene. Antibiotic therapy should be accompanied by culture and antibiotic susceptibility testing wherever feasible. This article provides insights about etiopathogenesis, diagnosis, and effective therapeutic management of canine pyoderma.

**Keywords:** *Staphylococcus pseudintermedius*, epidermal collarettes, *mecA*, antibiotic resistance, culture and antibiotic sensitivity

### Introduction

Canine skin and mucus membrane harbor a wide range of commensal resident microflora which can lead to infections of the skin, urinary and respiratory system in immunocompromised individuals. Pyoderma by definition is the cutaneous inflammation characterized by pus-filled lesions caused by pyogenic bacteria. Pyoderma is one of the most common dermatological disorders of canines. Canine skin is found unusually prone to *Staphylococcus* infection. The disease is usually secondary to underlying primary skin disorder but can also manifest as a primary infection on its own. Pyoderma can involve superficial or deep tissues of the skin. Staphylococcal deep pyoderma includes furunculosis, cellulitis, and pyogranulomatous dermatitis which can be difficult at times to treat. Recurrent pyoderma with an uncertain primary cause is termed as primary idiopathic recurrent pyoderma/ recurrent



pyoderma syndrome which includes recurrent superficial pyoderma, recurrent deep pyoderma, and German shepherd pyoderma (DeBoer, 1990).

This article provides clinical insights on bacteria involved in causing pyoderma, its diagnosis, and therapeutic management.

### Etíopathogenesis

*Staphylococcus pseudintermedius* is found to be the major causative agent of canine pyoderma. *Staphylococcus schleiferi* subspecies *coagulans* was isolated from suppurative skin lesions of canines (Bes, 2002). Gram-positive bacteria like coagulase-negative *Staphylococcus* (CoNS) species and *Streptococcus* species and Gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Serratia marcescens* were also isolated from canine skin infections (Nocera *et al.*, 2021). The pathogenesis of pyoderma is not well understood but immune dysfunction is believed to be the leading cause. The role of hypersensitivity reactions against staphylococcal antigens in the pathogenesis of canine superficial pyoderma is still debated and requires further validation (DeBoer, 1990).

### Clínical signs

All dogs are affected by pyoderma irrespective of breed, age, and sex. Clinical signs include pruritus, erythema, papules, pustules, epidermal collarettes, and crusts. Lesions are primarily concentrated in the inguinal, ventral abdominal, thoracic, and axillary regions. Deep pyoderma is characterized by furuncles and boils which may be found anywhere in the body. German shepherd dogs are found to be more susceptible to deep pyoderma of the interdigital space which is usually refractive and progresses even during treatment with antibiotics.

### Díagnosís

Impression smear from the lesions stained with Giemsa's reveals the presence of cocci and pus cells. Cytological examination of the pus or discharge from the pustules reveals the presence of suppurative to pyogranulomatous inflammation with degenerating neutrophils and neutrophils with phagocytosed gram-positive cocci. A sterile collection of exudates from intact pustule is used for culture and antibiogram. Methicillin-resistant *S. pseudintermedius* (MRSP) can be detected using polymerase chain reaction (PCR) targeting the *mecA* gene carried by the staphylococcal chromosome cassette *mec* (SCC*mec*) (Cain, 2013).

## Treatment

Systemic antibiotic therapy includes enrofloxacin at 5 to 20 mg/kg BW q24h, marbofloxacin at 2.75 to 5.5 mg/kg BW q24h (Caín, 2013), trimethoprim-sulpha combinations at 15 mg/kg BW q12h, ormetoprim-sulfadimethoxine at 27 mg/kg q24h, tylosin at 20 mg/kg, q12h, amoxicillin-clavulanic acid at 20 mg/kg q8h, cefadroxil and cephalexin at 20 mg/kg q8h (DeBoer, 1990). Topical therapy for canine pyoderma includes the application of ointments containing antibiotics such as mupirocin or fusidic acid, spraying solutions of chlorhexidine, or bathing with antiseptic shampoos like chlorhexidine, benzyl peroxide, or ethyl lactate (Borío *et al.*, 2015). Antimicrobial shampoos should be used twice weekly with a contact time of 5 to 10 minutes with lather until resolution of lesions. The treatment for superficial pyoderma requires 1 to 2 weeks past complete resolution of clinical signs whereas, deep pyoderma requires antibiotic therapy for a prolonged period of 8 to 12 weeks. Corticosteroids are contraindicated in the treatment of pyoderma as they dampen the inflammatory response giving a false appearance of resolution of the infection and cause immune dysfunction further aggravating the condition.

## Conclusion and discussion

Combined topical therapy with Chlorhexidine digluconate shampoo and solution at 4 percent concentration was found to be highly effective in resolving clinical signs caused by both methicillin-resistant and -susceptible staphylococci (Borío *et al.*, 2015). Clindamycin hydrochloride administered PO at 11mg/kg BW once daily produced excellent results compared to other systemic antibiotics used to treat deep staphylococcal pyoderma (Scott, 1998). Immunomodulators such as levamisole at 2mg/kg BW orally, every other day and cimetidine at 3 to 4 mg/kg BW orally twice daily can be used to counter the immune dysfunction (DeBoer, 1990).

Canine pyoderma is one of the most common dermatological disorders observed in small animal practice. Pyoderma may be deep or superficial occurring mainly as a secondary manifestation and occasionally as a primary dermatological disorder. The emergence of antibiotic resistance like methicillin- and multi-drug-resistant staphylococci has made the treatment of canine pyoderma an arduous task. Topical antibacterial therapy is found to be effective in counteracting antibiotic resistance and should be encouraged along with judicious antimicrobial treatment based on culture and susceptibility whenever possible.

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