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RAKSHA TECHNICAL REVIEW

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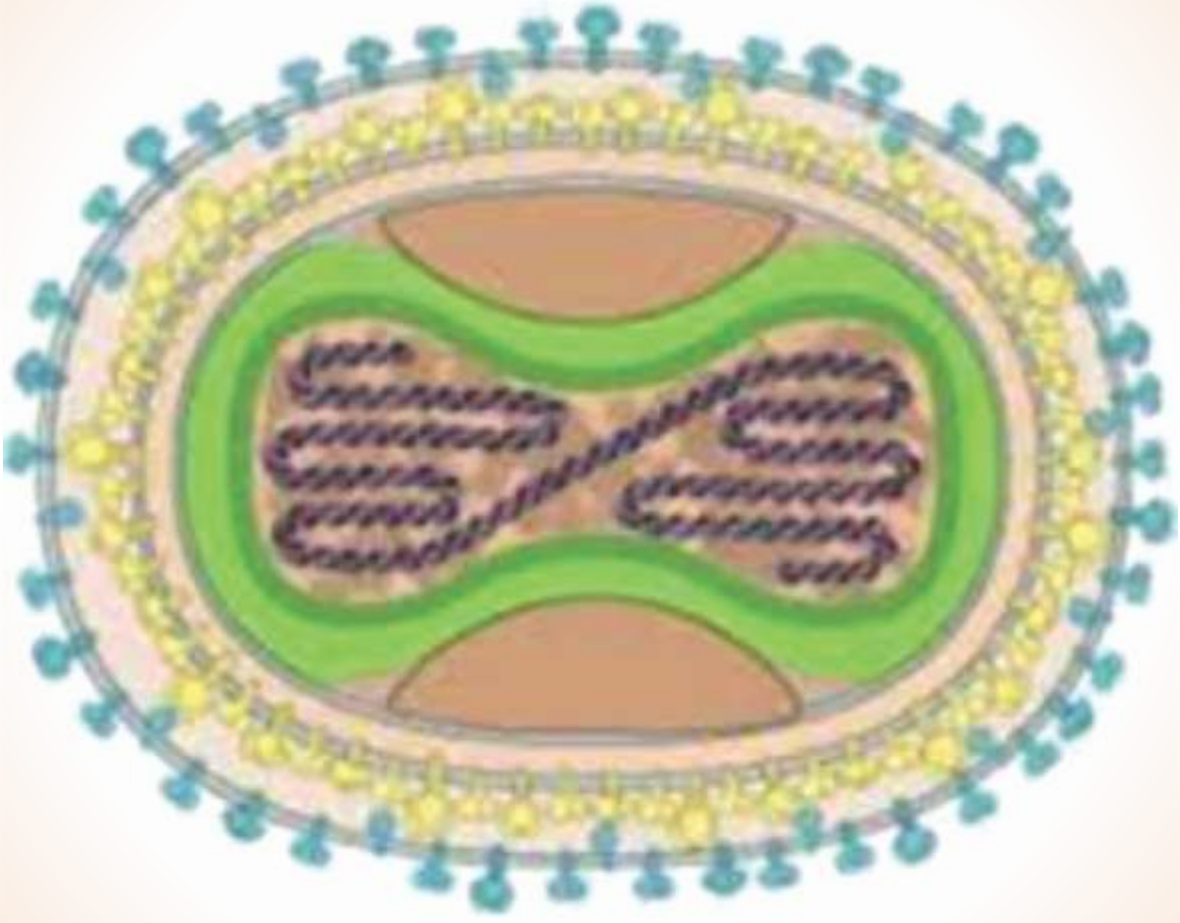
LSD Virus



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Contents

From the Editor's desk

Lumpy Skin Disease (LSD) is a highly contagious viral disease of cattle and buffalo. Infection typically includes symptoms like fever, 2-5 cm sized skin nodules, abortion in pregnant animals, depression and reduction in milk yield. The disease can result in animal welfare issues and significant production losses. LSD is spread by the movement of affected animals, by insects or parasites such as flies, mosquitoes and ticks, by contaminated equipment and directly from animal to animal in some cases. The disease and its impact on milk output can be arrested if cattle are vaccinated on time.

Large Animal Section brings an article *Lumpy Skin Disease: An Update* describing elaborately the aetiology, epidemiology, diagnosis, prevention and control of the disease.

The article *Brucellosis and Its Public Health Significance in India: A Review* explains the epidemiology, transmission, clinic signs, risk factors, pathogenesis, diagnosis, prevention and control measures.

The article *Paratuberculosis: The Mystic Killer* draws the reader's attention that the disease is a major health concern, causes losses to rural economy summarises its aetiology, transmission, diagnosis, treatment and prevention and control.

An Overview of Herpes Viral Infection in Horses article explains the viruses that cause the disease, vaccines to prevent and control and management practices to reduce the spread of infection.

Indigenous Breed Section in this issue brings an article *Kharai Camels of Kutch: Unique Germplasm of Gujarat.*

In Grazers and Browsers Section, the article *Therapeutic Management of Contagious Ecthyma in Goat: A Case Report* has given how the treatment and recovery of the goat were done and in the article *Management of Dystocia due to Lateral Deviation of Fetal Head and Neck in an Indigenous Goat: A Case Report* the author is cautioning the importance of specialist intervention to save both fetus and dam.

In Companion Animal Section, the article *Successful Medical Management of A Rare Case of Pica in Dog* stresses the need to constantly monitor of pica habits of a dog and educate the clients on the basics and the real health hazards to the pet.

The article *Successful Medical Management of Canine Juvenile Cellulitis (Puppy Strangles): A Case Report* suggests that successfully managing the condition by taking proper home care, hygiene, sanitation, restricted body movements and balanced food substantially contributes to the healing process.

The article *Surgical Management of Advanced Cystourolithiasis in a Spayed Female Dog* lists the surgical management procedure from identification to the removal of the stones and stresses educating the client on the importance of home food management with optimized protein content.

The article *Successful Surgical Excision of a Ventrolateral Neck Region Malignant Melanoma Mass in a Senescent Female Dog* describes the surgical procedure and recovery done.

In General Articles Section, the Articles *Virophages: Are They Future Antivirals; Potential Threat of Monkeypox: A Review; Housing Management of Pet Birds; and Asian Palm Civet: An Economic Importance* are of the reader's interest in knowledge.

Wishing all our readers, a very happy and prosperous New Year 2023!!

Dr Priyabrata Pattnaik
Dy Managing Director

Managing Director's Message

Dear Patrons,

Season's Greetings!!

Recovering from pandemic, the World is witnessing the war-related consequences on certain global economies. Forecast of recessionary trends is looming large across the globe. While the pandemic is receding, newer zoonotic diseases are posing challenges to us. Be it Lumpy Skin Disease or Monkey Pox Virus, conflict of human – animal interaction is more evident now than the past.

Silver lining to the dark cloud is that our company, Indian Immunologicals Limited, is rising to the occasion in support of the nation. IIL is working very closely with various Government Institutions e.g., IVRI, ICMR etc., in dealing with several zoonotic diseases.

Apart from veterinary and human vaccines, IIL recently forayed into Aqua business. IIL has tied up with various Aqua research Institutions e.g., Central Institute of Fresh Water Aqua Culture (CIFA), Central Institute of Brackish Water Aqua Culture (CIBA) etc., for developing fish vaccines. IIL is expecting to launch fish vaccines within the next two – three years.

WISH YOU ALL A VERY HAPPY AND PROSPEROUS NEW YEAR 2023!!

Warm Regards

Dr K Anand Kumar

Lumpy Skin Disease: An Update

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Abstract

Lumpy skin disease is a transboundary disease, caused by LSDV belonging to genus capripox of family poxviridae, characterized by onset of febrile condition and nodules on the body. It considered as a transboundary disease due to its significant impacts on trade and food security as well as its capacity to spread to other countries (1).The Office International des Epizooties classifies it as a "List A"- disease (2).It is diagnosed on the basis of peculiar clinical signs like raised, swollen, circumscribed skin nodules or lesions, swollen lymph nodes, lameness etc. In 2022, outbreaks are reported from Rajasthan, Madhya Pradesh, Gujarat, Punjab, Haryana, Maharashtra and Uttar Pradesh and severity is noticed on a higher scale than the cases reported for the first time in India in the year 2019. It is believed certain variants of LSDV have led to increased infectivity among the cattle. The disease being documented in various parts of the country has revealed considerable economic losses due to decrease in milk production, infertility, treatment costs, abortions and mortalities.

Introduction

The disease was observed for the first time in Zambia in 1929. Lumpy skin disease, also known as KNOPVELSIEKTE (3), belonging to the genus capripoxvirus has become one of the major dreadful conditions affecting the livestock abruptly. Cattle being the most susceptible species are always the target for viral proliferation. It is a transboundary viral disease and spreads very rapidly among other animals (4). Lumpy skin disease is manifested by distinguishing firm, circumscribed, few (mild forms) to multiple (severe forms) skin nodules, which sometimes involve mucous membranes of respiratory system, urogenital system and other internal organs (5). ICAR's National Research centre on Equines (NRCE) at Hissar, Haryana and the Indian Veterinary Research

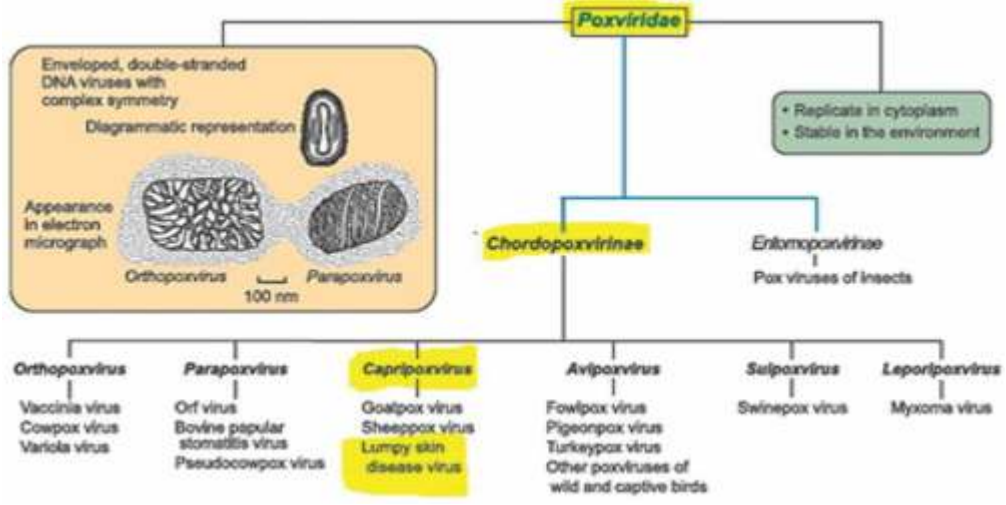
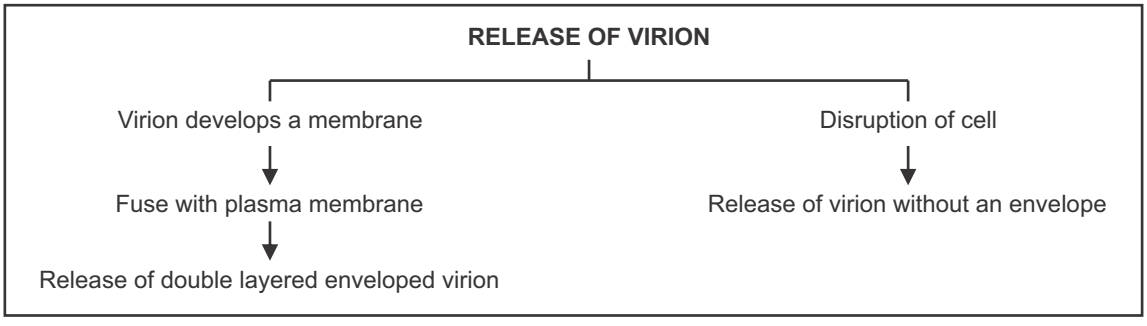


Fig. Poxviridae Diagrammatic Presentation (5)



institute(IVRI) developed a homologous vaccine. The vaccine, named Lumpi-ProVacInd, is live attenuated one. The culturing was done over 50 generations ("passages") and took about 17 months. Also, vaccine trial was conducted in cattle at IVRI.

Etiology

Lumpy skin disease is caused by Neethling prototype strain of poxvirus, belonging to the genus capripoxvirus and subfamily *Chordopoxvirinae.* Antigenically it's related with goat pox and sheep poxviruses which belong to the same genus. Poxvirus multiplies in the cytoplasm. Enveloped, dsDNA genome of LSDV is constituted of a virion which is uncoated by both cellular enzymes and enzyme products of an early transcribed mRNA. Cytopathic effect (CPE) of forming intracytoplasmic inclusion bodies is one of the characteristic features of the viral agent.

Phylogenesis

DNA analysis using restriction endonucleases on field samples and vaccine strains showed 80 % homology between strains of capripoxvirus (CaPV) (6). The different strains of capripox virus are very difficult to be differentiated on the basis of serology. Molecular studies have demonstrated that LSDV, SPPV and GTPV are phylogenetically distinct (7).

Epidemiology

A) Environmental Factors

The high incidence of cases is seen during late summer and during monsoon because of increase in insect breeding and their biting nature. Transportation has also a major impact for the disease occurrence. Introduction of new animal into the herd without quarantine measures can also be a contributing factor of this disease.

B) Host Factor

Mostly cattle are the ones affected but water buffaloes can also be considered as one of the susceptible host for Lumpy skin disease. Although genetic makeup has a role to play, immunity also predisposes the animal against the disease. Among all breeds, Indigenous breeds are considered to be more resistant to Lumpy skin disease (8) but in the present episode of 2022 outbreaks, severe infections were observed in indigenous breeds of cattle also. Wildlife species are not affected in natural outbreaks but can be a reservoir for lumpy skin disease. (3)

C) Pathogen Factor

Since the pathogen can survive for long durations under the normal temperature, it is not east to mitigate the virus. The virus can be inactivated at temperature of 55°C for 2 hours and 65°C for 30 minutes. It can be recovered from skin nodules kept at –80°C for 10 years and infected tissue culture fluid stored at 4°C for 6 months. It is susceptible to highly alkaline or acidic pH, but no significant reduction in titer when held at pH 6.6-8.6 for 5 days at 37°C (9).

Mode of Transmission

LSDV spreads with the aid of arthropods and hence is a vector borne disease. As the water logging condition harbors the growth of flies and mosquitoes, they are important mode of transmission during the rainy season. Peak summer also provides best temperature for the survival of certain ticks and mosquitoes and hence responsible for the transmission. Recent studies in ticks have shown transstadial and transovarial persistence of LSDV in *Rhipicephalus decoloratus, Rhipicephalus appendiculatus and Amblyomma hebraeum,* and mechanical or interstadial transmission by *Rhipicephalus appendiculatus and Amblyomma hebraeum (2).* Vehicles carrying the infected animal and healthier ones together also pave a way for disease transmission. Even the intrauterine transfer of the virus to the young ones has been reported. Improper hygiene is also an important reason for the disease to spread.

Source of Infection

Apart from the biting arthropods, LSDV also remains viable in dry scabs and nodules for long period of time and these dried skin lesions, nodules become a source of infection. Certain secretions of the infected animal like nasal, oral, lacrimal, ocular, milk, semen may act as a source of infection. Inanimate objects, fomites, contaminated needles and vaccines can serve as a source. The crusts that dry off and fall are the major source of virus to spread the disease.

Pathogenesis

Since Lumpy skin disease is majorly vector borne, the bite of the infected arthropod begins the mechanical transmission and once the virus enters the blood, initially febrile condition sets in followed by viremia. After the generalized infection sets in, there is endothelial and fibroblast damage, which leads to vasculitis, thrombosis, infarction and finally edema, causing inflammatory swellings or nodules (10). Intense hyperplasia sets up and is followed by degeneration of keratinocytes, which finally leads to infiltration of inflammatory cells into the dermis leading to deep ulcers. The lesions may dry and form scabs or crusts and carry the viral agent even if sloughed off. The inflammatory nodules prefer certain sites of predilection such as skin of head, neck, genitalia, udder, perineum and limbs.

Clinical Signs

- LSD can be classified into mild and severe forms based on the number of lumps (nodules) and occurrence of complications, dose of the inoculum as well as the susceptibility of the host and the density of insect population (5).
- Swollen superficial lymph nodes are pathognomic.
- Generalized cutaneous eruptions (0.5 cm to 5 cm diameter) which are raised and circumscribed in

appearance.

- Scabs develop in the center of nodules and after the scabs fall off, they leave large holes which become infected with secondary bacterial infections.
- Lumps develop in the skin after the onset of the febrile reaction.
- Marked drop in milk production is often seen.
- The affected ones become dull, depressed and are anorectic.
- There is purulent nasal discharge, conjunctivitis and excessive salivation.
- Animal becomes reluctant to move when ulcerative lesions or vesicles are present in the limbs and ventral part of the body.
- Lumpy skin disease may occur as acute, sub-acute and chronic forms.
- Lesions where skin is lost may remain visible for long periods. When lesions coalesce, large areas of raw tissue can be exposed, and these are susceptible to invasion with screwworm fly larvae (9).
- In addition, lactating cow's milk production may lessen, and mastitis occurs and possibly abortion in some pregnant cows; calves with extensive skin lesions, presumably acquired by intrauterine infection may be delivered. Swelling of the testicles and orchitis are also reported in infected bulls. Following lesions in reproductive organs, temporary or permanent sterility may occur in bulls and cows (3).

Gross Pathological Lesions

- Large irregular skin nodules seen, when incised has a greyish appearance.
- The nodules involved the epidermis, dermis, and subcutaneous tissue and may even spread to the musculature. Once the nodules necrotize, a deep scab is formed, known as 'sit fast'(11).
- Regional lymph nodes become enlarged (up to 10 times than their usual size), edematous, congested and having pyemic foci, in addition to local cellulitis (11).
- Pleuritis, bronchopneumonia are common findings.
- Haemorrhages in spleen, liver, rumen and in the GI, tract are also seen.
- Tracheal and Gall bladder ulcers are pathognomic for lumpy skin disease (10).
- Lesions in the skin of scrotum.

Histopathological Lesions

- The nodule is oedematous, and is composed of perivascular collections of lymphocytes, macrophages, plasma cells, and neutrophils and proliferating fibroblasts. There is acanthosis (thickened epidermis), parakeratosis (thickened stratum corneum containing pyknotic nuclei), and hyperkeratosis (thickened stratum corneum) of the epidermis followed by necrosis and vesicle formation (12).
- Large epithelioid macrophages are characteristic histopathological findings seen in the dermis and epidermis infiltrations.
- Endothelial proliferation is seen in the blood vessels of the dermis and subcutis, with lymphocytic cuffing of the blood vessels, which lead to the thrombosis and necrosis. Specific intracytoplasmic inclusions may be found in the various epithelial elements, sebaceous glands and follicular epithelium (13).
- Ulcerative lesions in the oral cavity.
- Ballooning degeneration of stratum spinosum with micro vesicles formation (11).
- Liquefactive necrosis is commonly found in the epidermal layer.
- Inflammation of blood vessels along with the wall thickening is also a common finding.
- Zenker's necrosis also seen in muscular layer.
- Aggregation of certain inflammatory cells like lymphocytes, neutrophils, red blood cells and macrophages could be seen.

Hematological and Serum Biochemical Changes

The haemato-biochemical analysis showed marked Leucopenia. Neutrophilia and Lymphopenia are also a common finding in LSDV infected animals. There is a decrease in total serum protein (14). The present study revealed significantly reduced serum GSH and elevated MDA in LSDV-infected cattle; these manifestations were attributed to increased oxidative stress due to free radical production and lipid peroxidation with the exhaustion of antioxidants in the blood, resulting in tissue injury (15, 16). There was an elevated IL-4 and TNF- concentration, which may be attributed to the stimulation of macrophages and lymphocytes to release different cytokines that initiate inflammation in different tissues during the viremic stage of the disease (17).

Diagnosis

1) Diagnosis is based on the clinical signs and various pathological lesions :

The major signs visible to be used as a diagnostic tool are:

- Large nodules on the whole body
- Enlarged or swollen lymph nodes
- Increased temperature
- Persistent emaciation and anorectic

- Atelectasis, pleuritis
- Nodules in the skin of scrotum
- Nodular lesions over the udder could also be seen
- Ulcerative lesions in the limbs prevent the animal from walking and hence laminitis is a common finding.
- Ulcerative lesions could be seen inside the buccal mucosa
- Same characteristic lesions appear in vagina and conjunctiva (18).
- Characteristic histopathological findings are :
- Lobular Atelectasis in lungs
- Pox like lesions in the pharynx, epiglottis, tongue
- Synovitis and tendosynovitis with fibrin in synovial fluid (13).

2) On the basis of laboratory confirmation of the viral agent: Using virus isolation technique, virus is grown bovine, caprine or ovine cell cultures; especially lamb testis cells (19).

3) Laboratory investigations and identification of the agent based on (9)

a) Polymerase Chain Reaction (PCR): Most peculiar diagnostic test is real time PCR for specific detection of capripoxvirus. Skin samples or blood samples can be used for PCR. Species-specific real-time PCR methods for differentiation between SPPV, GTPV and LSDV can be used (20).

b) Electron Microscopy: It's very useful in diagnosis of capripox virus.

c) Agar Gel Immunodiffusion

d) ELISA: mainly used to detect the accurate amount of antibodies.

e) Fluorescent Antibody Test: mainly used to detect a peculiar antigen.

f) Western Blotting: Can be used to detect the virus along with ELISA for the confirmatory diagnosis.

Differential Diagnosis

1. Pseudo lumpy skin disease ,Onchocerciasis, show almost similar skin lesions but can be differentiated from lumpy skin disease with the help of PCR.
2. In photosensitization, the skin lesions are mostly superficial, but the course of the disease is also very low.
3. In case of insect bites, there would be swelling in the region of bite or wheal formation is seen.
4. In urticaria, swelling over face, limbs and ventral side of abdomen are seen in urticaria unlike that of Lumpy skin disease and can be differentiated by PCR (21).
5. Dermatophilosis: early ringworm lesions, more superficial, clearly different, non-ulcerative surface structure of the ringworm lesion.
6. Demodicosis: dermal lesions predominantly over withers, neck, back, and
7. Flanks, often with alopecia present. The disease can be ruled out by detection of mites using skin scrapings.
8. Bovine papular stomatitis (Para poxvirus): lesions occur only in the mucous membranes of the mouth.

The disease can be ruled out by PCR testing.

Treatment

1. The main motive of treatment is to manage the symptoms
2. Long-acting antibiotic should be administered for at least 7 days
3. Antihistamines are a must
4. Vitamin supplements are to be given.
5. Since the scabs fall off, there are holes left behind which gets infected with secondary bacterial infection. So, use of topical ointments, antiseptics, are must.
6. Feeding soft and palatable feed is a must.
7. Ethnoveterinary Treatment of Lumpy Skin Disease:

- Neem oil 0.51g
- Ocimum sanctum (Fresh Tulsi leaves) 100 g
- Neem fresh leaves 100g
- Alium sativam 100g
- Curcuma longa 100g
- Aloe Vera 100g

Mix well and paint the animal for 10-15 days and the lesions will subside. (10)

8. Herbal mixture composed of

- Haldi,
- Aloe vera gel,
- Baking soda,
- Neem leaves,
- Betel leaves,
- Garlic & Peppers.

Prepare the mixture and can be fed orally to the infected ones. (10)

Prevention and Control

1. Since Lumpy skin disease is a vector born disease, the prime step in preventing the disease is by controlling the vector population. It's very important to prevent water logging conditions and there should be use of proper vector control measures like using mosquito nets.

2. The disease mostly spreads when the infected animals are in close contact, like during transportation. Proper quarantine measures are a must, before

transporting the animals and mixing them in a herd.

3. Once the animals are infected, routinely the farm should be disinfected with either sodium hypochlorite (2-3%), Formalin (1%), Iodine (1:33), or ether (20%), to avoid further contamination.

4. Unvaccinated cattle movement must be restricted.

5. Prophylactic measure to be obtained by proper vaccination of the herd in the endemic area. Annual vaccination is recommended in affected countries. Calves from naive mothers should be vaccinated at any age, while calves from vaccinated or naturally infected mothers should be vaccinated at 3-6 months of age (22).

Live Attenuated Lumpi-pro VacInd Vaccine

• A new vaccine is jointly being developed by ICAR's National Research centre on Equines (NRCE) at Hissar, Haryana and the Indian Veterinary Research institute(IVRI).

• The vaccine, named Lumpi-ProVacInd is live attenuated one. The culturing was done over 50 generations ("passages") and took about 17 months. Also, vaccine trial was conducted in cattle at IVRI.

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Brucellosis and Its Public Health Significance in India: A Review

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Introduction

Brucellosis is a re-emerging and neglected tropical bacterial zoonotic disease transmitted to humans by consumption of infected, unpasteurized animal milk or through direct contact with infected animals, particularly aborted fetuses. The livestock production losses resulting from these abortions have a major economic impact on individuals and communities. The disease is partly owing to a lack of knowledge and a lack of investment in surveillance and control methods (1). In a survey of 76 animal diseases and syndromes, brucellosis is ranked top ten disease in terms of their impact on poor people (2). Brucella are Gram-negative, intracellular, non-motile, non-spore-forming coccobacilli that primarily infect ruminants and wildlife. It is known as true zoonosis since almost all human diseases originate from animals (3). Human brucellosis is known as Remitting fever, Undulant fever, Cyprus fever, Crimean fever, Mediterranean fever, Maltese fever, Goat fever, Gibraltar fever, or Rock fever, and bovine brucellosis is known as Melitococcosis, Bang's disease, Contagious abortion, Epizootic abortion, or 'Kandruveechu noiy' (Tamil) (4). *Brucella abortus* was the most commonly reported Brucella species in domestic animals and wildlife, among wild animals highest incidence rate identified in American bison *(Bison bison)* (39.9%), followed by Alpine ibex *(Capra ibex)* (33%) (5). By consuming polluted forage, wild ungulates can become infected (6). Because of the massive increase in worldwide trade in animal products, rapid deforestation, unplanned and unsustainable development, urbanization, intensive farming, migratory/nomadic animal husbandry, and foreign travel, brucellosis' zoonotic importance is growing (7).

Each year, 500,000 new human cases of brucellosis are reported worldwide, making it the most common bacterial zoonosis. The global incidence of Brucellosis ranges from 0.01 to 200 per 100,000 people. In India also, human brucellosis prevalence varies across the states (3). The World Health Organization (WHO) estimates that the true incidence is at least one order of magnetite higher. Patients with Brucellosis, as well as their family members, should be tested on a regular basis in endemic locations. Surveillance, prevention of transmission, and control of the infection reservoir by various ways, including culling, are all effective control tactics for this illness (7).

History

The sickness was first identified as a distinct clinical entity on the island of Malta as Mediterranean remittent fever in 1863. Sir David Bruce, Hughes, and Sir Themistocles Zammit characterized the condition in detail in 1886. *B. melitensis* was first isolated from the spleen of a human patient who died of Malta fever in 1887(8). Bernhard Bang was the first to discover *B. abortus,* which is known to cause undulant fever in humans and abortions in cattle (9). *B. suis* was recovered from swine by Traum and Huddleson. Stoenner and Lackman isolated *B. neotomae* from rats (10). Carmicheal and Bruner discovered *B. canis* from dogs. *B. pinnipedialis* and *B. ceti* are newer Brucellae that have been isolated from marine mammals in the last decade and could pose a future zoonotic concern (11). *B. microti* has been found in terrestrial animals (12).

Epidemiology

Agent

Brucellae are Gram-negative *coccobacilli* (short rods) that are 0.6 to 1.5 m by 0.5-0.7 m in size. They are non-motile because they are non-sporing and lack capsules or flagella. The DNA has a guanine-plus-cytosine concentration of 55-58 moles/cm. Except for *B. suis* biovar 3, the genome has two circular chromosomes of 2.1 Mb and 1.5 Mb each, which contains a single 3.1 Mb chromosome.

Antigenic Structure

The most pathogenic and invasive species are *B. melitensis,* followed by *B. suis, B. abortus* and *B. canis.* Two main somatic antigens of brucellae, A and M are present in different amounts in the three major species. Antigen 'A' is dominant (about 20 times as much as 'M' antigen) in *B. abortus* and antigen 'M' is dominant in *B. melitensis* (about 20 times of 'A' antigen). *B. suis* has an intermediate antigenic pattern.

Host

The epidemiological evidence suggests that *B. abortus, B. melitensis* and *B. suis* show distinct host preferences, this only marks in general trend and the organisms can establish infection in a wide range of host species, including man. However, *B.neotome, B. canis* and *B. ovis* show greater host specificity. In cattle, the infection is predominantly caused by *B. abortus,* less frequently by B. melitensis and occasionally by *B. suis.* Pappas (13) demonstrate the detailed host preference of Brucella spp. **(Table1).**

The disease has been reported in wild animals in some African countries, including Kenya, South Africa,

Table 1: Zoonosis and Host Preference of Brucella Spp.

S.No.	*Brucella spp.*	Colony	Natural Host	Zoonoses
1.	***B. melitensis (bv. 1-3)***	Smooth	Goat & sheep	+++
2.	***B. abortus (bv. 1-6, 7, 9)***	Smooth	Cattle	++
3.	***B. suis (bv. 1&3)***	Smooth	Pig	++
	2	Smooth	Wild boar, Hare	+
	4	Smooth	Reindeer, Caribou	++
	5	Smooth	Rodent	-
4.	***B. ovis***	Rough	Sheep	-
5.	***B. neotomae***	Smooth	Desert Rat	+
6.	***B. canis***	Rough	Dog	+
7.	***B. ceti (B. delphini)***	Smooth	Dolphins	+
8.	***B. pinnipedialis***	Smooth	Seals	+
9.	***B. microti***	Smooth	Wild voles	(?)
10.	***B. inopinata***	Smooth	Human	++
11.	***B. papionis***	(?)	Baboons (Papio spp.)	(?)
12.	***B. vulpis***	(?)	Red foxes (Vulpes vulpes)	(?)

Zimbabwe and Tanzania. Among Tanzanian wild animals, Brucella infections have been reported in topi *(Damaliscus lunatus jimela),* buffalo *(Syncerus caffer),* impala *(Aepyceros melampus),* thompson gazelle *(Eudorcas thomsonii)* and wildebeest *(Connochaetes)* (1).

Distribution of Brucellosis

Global Distribution

Brucella, which spreads in more than 170 countries and regions around the world is endemic in India (14).Only 17 countries claim to be free of brucellosis (15). Though it has been eradicated from almost all of Europe, reports still indicate presence of human brucellosis in Greece, Spain and Turkey. Brucellosis free status has been granted by the European Union (EU) to Sweden, Denmark, Finland, Germany, the UK (excluding Northern Ireland), Austria, Netherland, Belgium and Luxembourg (16). Norway, Switzerland and France are also considered brucellosis free countries,as well as Canada, Japan, Australia, and New Zealand, are thought to be free of the agent(17,18). Whereas, the Middle East, the Mediterranean region, Sub-Saharan Africa, China, India, Peru, and Mexico have the highest rates. The countries in central and southwest Asia are currently witnessing the most instances (18).

Distribution in India

Bovine brucellosis

In organized dairy farm, the disease is mainly caused by *B. abortus* biotype I, whereas in the cattle under the traditional system of husbandry disease caused by *B. abortus* biotype III. In India the true prevalence of brucellosis observed in cattle and buffaloes were 8.3% and 3.6%, respectively. Punjab has the greatest illness frequency among the states, which is likely due to the state's ongoing screening programme and large bovine population. With the exception of Manipur, all states reported a higher incidence in cattle than buffaloes. RBPT and SAT tests revealed seroprevalence of 1.9 percent and 1.8 percent in 23,284 cattle and 7,153 buffaloes, respectively, in the first national report, which included screening from 23 states across the country (19).The seroprevalence rate ranged from 6.6 percent in Madhya Pradesh's (20) to 60 percent in Assam's north-eastern state (21). Progress reports of monitoring programs from 2012–2013 by the Indian Council of Agricultural Research also estimates that the current national seroprevalence of brucellosis in cattle is roughly 13.5% and at a stable, endemic equilibrium (22). *Shome et. al.*, (23) reported the true prevalence of brucellosis in cattle was 8.3 percent and in buffaloes was 3.6 percent. The state of Punjab had the highest prevalence of brucellosis in both cattle and buffaloes (23.51 and 10.2 percent). Cattle were found to have a higher prevalence than buffaloes in all states except Manipur. For cattle (Punjab, Maharashtra, Rajasthan, Karnataka, Madhya Pradesh, Tamil Nadu, Gujarat, and Kerala), and buffaloes (Punjab, Gujarat, and Manipur), true prevalence of more than 5% were reported in eight and three states, respectively **(Table 2).**

Brucellosis in Sheep and Goat

Shome et. al., (24) reported that sheep (11.55 percent) had a greater brucellosis sero-prevalence than goats (5.37 percent). In both sheep and goats, brucellosis seropositivity was much higher in females than in males. In both sheep and goats, seropositivity >5% was recorded in ten states (Karnataka, Gujarat, Haryana, Andhra Pradesh, Maharashtra, Uttar Pradesh, Jammu & Kashmir, Himachal Pradesh, Punjab and Telangana) and sero-negativity in two states (Mizoram and West Bengal).

Table 2: State wise seroprevalence of brucellosis in cattle, buffalo population and small ruminant (sheep and goat) population

State	Prevalence (%)in cattle	Prevalence (%)inbuffalo	Prevalence (%) in small ruminants
Punjab	23.51	10.20	5.44
Maharashtra	13.93	04.68	11.48
Rajasthan	13.60	03.73	0.86
Karnataka	07.37	00	23.46
Madhya Pradesh	06.92	0.20	-
Gujarat	06.41	09.50	17.72
Kerala	05	04.31	-
Andhra Pradesh	2.84	3.52	13.14
Odisha	01.95	00	-
Jammu & Kashmir	00	00	8.33

Brucellosis in Dogs

Brucellosis is an important contagious disease causing infertility and abortion in dogs. The rate of infection is higher in stray dogs than in pets. *Brucella canis, Brucella abortus, Brucella melitensis,* and *Brucella suis* are the four species of Brucella that can infect dogs. *Brucella canis* is only seldom linked to human illness.

Pillai et. al., (25) first reported about presence of *B. canis* infection in Tamil Nadu using *B. canis* antigen in Mercaptoethanol test (MET) on 640 dogs with 2.18% presence. These initial findings were reconfirmed in a similar serological survey of 460 dogs, which showed 2% infection (26). Upadhyay (27) recorded a prevalence of 7.69% in male dogs through RBPT and ELISA and none of the female dogs was positive by serological test. *Lingam et. al.*, (28) performed a study on seroprevalence of brucellosis in dogs of Telangana state and found that the overall prevalence of Brucella was 2.75% and 3.25% by RBPT and ELISA, respectively.

Human Brucellosis

Humans in India live in close proximity with the animals thereby stand at a greater risk to zoonotic infections. Human brucellosis is witnessed regularly with *B. melitensis* and *B. abortus,* of which the *B. melitensis* exhibits higher virulence and with much severe and extended illness with harsh consequences. Mathur (29) concluded that brucellosis occurred more frequently in villages than in cities. It was also inferred that most human infections occurred with *B. melitensis* in the geographic regions where *B. abortus* was primarily responsible for bovine brucellosis, indicating the role of sheep and goats as the source of infection. General prevalence in general population (Human and household herds) was 2.24%, 9.50%, respectively and in dairy farms (Occupationally exposed, animal level, farm level) was 6.60%, 16.80%, 39.60%, respectively (30).

Robustness of Brucella

Brucella can survive for over two months in water at 20°C, soil and on cool moist pastures Survival of *B. abortus* and *B. melitensis* in the environment is promoted by humid conditions and low temperatures (≤ 4°C), and up to eight months in liquid manure, as well as several months in dry substrates (hay, dust, fencing, etc.). It persists in milk for several days (till the milk turn sour), fresh cheese for 3 months, tap water for 57- 60 days, human urine for 1 week, dust for 6 weeks, damp soil for 10 weeks and animal faeces for 100 days. Brucella can be killed by heating at 60^0C for 10 minutes, in 1% phenol for 15 minutes, and in direct sunlight for few hours (31).

Transmission

Ingestion- The most common way of infection is ingestion of undercooked meat and unpasteurized / raw dairy products. Carnivores such as wolves *(Canis lupus)* and foxes *(Vulpes vulpes)* are thought to be exposed through the ingestion of infected animals, placenta or aborted fetuses. Camel milk is possibly a major source of human infections in the Middle East countries (32).

Inhalation - This risk is generally greater for people in laboratories that work with the bacteria. In addition, slaughter house and meat-packing employees have also been known to be exposed to the bacteria and ultimately become infected.

Direct Contact - Bacteria can also enter wounds in the skin/mucous membranes through contact with infected animals. This poses a problem for workers who have close contact with animals or animal excretions (slaughter house workers, meat-packing plant, employees, veterinarians)(10).

Person-to-Person-It is extremely rare but can be spread by Sexual transmission, tissue transplantation or blood transfusions, infected mothers to their infants (mainly due to breast-feeding).

Clinical Signs

In Humans

Fever is one of the most common symptoms across patients; intermittent in 60% of patients with acute and chronic brucellosis, while undulant in 40% of patients with subacute brucellosis. About 20% of the rural

population suffers from Pyrexia of Unknown Origin(PUO)and1-2% of such pyrexia are due to brucellosis. Nearly 80% of patients suffer from chills, and 20% of patients develop a cough and dyspnea without any active pulmonary involvement (33). In humans, brucellosis increases the chance of spontaneous abortion, early birth, miscarriage, and intrauterine infection with fetal death, as well as malaise, weariness, and arthritis. Brucellosis is not confined to the reproductive system but is also known to cause neuro-brucellosis with clinical manifestation of meningitis, encephalitis, stroke, radiculitis, myelitis, peripheral neuropathies, and neuropsychiatric features. Sensorineural deafness, spastic paraparesis, brisk tendon reflexes, bilateral ankle clonus, and extensor plantar responses have all been described in studies (34).

In Animals

In sexually mature female cattle, infection concentrates in the reproductive system, producing placentitis and abortion, as well as productivity losses. Most infected animals only have one abortion in their lifetime, although they may remain infected for the rest of their lives. In non-pregnant female cattle and after the first abortion, the illness is generally asymptomatic. Orchitis can affect adult male cattle, while brucellosis can induce infertility in both sexes. Hygromas can affect leg joints. Buffaloes, bison, and yaks can all contract bovine brucellosis, and the symptoms are identical to those seen in cattle (35). It has been estimated that annual loss in India is up to Rs 240 million in livestock because 7.60% of cattle and 5.80%of buffaloes have been found reactors. *Brucella* is responsible for fistulous withers (Suppurative atlantal bursitis) among horses.

Risk Factors

Animal Risk Factor

B. abortus infection also depends on farm size, herd size, vaccinated animal, and infected animal. Cattle remain less susceptible to *B. abortus* before reaching sexual maturity and become increasingly susceptible while they attain breeding age. Pregnant cattle are highly susceptible to the disease (36).

Pathogen Risk Factor

B.abortus can protect itself by living within neutrophil and macrophages from the attack of humoral and cellular bactericidal effect. Placenta is the ideal site for replication of the organism. *B. canis* is not transmitted to other species except for cat and man.

Immune Risk Factor

The serum of infected cows possesses a high level of IgM, IgG, IgG2 and IgA antibody. The half-life of colostral antibody to *B.abortus* in calves which might have received colostrum from either vaccinated, non-infected or infected dams is about 22 days. Thus, it is feasible that some calves remain immune sufficiently long to interfere with vaccination.

Managemental Risk Factors

The movement of the infected animal from one corner of the farm to the other, coming in contact with virgin animals are the important risk factors. Thus, unregulated movement of the cattle must be prevented. One of the most important factors for the spread of brucellosis, according to Tukana and Gummow (37), is normal animals sharing common water sources with Brucella-positive animals. In this context, brucellosis has persisted in India due to a lack of public awareness, proper husbandry methods, trading contaminated animals, and the high cost of diagnosis, immunization, and management (38).

Pathogenesis

Organisms gain access through mucous membrane of the oropharynx, upper respiratory tract, conjunctival mucosa, abraded skin and cervix of the genitalia. The bacteria following invasion localize in the nearby lymph-nodes and other lymphoid tissues (spleen, iliac and lymph nodes). The organisms thereby reach circulation, multiply and set up bacterimia. The organisms sometime colonize in the gravid uterus and placenta. They then multiply in large proportion in the placenta and fetal tissues and produce degenerative changes in the placenta. A substance known as erythritol is produced by the fetus. This is capable of triggering multiplication of *B. abortus.* Thus, there is heavy concentration of bacteria in the fetal fluid causing infection of uterine tissues leading to severe ulcerative endometritis. There is abortion during the last trimester of pregnancy. Non-pregnant adult may develop self-limiting immunizing infection and turn into a carrier. Congenital infection through uterine route in new born calf is possible. Milk of the infected udder remains as a potential source of infection to calves and human subjects-who consume such milk. Clinically, milk may appear normal, but the infectivity can be proved based on milk agglutination test.

In the male, the organisms multiply in large proportion and localize in epididymis, testes and other accessory sex organs. There is epididymitis and orchitis. The organisms are shed through semen. Thus, males remain as a cause of infertility in a herd (39).

Economic Impact of Bovine Brucellosis

The dairy business suffers substantial losses due to bovine brucellosis, however there are few detailed economic analyses available. Direct (e.g., decreased milk yield, higher mortality) and indirect (e.g., immunization, culling) expenditures can all have an economic impact. Direct consequences are further divided into visible (abortion, repeat breeding), invisible (reduced fertility), additional expenditures (treatment, vaccine), and revenue foregone categories (e.g. distress selling). Loss may only include parameters that reduce benefits (e.g., reduced milk yield, weight gain, fertility, replacement cost, mortality, etc.), whereas cost would include amounts spent on disease treatment and control (e.g., biosecurity, vaccination, movement control, disease

surveillance, research, etc.) (40).The loss caused by distress selling, the feeding and management loss of pregnant animals in the event of abortion, the loss of person-days spent treating animals, the cost of antiseptics and detergents, the cost of transportation related to treatment, the cost of diagnosis, and so on have all been overlooked in most economic estimates.

A total of US$ 3.4 billion was lost due to brucellosis & 95.60 percent of the total 5 losses were due to losses in the livestock and buffalo sectors. In cattle and buffalo, the median production losses owing to abortions, temporary infertility, and sterility in mature animals were US$ 735.7 million and US$ 985.4 million, respectively. These losses accounted for a considerable portion of the losses in the cattle and buffalo sectors. The loss in meat and milk resulted in a loss of US$ 292.9 million and US$ 557.1 in cattle and buffalo industries. The loss in meat resulted in a median loss of US$ 1.8 million in pig industry. Foregone milk, meat and draught power due to reduction in fecundity resulted in a median loss of US$ 131.7 million in the cattle industry. Foregone milk and meat due to reduction in fecundity in buffalo resulted in a median loss of US$145.8 million in buffalo industry. A median loss of 185.4 million and 210.8 million occurred due to death of adult animals and perinatal mortality in cattle and buffalo, respectively. The disease was found to be responsible for a loss of US$ 6.8 per cattle, US$18.2 per buffalo, US$0.7 per sheep, US$ 0.5 per goat and US$ 0.6 per pig in India (41).

Public Health Significance of Brucellosis

According to the Brucellosis 2003 International Research Conference, 500,000 human infections occur each year worldwide, with rates ranging from less than one case per 100,000 in the United Kingdom, the United States, and Australia, to 20 to 30 cases per 100,000 in southern European countries like Greece and Spain, and more than 70 cases per 100,000 in Middle Eastern countries such as Kuwait and Saudi Arabia. Humans can be infected by five of the nine *Brucella spp.* that have been identified. *B. melitensis, B. abortus,* and *B. canis* are the most harmful and invasive species for humans. Marine Brucella *(B. ceti)* has been proven to be a zoonotic organism. The most severe form of human brucellosis is produced by *B. melitensis,* which is followed by *B. suis, B. abortus,* and *B. canis* in decreasing order. Because three species are highly contagious and can be aerosolized, the disease control and prevention programme in the United States has classified them as possible bio weapons (42).

Diagnosis

The presumptive diagnosis of brucellosis can be done by the exfoliative cytological methods, where smears of placental cotyledon vaginal discharge or fetal stomach contents are used to detect organisms after staining with modified Ziehl-Neelsen or Koster's methods.

Isolation / Culture method- Detection of Brucella by culture method required BSL-3 laboratory. Enriched medium such as glucose serum, liver infusion broth, trypticase soybroth are used for isolation. Small transparent colonies develop after several days of incubation at 37°C in aerobic conditions. Buffered charcoal yeast extract, Thayer-Martin medium and Farrell's medium are used as selective media. CO2 is needed for the growth of *B. abortus.* It grows on blood culture (10% CO2) which must be incubated for at least 4 weeks.

Biochemical Test

Dye Tolerance, Slide Agglutination, Requirement of CO2, Tbilisi Phage Lysis, H2S test.

Serology Test

Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Milk Ring Test (MRT), 2-Mercaptoethanol Test (2-MET), Heat Inactivation Test (HIT), Fluorescence Polarization Assay (FPA) and Complement Fixation Test (CFT) (43). ELISA test is an OIE prescribed test and can be conducted on serum and milk samples.

Skin Test

Brucellergen (killed bacteria) are injected into the skin. After 24 to 48 hours, it shows >5 mm reaction.

Molecular tests – It includes PCR (Real Time PCR and Multiplex PCR), Sequencing of conserved genes (16S rRNA, recA, rpoB) and whole genome sequencing.

Prevention and Control

Vaccination

Vaccination is an extremely important and effective tool that has been used by many countries to control, eliminate and eradicate animal brucellosis from the country. Presently there are four vaccine strains for use in animals.

Common Smooth Vaccines

Brucella Abortus Vaccine Strain S19

The S19 vaccine is the first and widely used vaccine in cattle, and it is the reference vaccine in many countries including India, Argentina, and Brazil. It is an OIE - recommended reference vaccine. S19 vaccine contains the whole organism of *B. abortus* strain 19. The LPS of this strain contains O-polysaccharide that can continuously stimulate animal to produce anti-LPS antibodies, which interferes with conventional serological tests between immunized and naturally infected animals. Adjuvants are not used in S19 vaccine. It was utilized to vaccinate 6-month-old calves and achieved satisfactory results. The S19 vaccine strain initially was a virulent strain isolated from milk of a Jersey cow in the early twentieth century, and the smooth mutant obtained by spontaneously attenuated at room temperature for 1 year. The current used S19 is an erythritol sensitive S19 strain selected by American scientists. eryA, eryB, eryC, and eryD are the four open reading frames (ORFs) in the erythritol

(ery) gene. The new S19 vaccination strain includes a 703-bp nucleotide deletion that affects the coding areas of eryC (BAB2 0370) and eryD (BAB2 0369), and it is safer than the old S19 vaccine strains (14).

Rev.1 Vaccine

The Rev.1 vaccine is a type of streptomycin-resistant smooth *B. melitensis* strain (44). The formulation of Rev.1 vaccine is whole organism of the live attenuated *B. melitensis* strain derived from a virulent *B. melitensis* isolate. The *B. melitensis* Rev.1 vaccine was developed in 1950s and since then it is in use for the prevention of caprine and ovine brucellosis including Brucella ovis infections. Adjuvants are not used in Rev.1 vaccine. The *B. melitensis* Rev. 1 vaccine is not safe for human beings as it causes disease in accidental inoculations. Secondly, Rev.1 vaccine carries smooth Lipopolysacchride with Opolysacchride that elicits antibodies interfering in sero diagnosis, a major problem in differentiation between vaccinated and infected animals and also in eradication campaigns.

B. melitensis H38 Vaccine

B. melitensis H38 vaccine was developed in 1965 from *B. melitensis.* The vaccine is an emulsion created by mixing inactivated H38 strain with an emulsifier. In the H38 vaccine, Freunds emulsified oil adjuvant was employed. Although the vaccine stimulates goats to produce antibodies, it offers little protection to the host and induces local purulence following vaccination. The vaccine can no longer be used to vaccinate goats.

Brucella suis Strain (S2)

After years of serial transfer of a virulent *Brucella suis* biotype 1 strain on culture media in China, an attenuated strain *B. suis* (S2) was created. In guinea pigs, *B. suis* S2 of the smooth type demonstrates the same level of attenuation as strain 19 and does not revert to the virulent condition following repeated culturing.

Common Rough Vaccines

Brucella abortus Strain RB51 (Rb51)

The RB51 is a laboratory derived live-attenuated vaccine strain developed through successive in vitro passage of virulent *B. abortus* 2308 on media containing rifampicin and penicillin. It is a spontaneous rough mutant devoid of O. polysaccharide chain, hence, lacks the ability to produce antibody in animals/humans (45). It was licensed by USDA for use as a vaccine against brucellosis in bovines. Inability of Rb51 to produce antibody allows an easy differentiation between infected and vaccinated animals. This vaccine is useful in eradication program where identification and removal of infected animals is necessary.

B. abortus 45/20 Vaccine

The live *B. abortus* 45/20 strain is derived of the smooth virulent strain 45/0 of *B. abortus.* After 20 passages of the 45/0 strain in guinea pigs, the approximate features of *B. abortus* 45/20 were achieved. The 45/20 vaccine is made up of the entire organism of a live *B. abortus* strain 45/20 (14). The 45/20 vaccine does not contain any adjuvants. When administered as a live vaccination, the 45/20 strain is unstable and can revert to the smooth virulent variant.

Genetically Engineered Attenuated Vaccines

With the development of Homologous Recombination (HR) technology, some engineered attenuated strains were made as vaccine candidates. For example, disruption of per, pgm, wboA, and wbkA (genes involved in the LPS biosynthesis pathway) results in rough mutants.

Vector-delivered *Brucella Vaccines*

Because of the similarity in infection, various nonpathogenic infections, such as the attenuated *Salmonella* strain, *Lactococcus lactis* strains, *E. coli* (K12), and Semliki Forest Virus, can be used as a vector to transfer Brucella antigen to immunologically important locations (SFV) (46).

Control of brucellosis in human is directly linked to control of animal brucellosis. Humans are the end result of bacterial activity in animals, so any preventive measures aimed at human brucellosis has no impact on the presence of animal disease in an area. Nevertheless, surveillance of human brucellosis is important in the management of brucellosis control eradication programmes. In fact, the incidence of human brucellosis is a good index of the presence of infection in animal population, and thus, human can be a good sentinel of the infection in animal population in all cases, where a reduced or variable degree of testing is performed (Boral *et. al.,* 2009).

Control is designed to reduce the disease occurrence in populations and can be achieved by reducing or eliminating the causes to a level of little or no consequence and thereby aims to reduce the impact on human health and economy. It is implicit that some accepted level of infection remains. Control programmes therefore have an indefinite duration and will need to be maintained even after the acceptable level of infection has been reached, so that the disease does not re-emerge.

National Animal Disease Control Programme

The National Animal Disease Control Programme (NADCP) is a flagship scheme launched by the Hon'ble Prime Minister in September 2019 to control Foot & Mouth Disease and Brucellosis by vaccinating 100% of cattle, buffalo, sheep, goat, and pig populations for FMD and 100% of bovine female calves of 4-8 months of age for brucellosis with a five-year budget of Rs.13,343.00 crore (2019-20 to 2023-24). The National Animal Disease Control Programme for FMD and Brucellosis (NADCP) has a long-term goal of controlling FMD via vaccination by 2025 and eradicating it by 2030.

Conclusion

Brucellosis is one of the most common and highly contagious reproductive illnesses in dairy cows. The disease is endemic in our country's bovine population, resulting in annual economic losses of US$ 34 billion. Abortions, stillbirths, poor milk production, and infertility all result in financial losses. After rabies, Brucellosis is the world's second most common zoonotic illness. Because more than three-quarters of rural India's population is in direct touch with the bovine population, there is a higher risk of zoonotic infection transfer from animals to humans. As a result, efficient management and eradication of bovine brucellosis is a global concern that can only be achieved through early, reliable, and accurate illness identification in animals, which will prevent the disease from spreading.

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Paratuberculosis: The Mystic Killer

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Abstract

Paratuberculosis, caused by the bacterium Mycobacterium avium subsp. paratuberculosis(MAP)has become a major health concern for both large as well as small domestic animals. The disease is characterised by severe diarrhoea and progressive emaciation. It causes significant socioeconomic losses and jeopardises the rural economy due to reduced production performance, lowered slaughter value, premature culling, trade restrictions and mortality. Bioloads of MAP, ranging from high to very high have been documented in a wide range of ruminants as well as non-ruminants from various parts of the world. The presence of live MAP bacilli in commercial raw as well as pasteurised milk and milk products implies its public health significance. Higher bioload of MAP in the animals raises the risk of MAP exposure in the human population. This review summarizes the current knowledge on MAP infection in animals, including its aetiology, economic impact, clinical manifestations, diagnosis, as well as the potential for effective animal management and control strategies that could lower the risk of human exposure.

India is considered as one of the fastest growing economies of the world, with the agriculture sector being the backbone of Indian economy. The animal husbandry is a vital part of the agriculture sector and milk and meat production and marketing help to improve the livelihood of rural poor, augments women empowerment and thereby enhances the socioeconomic status of the country. The agriculture sector has been forced to expand at an exponential rate as a result of the population boom. Food derived from animals will play a crucial role in ensuring the food security and livelihood of the rapidly growing population, thereby contributing to the overall economic growth. Thus, improving the health and productivity of our dairy animals is critical for the development of animal husbandry sector. Reducing the morbidity and mortality by ensuring proper nutrition and growth are the two most important prerequisites for attaining this goal. However, it has been observed that a large number of animals have suboptimal production or rather dies at an early age. Paratuberculosis (PTB) could be incriminated as one among the major, yet under estimated infectious diseases that results in a reduced yield, heavy morbidity and mortality.

Paratuberculosis also referred to as Johne's disease, is a chronic, contagious bacterial disease of the intestinal tract characterised by a slowly progressive wasting of the animal and severe diarrhoea. The disease primarily affects goat, cattle, sheep as well as other ruminant species all over the world, resulting in huge losses due to mortality, culling, reduced productivity and trade disruption with profound socio-economic impacts. It is of global concern but has received only minimal attention in many countries, probably due to the subclinical and slowly progressing nature of the disease. The disease often remains obscure in subclinical form in a herd and moves slowly through ruminant population. Thus, unless management interventions are introduced, the accumulation of infection in the population will increasingly gather pace and emerges as a slow epidemic that may result in severe mortalities and a 'paratuberculosis crisis.'Therefore, rapid interventions are required to break the course of the disease, prevent mortality and to develop effective control strategies. A better understanding of the aetiology, pathogenesis and therapeutic approaches are essential for this. Hence, the present review summarizes the current knowledge on MAP infection in animals, including its economic impact, aetiology, pathogenesis and diagnosis, as well as effective disease management and control strategies.

Historical Background

The disease was first described in Germany over 100 years ago and at present it is being reported from all over the world, affecting both the developing as well as developed countries of Europe, North America, South America, Asia, Australia and Africa. The clinical symptoms and pathoanatomical picture of paratuberculosis were first elucidated during the nineteenth century. In 1826, d' Aroval described a kind of enteritis that caused persistent diarrhoea in some calves. Hansen and Nielsen in 1881 studied the pathological lesions of calves dying from this type of enteritis and reported the thickening and corrugation of intestinal mucosa. The disease is named after H. A. Johne and L. Frothingham, who in 1895, discovered the link between bovine enteritis and the presence of acid-fast bacilli from the intestinal sections of sick animals. Because of the presence of acid-fast bacilli that were

indistinguishable from tubercle bacilli and its ability to provoke granuloma formation, they assumed that the disease was an uncommon or unusual form of tuberculosis (1). Robert Koch also made similar findings during the same time.

Later on, in 1906, Bang distinguished tuberculous and nontuberculous enteritis and proposed renaming the latter as pseudotuberculous enteritis or Johne's sickness (2). Between 1902 and 1908, pseudo-tuberculous enteritis in cattle was reported in Denmark, Germany, France, Norway, Holland, Belgium, Switzerland, and the United States (3, 4). In 1912, F. W. Twort succeeded in culturing and describing the organism and identified the aetiological agent, which was later demonstrated to cause experimental enteritis in 1914. He termed it as Mycobacterium enteriditis chronicae pseudotuberculosis bovis johne. The disease was renamed paratuberculosis once Mycobacterium paratuberculosis was fully characterised as a distinct species within the genus Mycobacterium.

It was only towards the middle of this century that the prevalence and wide geographic distribution of paratuberculosis were realised, and its effect on the livestock economy became apparent. In MAP-infected dairy herds, it was estimated that about one per cent of gross milk revenue, or US$33 per cow, was lost yearly, with losses mostly driven by the reduced productivity. Johnes disease in dairy cattle costs the United States an estimated US$198 million each year, Germany US$75 million, France US$56 million, New Zealand US$54 million, and Canada, one of the smaller dairy-producing regions analysed, US$17 million to US$28 million (5).

Aetiology and Classification

Mycobacterium avium subsp. paratuberculosis (often abbreviated as MAP) is a fastidious, non-motile, aerobic, Gram positive and acid-fast bacilli that belong *Mycobacteriaceae* family under the order Actinomycetales. Except for MAP, all *Mycobacterium* spp. produce the iron-chelating substance mycobactin (6). Because of the iron requirement for replication, MAP is classified as an obligatory intracellular pathogen of mammalian cells where iron is freely available and mycobactin is not required (7). It is classified as a facultative intracellular pathogen because, upon entry into host cells, it preferentially infects macrophages (8,9). The strong, protective cell wall and inclination to form huge clumps of cells allow MAP to tolerate a variety of extreme conditions that helps them to persist in the environment for more than a year (10).

Mycobacterium avium subsp. paratuberculosis belongs to Mycobacterium avium complex (MAC) which comprises of four distinct subspecies: *M. avium subsp. avium, M. avium subsp. hominissuis,M. avium subsp. paratuberculosis,* and *M. avium subsp. silvaticum (11).* Being a subspecies of *M. avium,* they share about 90 per cent of the genome with other subspecies. However, the presence of multiple copies of an insertion element, IS900, absence of IS1245 in its genome (12), and its requirement for mycobactin supplementation in order to grow in vitro helps it to be distinguished from other subspecies.

Based on the phenotypic characteristics such as growth rate and pigmentation, MAP is classified into at least two major strains namely the type S (Sheep type with subtypes I and III) and type C (Cattle type or Type II). The type II further has another subtype, referred to as the type B or 'Bison type'. There are also two types of bison (B) strains, one in North America and other in Asia. The latter is known as "Indian Bison type," and is a sublineage of type II. It has been reported from cattle, water buffalo, sheep, goats, deer, bison, rabbits, wild boars, and other species, and it is the dominant strain in several parts of Asia (13).

Type S strains are predominantly found in sheep and goats although they have also been recorded in cervids, South American camelids, and camels, as well as in some cattle in close contact with sheep (14). In contrast, the type II isolates grow faster and has a broad host range, including both ruminants and non- ruminants (15). Type III isolates are intermediate growers have been isolated from sheep, goats, cattle and camels (16).Cross-species infection and sharing of specific strains between wild and domesticated animals have been shown in several studies (17,18).

Epidemiology

Host Range

The disease mainly affects ruminants and camelids. Cattle, sheep, goats, water buffalo, South American camelids, dromedary and bactrian camels, moose, reindeer, bison, wild relatives of small ruminants, and numerous species of deer, antelope, and elk have all been recorded to exhibit clinical symptoms. Infections with MAP also been documented in captive or wild ruminants and camelids that have not shown clinical symptoms. Domesticated ruminants are assumed to be the major reservoirs of the organism. Wildlife appears to be infected primarily by the farmed herds, while a few wild cervid populations may harbour this pathogen regionally. In wild rabbits, nonhuman primates, pigs, cats, and dogs, as well as experimentally infected horses inoculated intravenously, illnesses and/or physical lesions compatible with paratuberculosis have been documented, but a causative role was not always proven. *M. avium* subsp. paratuberculosis has also

been found in some birds, including members of the corvidae, starlings, house sparrows and gulls.

Susceptibility to infection

The disease mainly affects domestic ruminants such as cattle, goat and sheep. However, goats and cattle are more vulnerable to MAP infection and are more prone to develop clinical signs, whereas sheep are relatively more resistant to the development of clinical illnesses.

The susceptibility to infection is inversely related to the infecting dose being exposed. It is being proposed that the susceptibility usually decreases with age where in calves and other ruminants under the age of six months are likely to be the most vulnerable to MAP infection (13). Because of the increased permeability of the intestines in the first 24 hours of life, neonates are likely to be the most vulnerable. (19). Although there is evidence that adult cattle can be infected with MAP, (20) in most herd circumstances, calf hood infection is significantly more important.

Some ruminant breeds are thought to be more resistant to MAP infection than others; Merino sheep have been shown to be more vulnerable to MAP than Romney sheep (21). Farm goats from Uttar Pradesh were found to be more adapted to the Indian Bison Type of MAP than farm goats from Rajasthan (22). There is strong evidence that genetic susceptibility may influence the outcome of MAP exposure in cattle and sheep (23). However, for the time being, management and environmental factors are likely to outweigh genetic influences (24).

An important risk factor that determines the susceptibility to infection in a herd is the herd size. This might be because larger herd size allows for more effective interaction between individuals, as well as related management techniques that make infection easier once MAP is introduced (25).

Prevalence

The disease was first diagnosed in Oldenberg region of Germany in 1895. Later on, the disease has been reported from many European countries such as Germany (26), Italy (27) and France (28), as well as in Oceania, Asian and African countries. Studies undertaken in the last few decades showed that paratuberculosis is worldwide in distribution and highly endemic in the dairy cattle herds of the developing countries. Only Sweden and some states in Australia are currently proven to be paratuberculosis-free. In India, the first case of paratuberculosis was observed in Lahore (undivided India) in 1913. Thereafter, the disease was reported from different parts of the country.

Transmission

Mycobacterium avium subsp. paratuberculosis is transmitted either directly through consumption of MAP-contaminated faeces or indirectly through MAP-contaminated colostrum, milk, water, or feed(29, 30, 31, 32, 33). The level of faecal shedding by MAP positive cows varies greatly. Super shedders, defined as animals that shed more than 107colony forming units of MAP per gram of faeces, play a major role in transmission. There are two unique shedding patterns among infected cows - progressors and non-progressors. Non progressors are characterised by sporadic and low MAP bacterial shedding and a complete lack of a humoral immune response, whereas progressors are defined by continuous and progressive shedders. Only around ten percent of naturally infected cows become high shedders, of which more than 95 percent are culled or die within a year of being sampled (25).

In addition to faeces-associated transmission, the organism can be present in colostrum and milk of adult cows in advanced stage of illness, facilitating the transmission even with the strictest sanitary standards during colostrum and milk collection (31). Moreover, it is now well established that the sanitary practices such as commercial pasteurization or combined pasteurization and desiccation does not necessarily eliminate MAP from milk (34). Foetal infections were possible in late-stage disease, and possibly even in the preclinical stages of MAP infection (35). Transplacental transmission has also be recorded from an infected dam to its offspring (36). The infection is generally introduced in to an uninfected herd through herd expansion or replacement purchases of carrier animals (37).Although not an important route of transmission, certain studies have isolated MAP from the semen of infected bulls (38) and saliva of infected cows (39).

Pathogenesis

Mycobacterium avium subsp. paratuberculosis is an intracellular pathogen that can survive for extended periods of time in the environment. The majority of infections are caused by oral ingestion of MAP shed in the faeces of adult animals, but in utero transmission can also occur. Once the animal has been exposed to MAP orally, the organisms may invade the tonsillar crypts. From there, the MAP organisms may spread through two different entry points that is either hematogenous or via lymph to mesenteric lymph nodes and ileum(40). The killing of ingested MAP organisms by macrophages is critical for the first stage of host defense against the establishment of a MAP infection. The activation of macrophages by cytokines such as interferon-gamma, which is

produced by Th1-type T-helper lymphocytes, improves the killing of intracellular MAP organisms (41). Some exposed cattle are likely to be successful in eliminating the MAP infection through this mechanism and thus do not progress to clinical Johne's disease. Many exposed animals, however, are unable to eliminate MAP, and the organism persists within the macrophages. This ability of MAP organisms to survive within macrophages is unique and critical to the chronic, progressive nature of paratuberculosis (42).

Although the mechanism for intracellular survival is unknown, MAP seems to prevent maturation and acidification of the phagocytic vacuole within the macrophage, thereby preventing the organisms from being exposed to the bactericidal effects of lysosomal enzymes and oxygen-derived radicals (43). In general, even in susceptible animals that will eventually succumb, the host defenses are able to contain the infection early on, allowing only slow proliferation and spread of MAP within the gut and gut-associated lymphoid tissue. This early, "controlled" infection causes a prolonged "eclipse phase" characterised by a destructive granulomatous inflammatory response that eventually results in intestinal malabsorption and protein losing enteropathy. The granulomatous inflammatory response confines MAP-laden macrophages to the gut and lymphoid tissue associated with the gut. This process allows MAP and its host to coexist for many years with no outward signs of disease in the host, but despite being contained, MAP is not killed. During this period, the animal exhibits no outward clinical signs of infection, there is no discernible effect on production or weight gain, and faecal shedding of MAP and serum antibodies is usually not observed.

As the immune "control" over the infection is lost, the infected animal begins to shed MAP in massive numbers in the faeces, and MAP organisms spread to other tissues such as the uterus (causing in utero transmission), mammary gland, and other internal organs and muscle tissue. Although the animal may not be showing any outward clinical manifestations of Johne's disease at this point, studies have shown a decrease in milk production (40) and reproductive efficiency.

Table 1. Different stages of paratuberculosis (45)

Features	I: Silent infection	II: Inapparent carrier	III: Clinical disease	IV: Advanced Clinical disease
Replication of MAP	Slow proliferation in jejunal and ileal mucosa and spread to regional lymph nodes	Continued replication in infected tissues	Infection becoming disseminated. MAP presented in extra intestinal sites	Widespread proliferation and replication of MAP
Shedding	Intermittent shedding of the organism at low levels in faeces	Most animals shed the organism in faeces and possibly in milk	Shed increasing numbers of MAP in faeces and milk	Shedding large numbers of MAP in faeces and milk - >1000cfu/g faeces = super shedders
CMI Response	Th1 CMI responses initiated to control infection	Increasing CMI response. Gradual switch from Th1 to Th2	May be detectable	Possibly anergy
Humoral immune response	None	Increasing antibody response IgG2, IgG1	Predominantly strong antibody response	Predominantly strong antibody response
Clinical signs	None	None	Gradual weight loss and diarrhoea	Emaciation, profuse diarrhoea, bottle jaw, cachexia
Histopatho-logical changes	None detected	Detectable granulomas if multiple tissues examined	Abundance of lymphocytes, epitheliod macrophages and giant cells in infected tissues, blunted villi	Abundance of lymphocytes, epitheliod macrophages

Eventually, the overt clinical signs of Johne's disease become apparent. The average incubation period ranges from a few months to many years. The lesions in the intestines and mesenteric lymph nodes worsen as the MAP infection progresses and the granulomatous infiltrate become diffuse affecting the jejunum, ileum, caecum, and, to a lesser extent, colon. In the final stages of infection, despite a good appetite, decreased output, weight loss, diarrhoea, and hypoproteinemia are evident. Fecal shedding occurs before other clinical manifestations, allowing asymptomatic carrier animals to contaminate the environment and infect susceptible animals before they are identified as infectious.

Clinical Signs

The course of paratuberculosis could be divided in to four stages namely silent stage, inapparent or subclinical stage, clinical stage and terminal stage or advanced clinical stage (6, 44). The table below shows a representation of the various stages of paratuberculosis **(Table 1).**

The typical incubation period of MAP infection in dairy cattle is about five years (46). The incubation period is inversely proportional to the MAP dose - that is, the more MAP taken, the shorter incubation period. Some infected cattle may not show clinical indications for the rest of their lives (47). One of the challenges in controlling MAP infection is that animals commonly shed MAP in their faeces before the onset of clinical signs there by contributing to the spread of bacteria.

In cattle, the initial signs can be subtle and frequently go unnoticed. Weight loss, decreased milk production, and roughening of the hair coat are the most common clinical signs. Animals are usually alert and have a normal appetite. Intermittent diarrhoea, usually without blood, mucous, or epithelial debris, is common and gradually worsens over weeks or months (48). The vital signs, heart rate, respiratory rate, and temperature are all within normal limits. Emaciation and cachexia appear gradually, accompanied by a decrease in milk production.

Some cattle acquire subcutaneous oedema, particularly submandibular and/or ventral oedema, when diarrhoea becomes profuse, and hypoproteinemia develops (49). The condition of the animal progressively deteriorates in a short span of time, becoming progressively emaciated before dying from terminal cachexia and dehydration. Cattle at this stage of illness rarely survive more than a few weeks in the herd and are culled due to weight loss, reduced milk yield, and unresponsive diarrhoea.

While clinical signs in other ruminants and camelids are generally comparable, there are notable differences, particularly in the presence and degree of diarrhoea. In sheep and goats, weight loss and exercise intolerance are common symptoms, and the affected animals may trail behind the flock. Diarrhoea is less prevalent than in cattle, soft faeces is mostly manifested rather than frank diarrhoea. It can also be intermittent. Submandibular oedema ('bottle jaw') is a common manifestation in most small ruminants and the wool is frequently torn and easily shed. In both cervids and camels, the sickness may progress at an exceptionally fast rate.

Diagnosis

Early identification of paratuberculosis is crucial since it is a disease with considerable economic significance. The diagnostic tests employed should depend on the stage of infection and the immune status of affected animal. Animals in the early stages of infection are frequently asymptomatic or subclinical. Intermittent excretion, variable distribution, and potentially low MAP levels in faeces all results in delayed ante mortem diagnosis (47). As a result, to enhance the possibility of discovering shedders, periodic and regular sampling and testing are necessary. The progression of disease, clinical signs in affected individuals, non-responsiveness to therapy, and acid-fast positive lesions during post-mortem inspection provide reasonable diagnostic clues.

Microscopic Examination

The Ziehl - Neelsen method, which involves direct microscopic analysis of smears from the infected mucosa, faeces, and cut surfaces of lymph nodes, is a low-cost and rapid method to diagnose Johne's disease. Clumps of tiny (0.5–1.5 µm), highly acid-fast bacilli (three or more organisms) aid in the preliminary diagnosis. However, it has drawbacks, such as the inability to distinguish among other mycobacterial species and only a small percentage of cases can be confirmed by microscopic analysis of a single faecal sample.

A preliminary diagnosis can also be reached by histopathological analysis of haematoxylin and eosin-stained sections of affected mucosa or lymph nodes. The characteristic histopathological picture involves infiltration of the intestinal mucosa, submucosa, Peyer's patches and the cortex of the mesenteric lymph nodes with large macrophages, also known as epithelioid cells, and multinucleate giant cells, in both of which acid-fast bacilli are seen either as clumps or as single bacilli.

Culture Methods

M. avium subsp. paratuberculosis detection by cultivation of the organism from faecal or tissue

specimens on bacteriologic media has been the mainstay of paratuberculosis diagnosis for nearly a century and remains the most widely used 'gold standard' diagnostic test for the infection. Löwenstein-Jensen, Herrold's egg yolk medium (HEYM) with and without sodium pyruvate, and Middlebrook 7H11 including mycobactin J are the most often used culture media. However, isolating MAP is challenging due to the intermittent shedding of the bacteria and the low number of bacilli in faeces and tissues, respectively. MAP is a fastidious bacterium that takes weeks to months to develop in laboratory medium. The incubation of samples with antibiotics prior to culture to avoid overgrowth by other faster growing bacteria can result in the death of MAP bacilli in samples with a low number of bacteria. As a result, MAP culture from faeces and tissue samples is less sensitive than molecular techniques and histopathology of lesions for confirmation of paratuberculosis.

Molecular assays

Molecular tests, such as polymerase chain reaction, can be used to diagnose paratuberculosis from the faeces and blood of suspicious animals. The insertion sequence 900 (IS900) element, IS901, IS1245, or the dnaJ gene have been utilised to detect the MAP genome (50). To detect MAP in milk or cheese, an F57-based real-time PCR system was employed (51). Furthermore, a loop-mediated isothermal amplification assay (LAMP) targeting ISMap02 was employed to detect MAP in small ruminants in a quick and sensitive manner (52).

Serological tests

Agar Gel Immuno diffusion (AGID), Complement fixation test (CFT) and Enzyme linked immunosorbent assay (ELISA) are amongst the serologic assays used to diagnose paratuberculosis in small ruminants. These tests are especially relevant in small ruminants, since the faecal culture has the limitation of reduced sensitivity, is time consuming and expensive.

The current diagnostic techniques vary in accuracy. Because of the immunological complexity and protracted subclinical stage, it is difficult to identify a single reference diagnostic test. Hence, it is recommended to use two or three of them at the same time in the same animal to assess the stage of illness in both the animal and the herd. The diagnostic technique for MAP is complicated, and no single diagnostic instrument with high accuracy, sensitivity, and specificity is available. As a result, more attention is needed to comprehend the organism's features, pathophysiology, and the development of molecular diagnostic tools. Because existing diagnoses have limitations, selecting numerous laboratory tests to determine the condition can be a confirmatory method.

Treatment

MAP, like its renowned counterparts, M. tuberculosis, *M. leprae* and *M. bovis,* has a prolonged incubation period, persist in the host for years, causes granulomas, and is difficult to eradicate. Despite advancements in the field of medical research, no viable therapy of paratuberculosis has been recorded to date. As a result, the primary focus is now on modifications in management practices, vaccination, biosecurity measures and selective breeding.

Prevention and Control

The management practices such as provisions for segregated calving, removal of calves within two hours after birth, selection and hygienic collection of colostrum, feeding solely on pasteurised milk or milk replacer, segregation from the adult herd, culling of strong ELISA-positive animals and selection of replacement heifers from ELISA-negative cows, have shown to significantly reduce the prevalence of MAP among US dairy herds (53).

Within-farm management techniques for paratuberculosis include minimising interaction between sick adult goats, sheep, and others, as well as preventing exposure to possibly infected adult animals, their dung, and the contaminated surroundings. The farmer's knowledge and awareness are an important factor in determining the success of management strategies.

Another key method is the use of test and cull procedures to detect early MAP infection in animals, particularly before they begin faecal shedding. The 'test and cull' technique would be challenging in many nations due to the prolonged incubation period of the disease. However, combining vaccination with 'test and cull' was proven to be a cost-efficient and successful technique for controlling PTB in goat, buffalo, and cow herds.

When compared to other management techniques, vaccination is the most cost-effective. Vaccination, on the other hand, is not regarded the greatest option as a control measure and is even outlawed in some countries due to interaction with the tuberculin skin test. Fortunately, new potential techniques to avoid this interference have been successfully implemented in skin tests employing proteinic and peptidic mixtures rather than typical test reagents. Vaccination as a management technique to reduce paratuberculosis has, on the other hand, been popular in a number of countries, including Australia, New Zealand, Spain, India, and the Netherlands.

Live (non-attenuated and attenuated) and killed whole cell vaccines, as well as subunit vaccinations that have been utilised in a few cases with a lower level of protection, are the most widely available vaccines against PTB. It was reported that both inactivated (killed) and attenuated vaccinations were equally efficacious. Many nations, on the other hand, do not choose live vaccines because of the partial protection that may be offered by minimising the clinical risk and, maybe, because of the public health risk of infecting humans. Small ruminants should be vaccinated against PTB while they are young to prevent interfering with tuberculosis diagnosis.

Public Health Significance

The recovery of MAP from individuals with inflammatory bowel disease or Crohn's disease, as well as animal healthcare workers with chronic gastrointestinal difficulties, suggests that MAP is linked to chronic disorders that affects the human health.

Conclusion

Paratuberculosis is a severe threat to cattle health and productivity not only in India, but around the world. Despite the fact that the true burden of disease and associated economic losses are still underestimated, serological investigations have revealed a larger MAP bioload in both animal and human populations. There is currently no national policy for the diagnosis and control of animal paratuberculosis. The absence of indigenous diagnostic tests and kits has impeded paratuberculosis control in India. Long-term goals should include the eradication of paratuberculosis in domestic and wild ruminants. As the human population continues to be exposed to MAP, more research is needed to establish a link between MAP and Crohn's disease.

The quest for sensitive and reliable diagnostic tests that may detect early subclinical stages of infection or offer insight into the potential consequences of animals exposed to MAP at a young age is critical. Strategic interventions including changes in management factors important to paratuberculosis control must be implemented, since these give insights into national and maybe worldwide management initiatives. To raise awareness among rural people baffled by this insidious disease, strong leadership and good communication are necessary. This area warrants further research since vaccine effectiveness, safety and challenges with post-vaccination diagnostics and administration difficulties are frequently reported.

The identification of different immune and cellular profiles of sheep with different disease outcomes, which may one day provide a method of predicting disease outcome in an individual, as well as a better understanding of the importance of vaccination for improved long-term disease control, are all important aspects of global paratuberculosis management. Although the disease is also seen in small ruminants and wild animals, its prevalence and economic consequences are not well understood in these animals, mainly due to a lack of resources or technological feasibility and economic viability for study in this area. Above all, every producer should have a biosecurity plan in place to prevent MAP from entering and spreading within their facility.

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An Overview of Herpes Viral Infection in Horses

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Abstract

Equine herpes viruses are ubiquitous organisms affecting all members of family Equidae. EHV1 and EHV4 are the most important equine herpesviruses.Equine herpesviruses (EHVs) are a group of 11 viruses of the family Herpesviridae causing infections in equines, including nine equine herpesviruses (EHV1–EHV9) and two asinine herpesviruses (AHV4–AHV5). Herpes viruses have a unique four-layered structure: a core containing the large, double-stranded DNA genome is enclosed by an icosa-pentahedral capsid which is composed of capsomeres. Transcription and replication of viral DNA occurs within the nucleus. Infection spreads via nose-to-nose contact, contaminated equipment, respiratory secretions, aborted fetuses etc. and causes respiratory illness, abortion and Equine Herpesvirus Myelo-encephalopathy (EHM). Disease can be diagnosed by taking nasal swabs, serum and whole blood and performing cell culture, immunohistochemistry, ELISA, VNT, PCR etc. Immunization of animals can be done using inactivated and modified live vaccines and healthy managemental practices are mandatory to reduce the spread of infection.

Introduction

Equine herpes viruses (EHVs) are highly significant viral pathogens affecting all members of family Equidae globally. Five subtypes of herpes viruses (EHV1–EHV5) have been reported in horses, while donkeys are host to EHV6 to EHV8 (asinine herpes viruses, AHV1 to AHV3) and gazelles, giraffe and zebras are hosts to the newest member of the equine herpes virus EHV9 (gazelle herpes virus) with encephalitis (1). EHV1 and EHV4 are the most important equine herpes viruses that infect 80–90% of horses globally by 2 years of age, resulting in respiratory infection, characterized by fever, anorexia and nasal and ocular discharge (2). EHV1 causes upper respiratory tract infection in young horses at the time of weaning (2), abortion in pregnant mares, neonatal foal mortality and neurological disorders.

The family *Herpes viridae* includes more than 100 viruses infecting fish, amphibians, reptiles, birds and mammals including man. Their ubiquitous occurrence, evolutionary diversity and involvement in a range of important medical and veterinary diseases make this group of viruses one of the most important. The name is derived from the Greek word 'herpein' meaning 'creeping' in reference to the recurrence of vesicles in people infected with herpes simplex virus (3).

Abortion is economically the most crippling outcome of EHV1 infection with 95% of EHV1-associated abortions occurring in the last 4 months of pregnancy. Neurological disease associated with EHV1 is called equine herpesvirus myeloencephalopathy (EHM), which can cause serious economic loss in breeding horses (4). EHV3 is responsible for equine coital exanthema (ECE), a venereal and highly contagious disease. EHV3 infection causes the formation of papules, vesicles and pustules on the external genitalia of mares and stallions. The affected stallions may have stiff gait and loss of the libido and refuse to mate mares (5) EHV2 and EHV5 cause upper respiratory tract infection, inappetence and immunosuppression.

The viruses also cause keratoconjunctivitis, lymphadenopathy and general malaise (6). EHV5 is routinely detected in blood and nasal secretions of healthy horses and generally does not cause disease in the horse. However, recent reports associate EHV5 with lung infection leading to equine multinodular pulmonary fibrosis (EMPF) (7).

Etiology

Equine herpes viruses (EHVs) are a group of 11 viruses of the family *Herpesviridae* causing infections in equines, including nine equine herpesviruses (EHV1–EHV9) and two asinine herpesviruses (AHV4–AHV5). EHVs reported so far belong to subfamilies *Alphaherpesvirinae* and *Gammaherpesvirinae* and none belong to subfamily *Betaherpesvirinae* (Table1).

EHV1 and EHV4 have linear double-stranded DNA genomes that are approximately 150 (EHV1) and 145 (EHV4) kbp in size, respectively, with a unique long region (UL) and a unique short region (US). The US is flanked by identical pair of terminal repeats (TR) and internal repeat (IR) regions. Sequence analysis showed 55–84% DNA homology at nucleotide level and 55%–96% homology at amino acid level (8). The genomes of both viruses encode for 76 homologous genes, with three duplicated genes in EHV4 and four duplicated genes in EHV1 within the repeat regions, two more genes were reported for EHV1 genome as regulatory genes IR2 (ORF77) (9) and IR3 (ORF78) (10). Thus, EHV1 genome has a total of 78 ORFs, including six regulatory genes 64, 65, 66, 67, 77 and 78 that are present as duplicate genes both in the internal and terminal repeats of the EHV1 genome (11).

The expression of EHV1 and EHV4 genes takes place

Table1 Classification and diseases associated with equine herpesviruses

Species	Subfamily	Genus	Disease
EHV1	α	*Varicellovirus*	Rhinopneumonitis, abortion, myeloe-ncephalopathy
EHV2	γ	*Percavirus*	Respiratory tract infection, keratoconjunctivitis,
Malaise	EHV3	α	*Varicellovirus*
Coital exanthema	EHV4	α	*Varicellovirus*
Rhinop-neumonitis	EHV5	γ	*Percavirus*
Equine multinodular pulmonary fibrosis	EHV6	α	*Unassigned*
EHV8	α	*Varicellovirus*	Rhinitis
EHV9	α	*Varicellovirus*	Gazelle and equine neurological disease

in an orderly and tightly controlled cascade and is accordingly categorized into immediate-early (IE) or α-genes, early (E) or β-genes and late (L) or γ-genes. The regulatory genes playing an important role in coordinated gene expression in EHV1 include one immediate-early protein (IEP, ORF64), four early proteins (IR2, truncated ORF64; EICP0, ORF63; UL5; and IR4) and one late tegument protein (equine α-transinducing factor, ETIF) (12). EHV2 has a 184-kbp genome and 79 open reading frames (ORF) encoding 77 proteins, while EHV5 has a shorter, 179-kbp genome, both are distinct viruses with only 60% shared identity at DNA and amino acid levels between all the conserved EHV2 and EHV5 sequences (13). The EHV2 genome has unique central region (149 kbp) flanked at both ends by long (17.5 kbp) direct terminal repeats. The unique central region hasa pair of unrelated internal, short, invertedrepeats at separate locations. However, the 179-kbp genome of EHV5 lacks both internal and terminal sequence repeats (14).

Virus Structure

Herpes viruses have a unique four-layered structure: a core containing the large, double-stranded DNA genome is enclosed by an icosa-pentahedral capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the tegument. It is encased in a glycoprotein-bearing lipid bilayer envelope.EHV1 and EHV4 are considered to contain at least 13 glycoproteins. From DNA sequence analysis of EHV1 and EHV4 homologues for 10 of the 11recognized Herpes simplex virus type 1 (HSV1) glycoprotein have been identified. EHV1 possesses at least three other glycoproteins designated gp2, gp10 and gp21/22a (15).

Virus Replication

Cellular attachment of herpesviruses occurs via the binding of virion glycoprotein spikes to one of several host-cell receptors. Following attachment, the viral envelope fuses with the cell plasma membrane, the nucleocapsid enters the cytoplasm, and the DNA–protein complex is then freed from the nucleocapsid and enters the nucleus, quickly shutting off host-cell macromolecule synthesis.

Infection is initiated by its attachment to the cell membrane. Following attachment, the viral envelope fuses with the cell plasma membrane, the nucleocapsid enters the cytoplasm, and the DNA–protein complex is then freed from the nucleocapsid and enters the nucleus through nuclear pores (16). Transcription and replication of viral DNA occur within the nucleus (17). The transcription of herpes simplex virus (HSV1) genes occurs in an ordered pattern of gene expression namely immediate–early (IE), early (E), early–late (E/L), and late (L) (18).

In horses, EHV1 can infect various cell types, including endothelial cells of inner organs, epithelial cells of the respiratory tract, and mononuclear cells in lymphoid organs and the peripheral blood (PBMCs) (19). Cells are either infected by direct contact with an infectious EHV1 particle or by cell-to-cell spread following contact with an infected cell (20). Efficient infection is initiated by a relatively unstable attachment to heparan sulfate molecules on the proteoglycan cell surface, mediated by gC and gB, followed by binding of gD to one of the specific receptors on the cell surface (21, 22, 23).

EHV1 can enter permissive cells either by direct fusion with the host cell membrane or by cell-mediated endocytosis, producing a productive infection in both cases (24). Both entry pathways facilitate the release of viral nucleocapsid and tegument proteins into the infected host cell. Once the virus is released inside the host cell, the tegument proteins dissociate from the nucleocapsid and the capsid is transported along microtubules via dynein, a minus-end-director motor protein, to the nucleus of the cell (20).The inner tegument protein UL36 (ICP1/2) which bears a nuclear localization signal (25), together with nucleoporins Nup358 and Nup214 which both bind either directly or indirectly to the capsid, facilitate this process (26, 27).

Like for other herpesvirus, it is believed that most of the tegument proteins dissociate from the capsid, which associates with microtubules via dynein, a minusend-director motor protein. The capsid is therefore transported along microtubules to the microtubules organizingcenter, near the nucleus. This mechanism of capsid transport is important in the infection of cells such as neurons when the site of infection can be far from the nucleus. The nucleocapsid binds directly to

the nucleopore complex (NPC) and the viral DNA is translocated into the nucleus while the nucleocapsid remains in the cytoplasm.

The transcription of the EHV1 genome is sequentially ordered. The tegument VP16 (HSV) homologue protein of EHV1, brought into the cell by the virus, is a strong activator of immediate early (IE) gene expression. The IE protein is encoded by ORF 64 and synthesized by cellular RNA polymerase II [28, 1]. This gene is required for the transcription of the adjacent early and late genes. Early genes encode the proteins involved in stimulating virus replication. Late genes encode the viral structural proteins. Herpesvirus nucleocapsids are assembled in the nucleus around scaffolding proteins prior to viral DNA encapsidation. The nucleocapsid, surrounded by tegument proteins, leaves the nucleus by envelopment at the inner nuclear membrane that contains glycoproteins. This primary envelope is lost when the virus buds through the outer nuclear membrane. A second envelopment occurs at the cytoplasmic membranes (ER or exocytotic vesicles), which contain all the viral glycoproteins, before the migration of the mature virus through the secretory pathway (via the Golgi apparatus). The infectious virus can be released into the extracellular space or infect other cells via virus-induced cell fusion. In vitro, gB is absolutely essential for direct cell-to-cell spread of virions. EHV1 gD, gB and gK are involved in the cell-cell fusion process (20).

Replication occurs in the nucleus and protovirions derive their envelope from the inner lamella of the nuclear membrane. Virus particles bud from the cell surface and result in necrosis of the respiratory epithelial cells. Once the virus is within the white blood cell, it seems to be able to circulate without destruction despite high circulating antibody titers. In this location, the virus can disseminate to other tissues, including the central nervous system (29).Maturation involves the completion of encapsidation of virion DNA into nucleocapsids and the association of nucleocapsids with altered patches of the inner layer of the nuclear envelope. Complete envelopment occurs by budding through the nuclear membrane. Mature virions accumulate within vacuoles in the cytoplasm and are released by exocytosis or cytolysis. Virus-specific proteins are also found in the plasma membrane, where they are involved in cell fusion, may act as Fc receptors, and are presumed to be targets for immune cytolysis. Intranuclear inclusion bodies are characteristic of herpesvirus infections, both in animals and in cell cultures (30).

Transmission

Equine herpes virus (EHV1 and EHV4) is spread via nose-to-nose contact, contaminated equipment (water and feed buckets, tack and grooming supplies and shoes) and respiratory secretions within stalls/stables. Aborted fetuses and after birth can also contain the virus. EHV3 is spread through venereal transmission or contaminated equipment use for breeding. EHV is a contagious viral disease which can be spread via direct horse to horse contact and aerosol droplets over short distances (up to 5 meters) by coughing and snorting which can result in rapid spread through a group of horses. It can also be spread indirectly by shared equipment such as tack, feed bowls and people via their hands and clothing.When mares miscarry due to EHV, their foal associated fluids and discharges expelled are all sources of infection to other horses. EHV can lie dormant in the horse following first infection, meaning they will carry the virus as a 'silent' infection. The horse will often appear healthy and show no signs of disease, but occasionally the disease can reoccur at intervals throughout its life. The virus can be reactivated during stressful conditions, such as travelling, moving yards or attending a competition. The horse will shed the virus but may not show any signs of illness.

Pathogenesis

Following inhalation of infectious aerosol or contact with infectious fomites, EHV1 and EHV4 infect and replicate in mucosal epithelium of the respiratory tract. Within 24 hours, the virus is transported via infected leucocytes to lymph nodes associated with the respiratory tract and from there into the blood circulation in monocytes and T lymphocyte. Subsequently, the virus moves to different target organs and replicates in endothelial cells of target organs. EHV1 replicates in endothelial cells of the uterus, showing marked thrombosis and ischaemia, which is supposed to be the primary cause of abortion. The virus might cross the placenta and infects foetus leading to late-term abortion. In some cases, fetus may be born alive if EHV1 infection occurs at a later stage of pregnancy, but it does not survive more than 24 h (20).

Similarly in CNS, the virus induces myeloencephalitis by replicating in endothelial cells (and not in neural cells), leading to the development of nervous disorders due to equine herpes virus myeloencephalopathy (EHM). There is a strong association between EHM and the G2254 mutation in ORF30. However, this nucleotide substitution is not the only determinant of neurological disease. A number of EHV1 strains with A2254 genotype have been isolated from EHM cases. Similarly, G2254 genotype EHV1 isolates from numerous horses with no evidence of neurological symptoms have been identified. One of the possible reasons for this observation could be the fact that besides A2254→G2254 substitution, other non-synonymous nucleotide substitutions in ORF30 can also have effect on the production of neurological disease by either enhancing or attenuating the capability of viral replication rates in vivo. Furthermore, DNA polymerase is only one out of six proteins involved in 'elongation complex' of DNA replication machinery. Substitutions occurring in the ORF of any one of these proteins could have a considerable impact on viral replication rates, which will in turn have an effect on neuropathogenicity (31).

During latency, the virus reaches to the site (lymph nodes, PBL, trigeminal ganglia, etc.) and viral genome translocates to nucleus of target cells, circularizes and maintains as episome without integrating into the host genome. Horses with latent EHV1 infection periodically experience viral reactivation and shed the virus in respiratory tract secretions. Viral reactivation occurs due to stress during transport, weaning, racing or intensive management practices or corticosteroid treatment (32).The viral transcription and translation are blocked during latency, except for transcription of latency-associated transcripts (LATs) from the region antisense to immediate-early (IE) genes (14). These LATs have been thought to play a role in latency by promoting cell survival by inhibiting apoptosis or by downregulating the expression of viral genes. The molecular mechanisms by which LATs produce such function are poorly understood. The miRNAs are also thought to play a pivotal role in establishment and maintenance of latency. Currently, the role of miRNAs in alphaherpesviruses is known for six viruses- HSV1, HSV2, herpes B virus, BoHV1, BoHV5 and pseudorabies(33).

Pathology

Clinical Syndromes

- **Respiratory Illness**

Respiratory disease caused by EHV is widespread among young horses, in the period between weaning and 2–3 years of age. Exposure to EHV occurs through inhalation of aerosolized virus-infective respiratory secretions. The virus multiplies in the epithelia of the nasal cavity, pharynx, trachea and bronchi, causing primarily upper respiratory tract disease. The infection of the lower respiratory tract may also result from dissemination through airway surfaces or via blood vessels and cell-associated viraemia. The clinical signs include acute fever, inappetence, serous nasal discharge and cough.

- **Abortion**

It can be initiated either by exogenous infection or by reactivation of latent virus. EHV1 infection leads to late-gestation abortion, stillbirth and weak neonatal foals. EHV1 replicates in endothelial cells and induces thrombosis and ischaemia in the microcotyledons of the placenta, causing abortion (34). Sometimes, a live foetus may be born if infection occurs in later stages of pregnancies. Such foals die but soon after birth due to respiratory distress, pneumonia and other respiratory complications.

- **Equine Herpesvirus Myeloencephalopathy (EHM)**

Neurological disease can affect horses of all ages, including unweaned foals, and often horses exhibiting neurologic diseases can shed the virus in their nasal secretions and transmit the disease to in contact animals (4). Clinical signs of EHM usually occur 6–10 dpi following the onset of viraemia. It includes fever, ataxia, paresis/paralysis of hind limbs, bladder dysfunction, urinary incontinence and sensory deficit in the perineal area. In addition, ventral oedema, scrotal or preputial oedema in male horses and limb oedema are also noticed. The ORF30 spanning the nucleotide region 51522-55184 (3662 nt) in EHV1.

Genome encodes for a protein referred to as Pol, the putative DNA polymerase catalytic subunit, which possesses DNA synthesis activity. This gene is highly conserved throughout its length. Recently, a single nucleotide polymorphism (SNP) of guanine (G) for adenine (A) at 2254 nucleotide position of the ORF30 region resulting in an amino acid variation, from asparagine to aspartic acid (N/D752), has been proven to be associated with the neuropathogenic potential of the EHV1 strain (35). This DNA polymerase enzyme of EHV1 has two sets of identical protein subunits, each of which contains two catalytic pockets (36), serving as site for polymerase activity and the site for 3′–5′ exonuclease activity. In EHV1 neuropathogenic strains, the point mutation results in a switch from no charge to a negative charge and induces a conformational change within the viral polymerase structure and thereby increases the replicative capacity of the virus and produces significantly higher viral loads (35).

EHV1 infection as a mucosally acquired, infectious viral disease of equines with a multi-organ, systemic pathogenesis causing a spectrum of disease conditions including abortions, respiratory affections, paresis and perinatal foal mortality (37). It involves epithelium, leucocytes and endothelium in three separate systems/organs/tissues, viz. the respiratory tract, immune system and pregnant uterus. Foals infected experimentally through intranasal route develop distinct herpetic lesions in all parts of the respiratory tract. The lesions are characterized by the presence of intranuclear inclusions and necrosis of the respiratory epithelium and lymphoid germinal centers.

The gross lesions in the respiratory tract include hyperaemia of nasopharynx and tracheal mucosa, purulent mucus present in the trachea, oedema of the nasopharyngeal mucosa and little change in the pharyngeal lymphoid follicles (38). Lesions in aborted foetus differ depending on the stage of gestation. The early abortions are characterized by a severely autolysed foetus, with presence of numerous intranuclear inclusions without a local inflammatory response. In contrast, the prominent macroscopic lesions in late abortions include jaundice and petechiation of visible mucous membranes. In addition, subcutaneous oedema, excessive pleural fluid, pulmonary oedema and splenic enlargement with prominent lymphoid follicles and white foci of hepatic necrosis are observed.

Histopathological Changes

These include inflammation and necrosis of nasal, pharyngeal and occasionally tracheal epithelium along with the presence of intranuclear inclusion bodies (39) suggested that neonatal EHV1 infection

causes pneumonitis with extensive depletion and/or degeneration of lymphocytes in the spleen and thymus, which predisposes animals for local secondary bacterial infections. In EHV1-associated perinatal mortality grossly, lungs are voluminous and firm with massive atelectasis, and microscopic lesions include extensive non-suppurative histiocytic-type alveolitis, with acute focal necrotizing bronchitis and presence of intranuclear eosinophilic inclusions (40). The characteristic microscopic lesions include bronchiolitis, pneumonitis, severe necrosis of the splenic white pulp and focal hepatic necrosis with inflammatory cellular response.

The most consistent feature in aborted foetus is necrotizing bronchitis, interstitial pneumonia, focal hepatic necrosis and necrosis of germinal centres in all lymphoid tissues. In mares, the only gross lesion observed was distention of the regional lymphatics, whereas after abortion, the most consistent lesion observed was an intense perivascular infiltration of lymphocytes and plasma cells in association with those vessels located just beneath the glandular layer of the endometrium (41).

Smith et al. (1992) (35) reported that endothelial infection when associated with severe thrombo-ischaemic necrosis could result in abortion without viral infection of the foetus. Histological lesions in the endometrial include congestion, widespread vascular changes including perivascular oedema, ischemia associated with avascular necrosis and perivascular infiltration of lymphocytes, neutrophils andmonocytes (42) Neurological disease is associated with the thrombi formation in vessels in grey and white matter of the CNS and the leptomeninges. This is followed by focal vasculitis and necrosis of the brain stem, cerebrum and white matter of the spinal cord. Vasculitis lesions involved both arteries and veins, which led to ischemia of white matter in brain and left ventral white columns of the spinal cord (43).

Viral Diagnosis

Samples

Nasal swabs are collected for detection/isolation of the virus preserved in viral transport medium along with serum for serology and whole blood for detection of cell-associated viremia. Collect samples as early as possible in the acute phase of infection. Neutralizing antibody titers to EHV1 rises rapidly after natural infection and by 5-8 days after experimental infection.Isolation and identification of EHV1 from nasal or nasopharyngeal swabs or buffy coat samples are strongly supportive of diagnosis of EHM in a horse and considered as the gold standard test for a laboratory diagnosis of EHV1 infection.

Cell Culture

Suitable cells for both viruses include primary equine kidney, equine dermal fibroblasts and equine lung fibroblasts. A wider range of cell lines will supportthe growth of EHV1 including rabbit kidney(RK-13), baby hamster kidney (BHK-21) andMadin–Darby bovine kidney (MDBK). Acharacteristic herpesvirus CPE of focal rounding,increase in refractility and detachment of cells isusually seen within a couple of days (44).

Immuno histochemistry

Viral antigen may be demonstrated in cryostatsections of tissues such as lung, liver and spleen collected from aborted fetuses using immuno fluorescence (45). Immuno histochemical methods have been applied successfully to the detection of viral antigen in paraffin-embeddedtissues from aborted fetuses (46) and from neurologically affected horses(47).

Serology

Serological testing of paired serum samples and demonstration of a fourfold rise in antibody titer is useful in confirming a diagnosis retrospectively. Suitable serological tests include virus neutralization, complement fixation test and ELISA (48). Complement fixing antibody titers become negative within a few months of recovery from infection. Most serological tests are unable to distinguish between infection with EHV1 from infection with EHV-4 due to antigenic cross-reactivity between the two viruses. However, virus specific ELISAs based on discriminating monoclonalantibody or on recombinant glycoprotein G antigensare capable of distinguishing between the twoinfections (49).

ELISA and Virus Neutralization Test (VNT)

These are highly recommended by OIE, either for EHV confirmation of clinical cases or prevalence of infection surveillance. Peptide-based ELISAs provide a simple, very specific, rapid, sensitive and relatively cheap diagnostic alternative and have been widely used for the serological diagnosis of multiple veterinary parasitic82, bacterial83 and viral infections51,84. Serology that demonstrates a 4-fold or greater increase in titer, using acute and convalescent samples, provides evidence of infection. Many horses with EHM, however, do not exhibit a 4-fold rise in Serum Neutralization (SN) titer, since titers rise rapidly and may have peaked by the time neurological signs appear.

PCR

Polymerase chain reaction (PCR) testing has become the diagnostic test of choice due to its high analytical sensitivity and specificity. Due to the leukocyte associated nature of the EHV1 virus, whole blood (anticoagulated) buffy coat sampling provides an additional sample for viral PCR testing. Detection of EHV1 by PCR is routinely performed on secretions from nasal or nasopharyngeal swabs or from uncoagulated blood samples.

Advances in technology and the use of novel PCR plat- forms, such as real-time PCR (RT-PCR), enable the quantification of viral loads for equine herpes viruses. Serological surveys of EHV1 and EHV4 have always been complicated by the extensive antigenic

cross reactivity, the virtual absence of type specific antibodies and widespread use of EHV1 vaccination. The amount of sequence identity between EHV1 and EHV-4, at the amino acid level, ranges from 55-96% across the genome (50). This means that the two viruses cannot be distinguished antigenically using polyclonal antisera.

Prevention and Control

Prevention of equine herpes virus infections can be done by vaccination. available ehv1 vaccines are licensed for prevention of EHV respiratory disease and/or abortion. in addition, there are at least 12 multivalent ehv1 and ehv4 inactivated and modified live vaccines available in the market, which only provide protection against respiratory diseases due to EHV1 and EHV4. EHV1 is considered to be genetically and antigenically stable, and no impact of strain variation on vaccine efficiency has been demonstrated. for preventing respiratory infection, vaccination of foals is done around 3–5 months of age, with a second immunization within 4 to 6 weeks, followed by booster vaccination every 3 or 6 months, depending on type of vaccines. to avoid EHV1-induced abortion, it is recommended to vaccinate pregnant mares at fifth, seventh and ninth months of pregnancy. The various vaccines available are as follows:

(A) Inactivated Vaccines

Majority of commercially available vaccines have been inactivated whole virus or subunit vaccines. Viruses are usually inactivated by formaldehyde or β-propriolactone. Many subunit vaccines and recombinant vaccines targeting gb, gc, gd or gh of ehv1 have been tested in experimental animals, and they produce an antibody and afford protection (20). ICAR - National research centre on equines has developed an inactivated oil adjuvant vaccine using an indigenous isolate, which is very effective in controlling abortions mediated through ehv1. The vaccinated pregnant mares show good immune responses as estimated through serum neutralization assay.

(B) DNA Vaccines

Studies on antigenicity and efficacy of DNA vaccination against ehv1 infection have shown promising results in mice. a DNA vaccine encoding gd has elicited humoral and cell-mediated immune responses and reduced respiratory lesions, virus shedding, and abortion induced on challenge infection (51). However, results on vaccine trials in ponies have not shown much protection against challenge in one trial. The glycoprotein gene-vaccinatedponies showed gD- and gC-specific antibody responses. However, following challenge infection, vaccinated ponies showed clinical signs of disease, indicating EHV1 DNA vaccination-induced limited immune responses and protection (52).

(C) Live Attenuated Vaccines

Immunization with a live attenuated virus is expected to stimulate an immune response similar to those induced by infection. Two principal types of EHV1 mutants have been used as live attenuated EHV1 vaccines, namely, thymidine kinase-negative (TK-) and temperature-sensitive (Ts) mutants. A Ts mutant of EHV1 (clone 147) was derived from a German abortion isolate of EHV1 (strain M8). On intranasal immunization with this attenuated vaccine, ponies developed mild or no clinical signs and had an increased level of VN antibody 6 weeks after the inoculation. After challenge infection, vaccinated ponies developed only mild clinical signs of disease and shed virus, but none of them developed a cell-associated viraemia (53). However, there are safety concerns of virus shedding and cell-associated viraemia after vaccination with this vaccine (54).

There are two currently licensed modified live vaccines (MLV): one is based on RacH strain, which has been passaged 256 times on primary swine kidney cells, resulting in genomic alterations, gp2 gene sequence alteration and deletion of IR6 gene. The resultant attenuated RacH gene afforded protective immune response and safety in equines. Another MLV is derived from Kentucky KyA strain that was modified by passage in murine L-M cells, resulting in deletion of gene 1 and 2 and also deletion of Us region deleting gE and gI (55). The introduction of bacterial artificial chromosome (BAC) technology has facilitated the rapid construction of recombinant attenuated equine herpesviruses (56). BACs have been generated and stably maintained in E. coli, in which genetic mutations such as point mutations, deletions and insertions can be easily introduced by different mutagenesis methods including RecA- and Red/ET-mediated recombinations. Such mutations can be introduced in BACs for generating attenuated live EHVs for use as vaccine candidates. Functions of different genes like IR6, gE, gI and gp2 have been studied in detail for the attenuation of EHV1 employing BAC technology (59, 60). ICAR-NRCE has also ventured into developing BAC of indigenous isolate of EHV1 and has developed deletion mutant for gE, gI and IR6 which are being studied further for developing modified live vaccine.

(D) Vectored Vaccine

Recombinant poxviruses have been widely used for vaccination and poxviruses derived from Canarypox or Fowlpox virus are commercially available. A modified vaccinia Ankara (MVA) vaccine coding for EHV1 gC was evaluated in hamsters in combination with a DNA vaccination coding for the same protein, which induced both humoral and cellular immune responses, including proliferation and cytotoxic T-lymphocyte (CTL) activity (61). The immunizations of ponies with canarypox-based constructs coding for 4 Equine Herpesviruses gB, gC and gD glycoproteins of the Kentucky strain of EHV1 resulted in marked reduction of virus shedding on challenge with EHV1 (62). An immediate-early (IE) gene of EHV1 has been identified as a potent stimulator of virus-specific CTL

responses in ponies. A recombinant poxvirus vector (vaccinia derived NYVAC strain) or a recombinant modified vaccinia Ankara (rMVA) coding for the IE protein has been used for vaccination in horses in two different studies. Multiple immunizations increased CTL activity and IFNγ synthesis specific for EHV1 compared with unvaccinated ponies. Vaccination conferred significant clinical protection and a significant reduction in EHV1 viraemia (20).

Conclusion

Equine herpes virus infection is still persistent among domesticated horses around the world and the vaccines currently available are not completely protective, especially against EHM. An outbreak of any form of the disease has a significant economic impact on the farm, veterinary hospital or other venue where the outbreak occurs.EHV1 is considered as one of the most important causes of respiratory disease in horses and can also causes abortion and nervous manifestations with frequently fatal outcome. It is highly contagious and usually transmitted by direct contact, mainly through infected nasal discharge between infected animal oranimals and infected object.There is no specific treatment for the disease although supportive care, antivirals, non-steroidals and antibiotic therapy for secondary infections may be useful.Further investigations are necessary to determine the role of the mutation in the pathogenicity of the virus.

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Kharai Camels of Kutch: Unique Germplasm of Gujarat

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Introduction

The Indian camel population is rarely found beyond the Aravalli range and breeding is mostly confined to western states like Rajasthan, Haryana, Punjab and Gujarat. India is proud owner of nine registered camel breeds. Two camel breeds, viz Kutchi and Kharai are native to state of Gujarat. Kharai Camel breed is also one of those registered by National Bureau of Animal Genetic Resources (NBAGR), Karnal. Gujarat's diverse geographical landscapes are home to important indigenous livestock breeds and several indigenous communities pursuing traditional occupations (1). The Kachchh region of Gujarat has large camel herders' population mainly reared on common grasslands.

Origin

Kutch district of Gujarat. They are also found in Bharuch, Vadodara, Anand, Ahmedabad and Bhavnagar districts of Gujarat and only in those talukas of them which are having coastal area/bet within. Kharai camel is unique germplasm as it has survived since years having dual ecosystem of dry land and coastal.

Synonyms

The name of the breed is derived from the local word "khara" meaning saline. These camels are also known as "Dariyataru" due to its ability to swim in deep waters of the sea.

Utility

It is important livestock species contributing significantly to rural economy and livelihood of desert dwellers in Kachchh region of Gujarat. The kharai camels are bred by two distinct communities - the Fakirani Jats, who are the handlers, and the Rabaris, who own the animals (2). The male animals are used for draft purpose while the female animals are used for milking purpose.

Population

A sharp decline in camel population has been a pan-India phenomenon and needs urgent attention of the policymakers of the country. The state of Gujarat reported a decline of 9.10 % in the camel population from 2012 to 2019. Kharai camels were only recognized as a separate breed in 2015. According

to 20th livestock census the total population of Kharai camels in India is 4266, which is a meagre 1.7 % of the total camel population in country.

Physical Characteristics

The Kharai camels have medium body size with comparatively large head. The animals are brown-white mix and dark black in body color. The ears are erect with tip slightly curved inside and the neck is thick. The tail is short and thick, legs are strong and have medium footpad whereas, chest pad is small and short (3).

Unique Characteristics

It can survive on dry land mass as well as coastal ecosystem. Kharai camels have a special ability to swim in seawater, mangrove areas, high TDS water and feed on saline trees. Their gently padded hooves help them navigate the wet and salty coastal land with ease and they can swim up to three kilometres. They go by swimming in search of feed

for 2-3 days & return to fulfill their thirst with rainwater.

Threats

The livestock-based economy has always been one of the most important sources of livelihood for people there. This breed in particular is under threat and enlisted as 'Endangered' As per IUCN also come in Schedule I of Wildlife Protection Act, 1942. (2). Because of the salt content in the mangroves, the camels immediately need to drink water after grazing. So low rainfall spells a crisis for them and the steadily decreasing mangroves because of heavy industrialization along the coast have affected the traditional grazing routes. Rapid industrialization has blocked the tidal waves and so why slowly drying up the mangroves.

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Therapeutic Management of Contagious Ecthyma in Goat: A Case Report

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Abstract

A goat of 8 months of age was presented at veterinary clinical complex, college of veterinary science & A. H., NDVSU, Jabalpur, with the history of lesions around the mouth and on the lips, salivation, anorexia and depression since 5 days. On the basis of history, physical examination, clinical sign and presence of lesions the case was diagnosed as contagious ecthyma (ORF). Symptomatic treatment was instituted with intramuscular injection of enrofloxacin @5mg/kg body weight once daily for 5 days to combat the secondary infection. Simultaneously to alleviate pain, pyrexia and inflammatory changes intramuscular injection of meloxicam @0.2mg/kg body weight daily for 3 days. The case was recovered uneventfully.

Introduction

Contagious ecthyma is highly infectious viral dermatitis of sheep and goat caused by parapox virus of the family poxviridae, primarily affecting the skin around the mouth, udder and cornet (1). Contagious ecthyma is also known as Orf, contagious pustular dermatitis, scabby mouth and sore mouth (2). The disease is worldwide in distribution and is most common in late summer, fall and winter seasons on pasture and in winter in feedlots (1). Goats are more commonly affected with contagious ecthyma than sheep (2). Young animals are more commonly affected than adults (3). The morbidity of the disease can be as high as 100%, however the mortality rate in uncomplicated cases rarely exceeds 1% (4). The death in complicated cases occurs due to extension of lesions in the respiratory tract. The affected animals are characterized clinically by the presence of pustular and scabby lesions on the muzzle, lips and cornet, which may become extensive and develop into proliferative wart like lesions. Lesions may also develop on ears, vulva, around anus, scrotum, teats and feet, usually in the interdigital space. Lameness in the affected animals may result from extensive lesions on the feet. Other clinical signs include anorexia, salivation, lameness, fever, diarrhea and pneumonia (5).

Case History and Clinical Observation

A goat of 8 months of age was presented at Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, with a history of lesions around the mouth and on the lips, salivation, anorexia and depression since 5 days **(Fig. 1).** Clinical examination revealed high rectal temperature of 104.3^0 F, heart rate was 65 beats/minute and respiration rate were 23 breaths/minute. Owner revealed that vaccination and deworming of the animal was not done. Thorough investigation revealed that there was no diarrhea, no nasal discharge and no involvement of respiratory system. On the basis of history, physical examination, clinical signs and presence of typical lesions of orf, the case was diagnosed as contagious ecthyma (Orf).

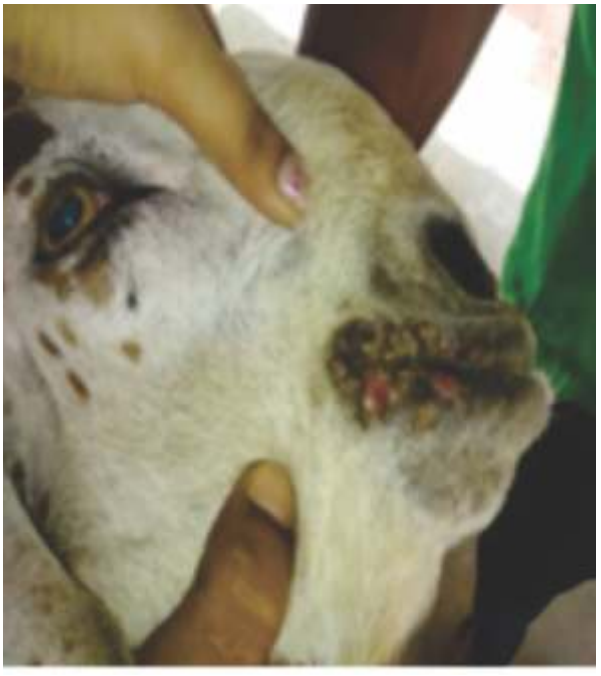

Fig. 1 Goat with dry, ulcerative and scabby lesions around mouth and on lips

Treatment

As Orf is a viral infection, so no treatment is available. However, Symptomatic and supportive treatment is administered to ease the animal. In this

case, firstly dry scabs and crusts were removed gently by washing it with potassium permanganate solution (1:1000) followed by application of boroglycerine to the lesions and affected area. Owner was advised to wash the lesions with 1% Povidone Iodine and application of boroglycerine ointment topically over the lesions twice daily for 7 days. Symptomatic treatment was instituted with intramuscular injection of enrofloxacin @ 5mg/kg body weight once daily for 5 days to combat the secondary bacterial infection. Simultaneously, to alleviate pain, pyrexia and inflammatory changes intramuscular injection of meloxicam @ 0.2mg/kg body weight was administered once daily for 3 days. 500ml of normal saline was also admin is teredin travenously for 3 days. Owner was advised to follow good hygienic measures to prevent the further transmission of the virus. After 3 days of treatment, remarkable improvement was seen, and the case recovered uneventfully after 7 days **(Fig. 2).**

Fig. 2 Goat after recovery from contagious ecthyma

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Management of Dystocia due to Lateral Deviation of Fetal Head and Neck in an Indigenous Goat: A Case Report

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Abstract

The present case study reports dystocia in an indigenous goat in its first parity due to lateral deviation of the fetal head which was delivered with one live fetus after repulsion and correction of posture.

Introduction

The incidence of parturition related disorders in goats was 38.6% (1), whereas the incidence of dystocia was 8.23% (2) and about 7% (3) and the fetal causes of dystocia are 44.44% in goats (4). Lateral deviation of the head and neck, and flexion of the carpus and shoulder being the most common in goat (5,1), although other maldisposition may occasionally occur. In another study in fetal cause of dystocia 5.26% are due to only lateral deviation of head (6). Mutation involves obstetrical maneuvers for relieving dystocia. The ability of the veterinary personnel to distinguish kidding difficulties is considered as an important step in treating dystocia. In this communication, a case of dystocia due to fetal head and neck deviation in an indigenous goat is reported.

Case History and Observation

An indigenous doe of about 1.5 years of age with full term pregnancy in its first parity was presented to the Gynaecology clinics, College of Veterinary and Animal Science, Bikaner with the history of dystocia. The water bag was ruptured 3 hours before and both the fore limbs were protruding from the vulva but further no progress was observed (Fig. 1). The goat owner tried to resolve the dystocia at home by local quake but failed due to insufficient space. The general condition of doe was good and rectal temperature of 102.5° F. Per vaginal examination following epidural anesthesia with 2% lignocaine hydrochloride revealed that cervix was fully dilated, and the fetus was presented in anterior longitudinal presentation, dorso-sacral position with right lateral deviation of head and neck.

Treatment

After proper lubrication of birth canal with liquid paraffin correction of the posture of fetus was performed using the repulsion (The fetus was pushed back into the uterine cavity to correct the head and neck deviation) and traction method and the live fetus was then pulled out manually by applying gentle traction on forelimbs (Fig. 2). Placenta got expelled 30 minutes later. The doe did not exhibit secondary complications like uterine straining or prolapse after relieving dystocia. The doe was treated with intra muscular injection of Megludyne- 1.5 ml, Chlorpheneramine maleate 3ml and Oxytetracycline8ml and intrauterine dispensing of 2 Furex bolus (Vets Farma Ltd.) were given to hasten the involution of uterus and control the infection. The animal was treated for further three days with antibiotic, NSAID, fluid (DNS, Metronidazole, Calcium borogluconate) and vitamin (B complex) along with administration of intrauterine bolus for three days. The animal was found to respond well to the treatments and was followed for further 2 weeks telephonically after treatment, and the animal recovered successfully.

Discussion

Dystocia or difficult birth is always emergent conditions and require urgent care. There are several factors affect the reproductive performance of the goat lead to decrease their numbers which result from the death of the fetus and the dams. One of the most important factors which lead to great economic losses was the dystocia (7).

Deviation of the head may vary in degree. It is most commonly deviated slightly but sometimes it may be deviated laterally to the lamb's body. Rarely may it be deviated downwards with only the forelimbs presented. In cases presented after sufficient delay fetal fluids are lost and the uterine wall tightly wrapped around the fetus. Great care must be exercised in correction of such cases to avoid damaging the uterine wall (8). Deviation of the head may be sometimes coupled with flexion of the extremities. Manual correction of the deviation is possible in small ruminants with sufficiently dilated birth canal and in animals presented early with live

fetuses. In small ruminants, due to small diameter of pelvis, only limited manual manipulation of the fetus to relieve dystocia is possible (9). It may be difficult in cases presented beyond 24 hours of 2nd stage of labor (2) which may require removal of one of the limbs by fetotomy or in some cases even caesarean section when fetus is dead and emphysematous.

Survival of the animals (and their fetuses) presented for treatment of dystocia irrespective of whether they are managed manually or surgically is directly related to their clinical status. Prolonged duration of dystocia and mishandling by quakes and inexperienced persons resulted in deterioration of the clinical status (6).

More number of dystocia cases during 1st delivery is probably due to the narrow pelvis of the dams because of breeding the animals at young age and/or to poor management(6). Mutation involves obstetrical maneuvers for relieving dystocia and is particularly beneficial under field conditions. However, caesarean is the only option when vaginal delivery is not possible or not indicated (10).

Conclusion

It is concluded that goats suffering from dystocia should be presented for treatment to specialists without any delay to save both fetus and dam. Emphasis should be given in attainment of proper body weight and/or their pelvis size during breeding. Extra care should be taken in the maiden one during delivery.

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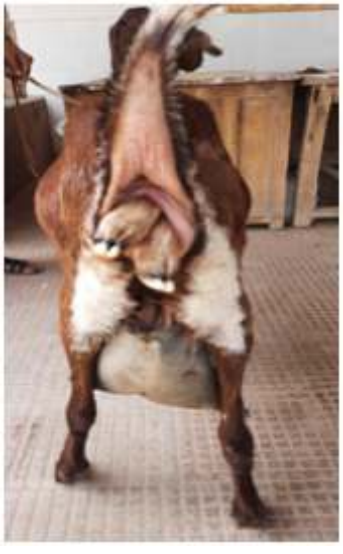

Fig.1: Protrusion of both fore limbs through vulva in doe

Fig. 2: Doe with one live kid

Successful Medical Management of A Rare Case of Pica in Dog

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Abstract

A female spayed 8-year-old(30kg) Labrador mix-breed dog was presented in the Milford Veterinary Clinic (MVC) with a history of continuing vomiting episodes, but no diarrhoea, coughing, or sneezing. Anamnesis revealed the pet's indiscriminate gulping predisposition. In-house survey radiographs showed opacities indicating the presence of some intra-luminal metallic objects. Since referral to veterinary emergency care was initially declined, appropriate remedial strategy, based on the imaging evidence, was planned and executed in the home clinic, MVC. To promote smooth passage of the entrapped intra-luminal foreign material, packaged enema, oral antiemetic, fluid therapy, and optimized symptomatic treatment were scheduled. However, in the next visit to the clinic the owner's fallacious contention that the pet was unable to defaecate continuously for the last five days raised serious clinical concerns prompting referral to the 24x7 Emergency Care. The repeat radiographs established that the dog's bowel was absolutely clear, and the treatment regimen in the home clinic was highly effective, obviating the need for surgical intervention. In point of fact, the ongoing unnoticed ingestion of rusted iron pipe debris in the house backyard was the root cause of the malady. The present communication underscores the need for educating the clients on pica habits, highlighting the preventive measures with emphasis on proper home management, balanced diet and the optimized micro environment.

Introduction

Pica, signifying depraved appetite in animals and humans is ingestion of totally non-edible and potentially harmful substances (1) is classified in clinical psychology parlance under obsessive compulsive disorder(OCD) (2,3). The aetiology is associated with minerals deficiency, anxiety, boredom and unsatisfied hunger (4). Companion dogs may accidently eat up, while playing with such entities as toys (5). The common non-food items, retrieved from the affected dogs gastrointestinal tract include sand, stones, metals, grasses, polythene, and fabrics (6-9). Whereas some of these items.

May pass through the GIT uneventfully, others may induce severe obstruction/impaction/perforation leading to emergencies like pneumothorax (10) with high morbidity and mortality rates (11). In some instances, dearth of experienced veterinarians and diagnostic facilities hampers early intervention (12). The incidence of pica cases in companion animals is increasing globally, primarily because of owner's lack of awareness of the proper management practices: habitat, hygiene and sanitation, balanced diet, restricted use of the commercial foods, and regular exercise (13). This communication supports this contention.

Case Description

A female spayed Labrador retriever mix breed (DOB 6.1.2014) weight 30 kg was presented on March 9,

2022,in the Milford Veterinary Clinic (MVC) with the complaint of vomiting repeatedly dark coloured ingesta over the last two days. No diarrhoea, coughing or sneezing was noticed. Physical examination: rectal temperature 100.80F, heart rate 96 beats/ minute, respiratory rate could not be recorded because of the panting, capillary refill time (CRT) <2 seconds, visible mucous membranes pink, body condition score (BCS) 3/5. Increased pain perception noticed on abdominal palpation, and per rectal examination revealed trapped gravel-like gritty material.

The owner informed that the pet indiscriminateLy grabs anything and may have ingested garden soil. Abdominal survey radiographs (Fig. 1 a, 1 b)clearly revealed prominent opaque patches in the entire stretch of intestines, and stasis in the bowel. Referral to 24x7 Emergency carewas advised but the owner declined, and treatment in the MVC was mandated.

A compendium of enema, antiemetic and symptomatic treatment was scheduled.

Enema did not eliminate any deleterious item(s). Repeat bolus per rectum failed to elicit the expected bio response. In the absence of normal defaecation, rectal lavage in the clinic on the following day was scheduled. Cerenia® (antiemetic) injected S/C @ 2mg/kg, and@1mg/kg PO SID for 2 days dispensed for home use ifvomiting persists. Antibiotic ampicillin (Polyflex®, Boehringer Ingelheim, USA @ 10mg/kg S/C. Famotidine (acidinhibitor) @ 0.25mg/kg S/C, Sucralfate@ 1gm TID x 3 days, 2 hr. after oral medication, or feeding. Lactulose (laxative preparation) dispensed for home use. Metoclopramide @0.2mg/kg S/C was administered to push out the noxious intestinal contents. Isosmotic electrolytes solution, injected S/C.Patient's overnight life support care in the Emergency referral was advised.

The owner called 5 days later to get the patient rechecked. The owner did not provide factual information about the pet's passing faecal excreta. Since chronic intestinal impaction necessitates urgent surgical intervention, the pet was shifted to Emergency care by the well-informed owner. The emergency clinic's attending DVM monitoring the fresh set of radiographs (Fig. 2 a, 2b) was pleasantly surprised that the bowel had cleared completely, testifying the highly effective treatment regimen in MVC. The owner, not aware of the dog's passing motions with smooth evacuation of the deleterious intra-luminal metal foreign body in the backyard, entertained the fallacious notion of no defecation continuously, raising clinical concerns, not based on facts.

Discussion

Pica,or depraved appetite in the companion animals may result from chronic deficiency of essential minerals, e.g. zinc and iron, social restrictions imposed on family dogs, and psychological stress like anxiety and boredom, or in some cases idiopathic (3). Pica may create high-risk GIT emergency situations documented in a series of case reports. For example, two dogs of different breeds, and households ate large pieces of garbage towel culminating in severe bowel impaction and stasis of the in gesta, multiple perforation wounds and fatal peritonitis (13). Early

Haematobiochemical Profile

Table1. Patient's Haemogram on Presentation

Auto Cell Counter H=High L=Low

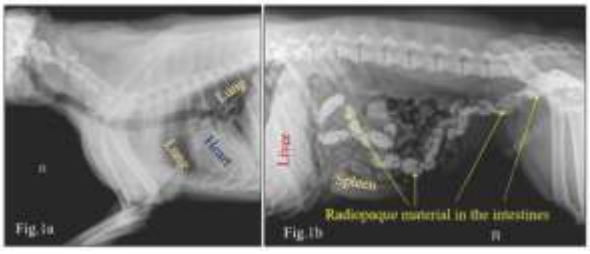
Parameter (units)	Value	Normal Range
TEC (1x106/µl)	9.2 H	5.65-8.87
Hb(g/dl)	21.4 H	13.1-20.5
HCT (%)	60.9	37.3-61.7
MCV (fl)	66.2	61.6-73.5
MCH (pg)	23.3	21.2-25.9
MCHC g/dl)	35.1	32.0-37.9
RDW (%)	19	13.6-21.7
Reticulocyte (1x103/µl)	57	10.0-110
Reticulocyte (%)	0.6	
Reticulocyte-Hb (pg)	25.7	22.3-29.6
TLC (1x103/µl)	9.63	5.05-16.8
Neutrophil (%)	78.8	
Lymphocyte %)	12.9	
Eosinophil (%)	3.9	
Monocyte (%)	4.2	
Basophil (%)	0.2	
Neutrophil (1x103/µl)	7.59	2.95-11.6
Eosinophil (1x103/µl)	0.38	0.06-1.23
Lymphocyte (1x103/µl)	1.24	1.05-5.10
Monocyte (1x103/µl)	0.4	0.16-1.12
Basophil (1x103/µl)	0.02	0- 0.10
Platelet (1x103/µl)	135 L	148-484

Table 2.Patient's Blood Chemistry Profile.
Blood Chemistry Analyzer

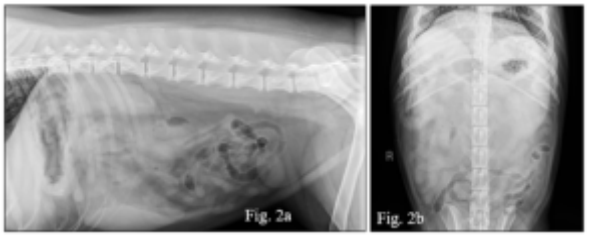
Parameter (units)	Value	Normal Range
Glucose (mg/ dL)	110	70-143
SDMA (µg/ dL)	13.0	0-14
Creatinine (mg/ dL)	0.9	0.5-1.8
BUN (mg/ dL)	9.0	7-27
BUN/ Creatinine ratio	10.0	
Calcium (mg/ dL)	10.3	7.9-12.0
Phosphate (mg/ dL)	2.8	2-5-8.2
Total protein (g/ dL)	7.7	5.2-8.2
Albumin (g/ dL)	3.6	5.2-8.2
Globulin (g/ dL)	4.1	2.5-4.6
A/G ratio	0.9	
ALT (U/L)	63.0	10-125
ALP (U/L)	66.0	23-212
GGT (U/L)	2.0	0-11
Amylase (U/L)	870	500-1500
Lipase (U/L)	887.0	200-1600
Total bilirubin (mg/ dL)	0.4	0-0.9
Cholesterol (mg/ dL)	240	110-320
Na+ (mmol/ L)	157	144-160
K+ (mmol/ L)	4.1	3.5-5.8
Cl-mmol/ L)	118	109-122

presentation in the clinic for prompt medical treatment/ judicious surgical intervention are the key factors in fast recovery, and. ultrasonography facilitates diagnosis and helps in planning the appropriate remedial strategy: medical, and / or surgical (14). These diagnostic aids are still not forthcoming in many locations. This mandates

Abdominal Survey Radiographs



(Fig. 1a) R/L thoracic survey radiographs
(Fig. 1b) R/L Abdominal view showing continuous band of opacities indicating impaction with metallic foreign body (clustered rust).



(Fig. 2a)R/L abdominal survey radiographs
(Fig. 2b) V/D view with no radiopaque intestinal content, indicating complete evacuation of the metallic ingesta from the patient's intestine.

effective preventive measures.

Conclusion

Companion dogs should be constantly monitored for pica habits. Pet practitioners should educate the clients the basics of pica habits and the real health hazards to the pets.

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Successful Medical Management of Canine Juvenile Cellulitis (Puppy Strangles): A Case Report

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Abstract

A male Sheepadoodle puppy was presented for health recheck in the Milford Veterinary Clinic (MVC) following emergency referral (ER) for diagnostics and treatment of multiple health issues. The clinical signs on presentation strongly suggested Canine Juvenile Cellulitis, 'Puppy Strangles' in popular parlance. Right hind limb affliction, presumably immune-mediated and otitis were also detected. The puppy was placed under long-term oral combination therapy: clavamox-meloxicam-gabapentin, prescribed in anemergency facility. Additionally, in the home clinic (MVC) the anti-inflammatory glucocorticoid, prednisonewas highly effective in resolving the primary health issue, puppy strangles. Anthelmintic, Panacur®eliminated Toxocara spp. infestation. Topical antibiotic preparation, Animax® was useful in resolving the chronic otitis. Recovery of normal body weight in the growing puppy attests to successful therapeutic management of the multiple ailments.

Introduction

Canine juvenile cellulitis, a cutaneous disorder also named 'puppy strangles',affects puppies mainly in the 3-24 weeks age group. Clinically manifested as sterile postulated granulomas, pyogranulomatous dermatitis and lymphadenitis (1-3),the disease syndrome is of ill-defined aetio-pathology (4-7). However, since the skin lesions respond well to glucocorticoids, involvement of an immune-mediated component appears logical. The classic symptoms on presentation: acute swelling of the muzzle, lips and eyelids associated with the sterile cutaneous pustules are the landmark. Otitis externa is also common: the ear pinnae appear indurated and oedematous. Small ulcers, draining tracts, seropurulent exudates, or crusts may develop in some of the ruptured pustules. Submandibular lymphadenopathy is often noticed (7). Occasionally, nodules may develop over the trunk and preputial / perineal areas. Patchy alopecia and scarring may result from the consortium of cutaneous lesions. Epithelioid macrophage is the dominant inflammatory cell type on microscopic examination (8-9). The cutaneous disorder may be promoted by concurrent distemper virus infection, hypersensitivity, poor hygiene and sanitation, malnutrition, endoparasite(s) infestation, and stress (10).

Case Description

A male 14-weeks old Sheepadoodle pup (DOB April 4, 2021) with persistingear infection, was presented in the Milford Veterinary Clinic (MVC) on July 6, 2021,for health recheck, following diagnostics and treatment in 24x7 Emergency Referrals, primarily focused on canine juvenile cellulitis and suspected

immune-mediated affliction of the right hind limb. Physical examination MVC: visibly increased lacrimation in both eyes with erythema, ear infection (otitis), non-reducible umbilical hernia, rectal temperature 103.2^{0}F, Heart Rate 110 beats/ minute, respiratory rate not recorded because of panting, capillary refill time (CRT) <2 seconds, visible mucous membranes pink, body condition score (BCS) 3/5. Increased pain perception was noticed during ear examination. The inner margin of lower eye lids, the lower lip, and the submandibular lymph nodes appeared moderately swollen. Small nodules were noticed in the skin round the neck, and near the base of ears, and interdigital spaces in the right front paw **(Fig. 1).**

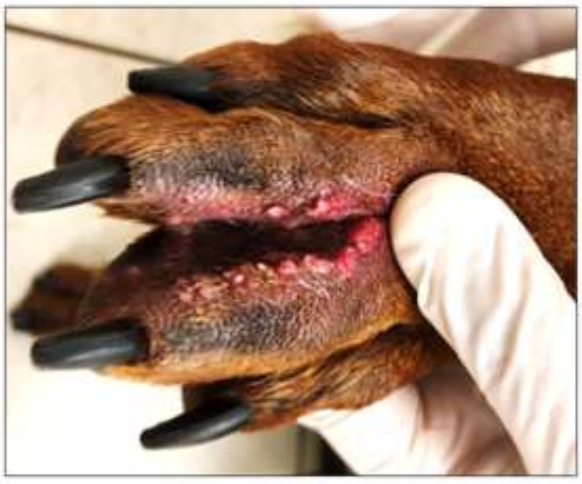

Fig.1 Interdigital Skin Lesions

A brief resume´ of diagnostics and treatment given to the patient in the emergency/ urgent care facilities will be pertinent. Complete blood count (CBC) in the Veterinary Care Specialists (VCS), Milford, MI on June 27, 2021,revealed leukocytosis associated with neutrophilia and monocytosis, presumably because of infection. Ear cytology profile confirmed the presence of bacteria and yeast. Inference: ear infection, pyrexia of undermined cause, stiff gait and lameness in the right hind limb. Treatment for otitis: Osurnia®solution (florfenicol, terbinafine, betamethasone acetate combination) 1 tube placed in each ear repeated in 7 days with no cleaning instructed during this interval. Home pain management treatment: gabapentin [250 mg/ml], 1 ml orally every 8-12 hr intervalx 7 days. Meloxicam non-steroid anti-inflammatory drug (NSAID) 1.5 mg/ ml, administer 0.5 ml OD x 7 days with food. Clavamox® (amoxicillin-clavulanate combination antibiotic) [125 mg] one tablet BID x 14 days. Diet: normal. Advisory on the patient's activity restrictions was issued for the owner's strict compliance on discharge the same evening. On June 29, 2021, the companion animal could not stand up without manual support. Radiography in the MedVet Specialty Healthcare for Pets, Commerce, MIpointed to comorbidity, juvenile bone disease: panosteitis / hypertrophic osteodystrophy (HOD)/ immune-mediated polyarthritis (IMPA). Clavamox, meloxicam and gabapentin combination regimen, prescribed earlier in the VCS was advised to be continued uninterrupted.

Concerned on observing the puppy scratching the ears and eyes, the owner approached the home clinic, MVC for a second opinion. No change in the line of treatment, prescribed in the emergency referral, was recommended. With no tangible relief, the patient was brought for recheck in the MVC on July 10, 2021. Anamnesis revealed the pet continuing to shake the head and eating and drinking less than normal. At this point of time, the comprehensive review of multi-dimensional canine juvenile cellulitis in the VIN Cyclopedia of Diseases-canine juvenile cellulitis, or puppy strangles (11) was highly informative in deciding the further treatment option. In the case under report, the prominent clinical features were vesicles and pustules in the oral cavity **(Fig. 2),** hips, ear pinna, and mandibular lymph adenomegaly with ulcerations on the right ventrolateral aspect of the neck.

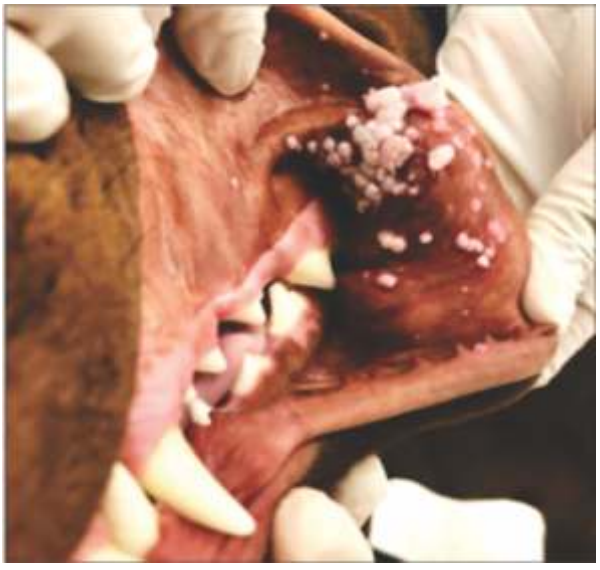

Fig. 2 Prominent Nodules in the Oral Cavity.

The cutaneous lesions were clipped gently, sanitized with antiseptic solution, and Animax® ointment was applied topically. Depo-Medrol® (methylprednisolone) [20 mg/ml] 0.4 ml and Polyflex® (ampicillin antibiotic) 1.5 ml, injected S/C.Clavamox125 mg tablet 1 BID x 10 days, Animax topical application and Prednisone 10 mg tablet 1 BID x 3 days, and ½ tablet x 14 days were dispensed under advisory for home medication. Recheck in the clinic after 4-5 days was recommended. A lone episode of increased pain perception was successfully managed by the owner. The puppy was apparently recovering unevent fully. August 31, 2021: the faecal sample tested positive for Toxocara spp. The patient was given Panacur® (fenbendazole) tablet OD x 3 days, initially. September 16, 2021: the patient was closely examined in the clinic. The body weight had increased noticeably (19.95 kg). Anal gland issues and licking of penis with some exudate and a non-reducible hernia (1.5 cm x 2 cm) were noticed. Pustules on the face were obliterated indicating complete resolution of the main health issue: puppy strangles. Repeat dose of Panacur (1 tablet OD x 3 days) was dispensed. Scheduled DA2PP

(Distemper, Adenovirus, Parvo and Parainfluenza) and Lepto vaccines were given. October 7, 2021: The pet recovered completely from puppy strangles. The clinical signs of concurrent bone diseases: panosteitis, HOD, IMPA too could not be detected. Repeat dose of DA2PP and Lepto vaccines given. The puppy did not require any further treatment in the clinic thereafter to the entire satisfaction of the well-informed owner.

Discussion

Pathogenesis of canine juvenile cellulitis remains enigmatic (2, 7). Special stains and high-resolution electron microscopic examination of tissue biopsies consistently failed to reveal any causative microorganisms, microbial cultures negative, and experimental transmission to the skin of healthy puppies through topical application of lesion tissues remained uniformly unsuccessful (12). In perspective, exponential development of the typical sterile granulomatous pustules that respond dramatically to glucocorticoid preparations (2, 3, 13) indicates some unidentified patho-biomechanism, whereby the innate immune system targets the puppy's own skin: autoimmune reaction. Further, the early age of onset coinciding with the mandatory vaccination schedules aroused serious concerns in the veterinary fraternity regarding possible vaccination reaction. However, the redeeming feature is the unique self-limiting feature of the malady; the affected puppies often recover within 4-12 weeks. The prognosis is favourable if adequate treatment with home care is initiated early (14-16). Critical appraisal of the scanned published reports revealed the treatment of choice: anti-inflammatory agent, glucocorticoid, used singly or in combination with a proven broad-spectrum antibiotic. The therapeutic efficacy of Prednisone @ 2.0 mg/ kg OD, orally or Dexamethasone @ 0.2 mg/ kg OD, PO for 2-3 weeks is well-documented (1,6,17-21). Concurrent Cephalexin/ Cefadroxil/ Amoxicillin-clavulanate antibiotic regimen is recommended to preempt fresh/ eliminate existing subclinical bacterial infection. To combat canine juvenile cellulitis systemic antibiotic alone is not effective(1,2,12,22-25). Anti-microbial drug, Griseofulvin @ 14.2-34 mg/kg BID, administered orally proved effective within 3 weeks' time (1, 22). Topical wet soaks of aluminum acetate/ magnesium sulphate are beneficial in ameliorating the discomfort through facilitated removal of the deleterious surface skin debris (26, 27). In the instant case, within a few days of bringing home, the neonatal male puppy was taken by the caring owner to the veterinary emergency facilities (VCS, MEDVET) for a variety of ailments, including persistent irritating otitis. The pet was under long-term combination therapy: clavamox-meloxicam-gabapentin. In the home clinic, the wide range of cutaneous lesions, with comorbidities like orthopaedic problems (lameness/pain inthe right hind limb), conjunctivitis, otitis, neurological involvement (inability to stand up),oedema in different body parts, and anorexia confirmed the suspected canine juvenile cellulitis (puppy strangles) syndrome. In the home clinic, additional anti-inflammatory Prednisone and broad-spectrum antibiotic Polyflex, and anthelmintic Panacur (aimed to eliminate the Toxocara spp. infestation) proved highly effective.

Conclusions:

Quick response to oral combination therapy (immunosuppressive glucocorticoid and broad-spectrum antibiotic) resulted in complete recovery of the patient with no recurrence of the skin lesions. Proper home care, improved habitat hygiene and sanitation, restricted body movements for the specified period, and balanced food contributed substantially to the healing process..

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Surgical Management of Advanced Cystourolithiasis in A Spayed Female Dog

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Abstract

A 5-year-old spayed female Welsh-Corgi dog was presented in the Milford Veterinary Clinic with the complaint of passing abnormal stone-like objects in the urine. The patient's haematobiochemical profile was normal. In-house urinalysis results and abdominal survey radiographs confirmed numerous bladder calculi, resembling a small bag packed with stones. Laparotomy-cystotomy was performed in the clinic on November 11, 2021, and sample submission to the Minnesota Urolith Center revealed the chemical identity:100% magnesium ammonium phosphate (struvite). The recovery was remarkably fast: virtual normalcy restored on day 1 post-surgery. To preempt recurrence of bladder stones, Hill's S/D therapeutic diet was advised for life-long use.

Introduction

Uroliths / calculi, or 'stones' in popular parlance refer to abnormal mineral deposits in different parts of the urinary system, clumped into a solitary big mass or a cluster of entities, varying in size, shape and chemical composition. Deposits in the urinary bladder are named cystouroliths. In dogs, most of these deposits are struvite (magnesium ammonium phosphate) and calcium oxalate.The clinical signs in the ongoing pathobioepisode include blood-stained, or discoloured pungent urine, polyuria, incontinence, abnormal licking of the genitalia, lethargy, anorexia, and vomiting.

Most bladder stones are identified with abdominal survey radiography, and/ or ultrasonography. Urine analysis, microbial culture and antibiotic sensitivity test may also be recommended for definitive diagnosis in individual cases(1). Haematuria is the consistent clinical sign (2). According to the Veterinary Information Network, VIN (USA), 85% of dogs with struvite uroliths are young females (average age 2.5 years), and Shih-tzu, Schnauzer, Yorkshire Terrier, Labrador Retriever and Dachshund are at increased risk. To promote accelerated dissolution of the stones special formulated dog food, e.g., Hill's S/D is recommended. In concurrent UTI, antibiotic support is also necessary. Lithotripsy, using high frequency sound waves to disintegrate stones in situis a less invasive, innovative option. Surgical removal of the bladder stone(s) is inevitable in life-threatening urinary obstruction (3).Calcium oxalate uroliths are more common in the male dogs, especially in the higher age group (average 9.3 years); some breeds, e.g., Norfolk terrier and Pomeranian at increased risk. These deleterious objects may be removed through surgical intervention, or the novellithotripsy / urohydropropulsion technique. For diagnosis and treatment options on bladder stones in the dog patients Minnesota Urolith Center is the leading referral agency in the USA(1).

Case History

A 5-year-old female spayed Welsh-Corgi (13.2 kg) was presented in the Milford Veterinary Clinic on November 8th, 2021,with the complaint of passing stone-like entities in the urine. All the vitals were WNL: rectal temperature 102.3°F, heart rate 110 beats/ minute, respiration rate not recorded because of panting, capillary refill time (CRT) <2 seconds, mucous membranes pink, and body

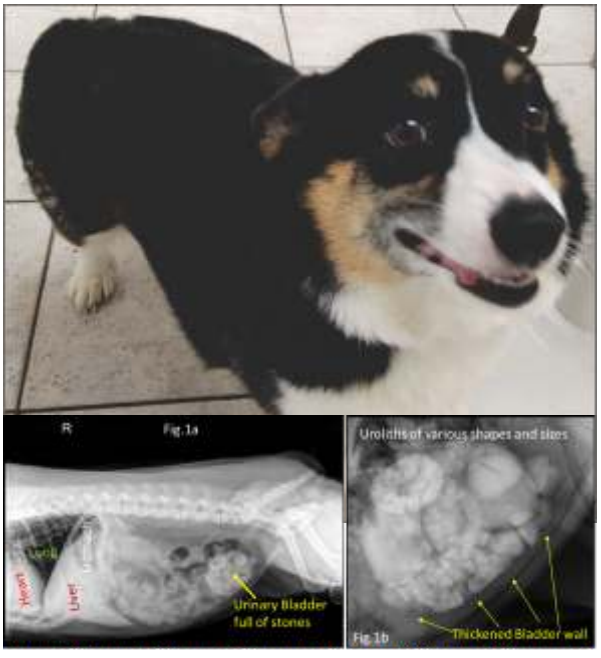

Fig. 1a. Abdominal survey radiograph: numerous cystouroliths. Fig. 1b. Bountiful uroliths inside the indurated bladder wall.

condition score (BCS) 3/5. A gritty feel and acoustics on abdominal palpation indicating the presence of numerous cystouroliths, is corroborated by the radiograph images.

Haemato-biochemical profile (Table 1& 2) was normal. Urinalysis (Table 3) suggested haematuria.

Table 1. Patient's Haemogram on 09.11.2021

Parameters (Units)	Value	Reference Interval	Status
TEC (1x106/µL)	8.37	5.65-8.87	Normal
Hematocrit (%)	61.4	37.3-61.7	Normal
Hemoglobin (g/dL)	20.9	13.1-20.5	High
MCV (fL)	73.4	61.6-73.5	Normal
MCH (pg)	25.0	21.2-25.9	Normal
MCHC (%)	34.0	32.0-37.9	Normal
RDW (%)	17.9	13.8-21.7	Normal
Reticulocyte(1x103/µL)	120.5	10.0- 110.0	High
Reticulocyte Hb (pg)	26.1	22.3- 29.6	Normal
TLC (1x 103/µL)	11.49	5.05-16.76	Normal
Neutrophil (%)	65.1		
Lymphocyte (%)	22.3		
Monocyte (%)	8.0		
Eosinophil (%)	4.4		
Basophil (%)	0.2		
Neutrophil (1x103/µL)	7.48	2.95-11.64	Normal
Lymphocyte(1x103/µL)	2.56	1.05-5.10	Normal
Monocyte (1x103/µL)	0.92	0.16-1.12	Normal
Eosinophil (1x103/µL)	0.51	0.06-1.23	Normal
Basophil (1x103/µL)	0.02	0.00-0.10	Normal
Platelet (1x103/ µL)	322	148-484	Normal

Auto CBC Analyzer; Blood Chemistry Auto Analyzer

Table 2. Patient's blood chemistry profile on 09.11.2021

Parameters (Units)	Value	Reference Interval	Status
Glucose (mg/dL	105	74-143	Normal
SDMA (µg/dL)	14	0-14	Normal
Creatinine (mg/dL)	0.9	0.5-1.8	Normal
BUN (mg/dL)	14	7-27	Normal
BUN/ Creatinine	16		
Phosphate (mg/dL)	5.0	2.5-6.8	Normal
Calcium (mg/dL)	9.3	7.9-12.0	Normal
Total protein (g/dL)	6.9	5.2-8.2	Normal
Albumin (g/dL)	2.6	2.3-4.0	Normal
Globulin (g/dL)	4.3	2.5-4.5	Normal
A/G ratio	0.6		
ALT (U/L)	28	10-125	Normal
ALP (U/L)	55	23-212	Normal
GGT (U/L)	8	0-11	Normal
Amylase (U/L)	741	500-1500	
Lipase (U/L)	719	200-1800	Normal
Bilirubin (mg/dL)	0.2	0.0-0.9	Normal
Cholesterol(mg/dL)	270	110-320	Normal
Na+ (mmol/L)	153	144-160	Normal
K+ (mmol/L)	4.9	3.5-5.8	Normal
Na/K	31		
Cl- (mmol/L)	111	109-122	Normal

Blood Chemistry Autoanalyzer

Table 3. Urinalysis* on 13.11.2021

Parameter	Status	Inference
Color	Red	Haematuria
Clarity	Turbid	
Specific Gravity	1.011	Dilute
pH value	5.0	Acidic
Total Protein (mg/dL)	2+	High
Glucose (mg/dL)	Negative	mg/dL
Ketonebodies(mg/dL)	Negative	mg/dL
Bilirubin (mg/dL)	Negative	mg/dL
Urobilinogen (mg/dL)	Normal	mg/Dl
Erythrocytes /µL	>100	Haematuria
Leucocytes /µL	0-2	Normal

Comments: * Free Catch

The patient was put on broad-spectrum Penicillin. G @ 24,000U /kg, S/C, followed by home care oral medication: Clavamox @ 10 mg/ kg; 62.5 mg tablets 1 tab BID for 10 days, prior to surgical intervention.

Surgery

I. Pre-surgery Protocol

Premedication with butorphanol [10mg/ml] @ 0.2 mg/kg,0.2ml and acepromazine [10mg/ml] @ 0.025- 0.2mg/kg, 0.1ml, injected S/C. Sterile lactate Ringer's solution infused with the I/V catheter @ 200 ml/ hr. Anaesthesia was induced I/V with ketamine [50 mg/ml] @ 2-10 mg/kg, and midazolam [5mg/ml] @ 0.1-0.3 mg/kg, 0.6ml each. The patient was transferred to isoflurane gas, hooked to automonitoring of the vital body functions. The caudal abdomensurgical site was clipped,gently scrubbed and sanitized.

II. Laparotomy-Cystotomy

In the suitably prepared site, a midline incision was made with #10 sterile scalpel blade, caudally from umbilicus to pubis. The urinary bladder was carefully exteriorized. Sterile lap sponges were implanted around the bladder. Care was taken to prevent seepage of the contents of the bladder into the open abdominal cavity. For manipulative convenience of the technician two stay sutures were tagged into the bladder wall. Urine was completely drained out with a sterilized syringe and needle (Cystocentesis), and numerous stones of varying size were cautiously retrieved **(Fig. 2a, 2b)** through a stab incision into the bladder. Urolith samples collected in a vial were labeled and referred for chemical identification.

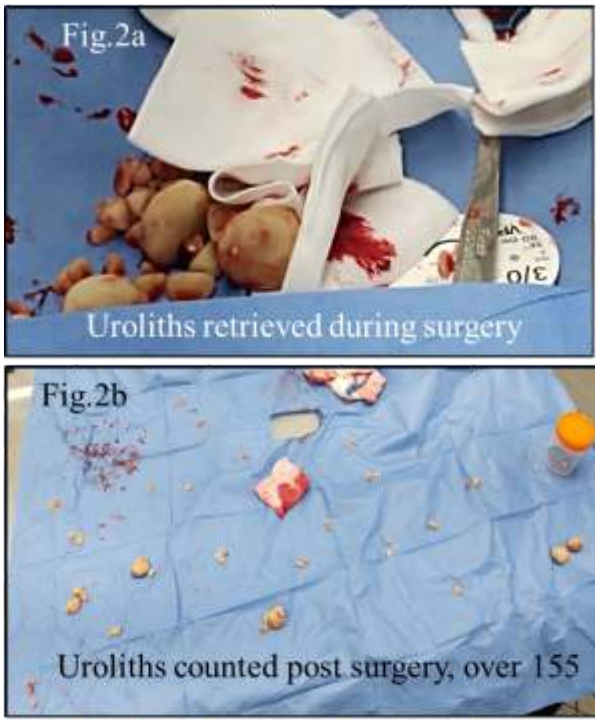

Fig. 2a & 2b.Uroliths collected during and counted after surgery

A sterilized urinary catheter was passed from the bladder to flush the lower chamber with normal saline solution, passing into the urethra to gently wash out any residual small uroliths, till clear fluid emerged. The bladder mucosal lining appeared markedly inflamed. The visible blood clots were removed manually, and the remaining flushed out.

The bladder was closed tightly with two layers of 3-0 absorbable sutures, scrupulously avoiding contact with the mucosal lining: the first in a simple continuous pattern, and the second in Cushing/ Lambert pattern, inverting to over-sew the first layer. To locate any seepage at the incision site a leak test was performed by slowly pushing isosmotic saline solution into the bladder and squeezing gently. After retrieving all the pre-countedlap sponges, the bladder was gently replaced into the abdominal cavity. The lineaalba was closed with 3-0 absorbable sutures in the simple continuous pattern, and thereafter the subcutaneous tissues with 3-0 absorbable sutures in the same pattern. Finally, the cutaneous layer was closed with non-absorbable 3-0 sutures in the simple interrupted cruciate pattern. Complete removal of the bladder stones was apparent in the post-operative radiographs **(Fig. 3a& 3b).** The owner was briefed on the harmless presence of some blood drops in the dog patient's urine for 2-3 days.

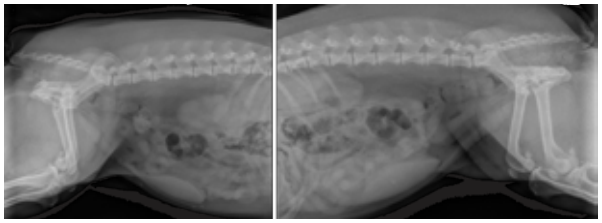

Fig.3a & 3b. Post-operative abdominal survey radiograph: no radiopacity in the urinary bladder corroborates total clearance of uroliths.

Post-operative protocol: Broad-spectrum Polyflex @ 10-30mg/kg, 2.0ml and pain medication,Buprenorphine[0.3mg/ml] @ 0.005-0.01mg/kg 0.64 ml, injected S/C. Take home antibiotics: Clavamox®125 mg tablet @ 10 mg/ kg dose, 1 tablet BID for 14 days, and Rimadyl oral 25mg @2.2mg/kg with food BID were dispensed for 4 days. Gabapentine pain medication 100mgtablets and one tablet BID were dispensed for 3 days. Home care advisory: constant use of e-collar and restricted movements for minimum 10-14 days. The sutures were removed on day 14 post-surgery. Follow-up phone call next day, patient very comfortable, and relaxed.

Discussion

Chemical composition, size, location, numbers, and surface morphology of the uroliths are of logistic clinical concern in pet practice. Food, genotype and gender of the companion dog are importantly involved. Notably, magnesium ammonium phosphate (struvite) uroliths, more common in the younger females, result from unabated bacterial infection in the bladder, causing abnormal release of ammonia gas from degradation of urea. On the other hand, calcium oxalate uroliths, most common in the aged male dogs, result from excessive urinary oxalate synthesis, following compromised renal function. Dalmatians with genetic deficiency of uricase enzyme in the liver and kidney are prone to hyperuricemia with stone formation. In portosystemic shunt, a congenital vascular

anomaly, the liver cells are unable to clear uric acid from the blood circulation, resulting in significantly increased chances of urolithiasis. Partial or complete urinary obstruction inmulti-factorial urolithiasis in dogs' mandates early definitive diagnosis for effective surgical and/or medical therapy(3-5). Haematuria, stranguria (difficult urination), and oliguria are the common signs of lower urinary tract disease, not specific to urolithiasis (6). These stones are difficult to localize through digital palpation. On presentation, the patient's signalment, case history, clinico-haemato-biochemical profile, and urinalysis reports are not conclusive, and only diagnostic imaging is definitive (2). Abdominal survey radiography, in tandem with ultrasonography is often used for the diagnosis and localization of uroliths in the urinary tract. Contrast radiography may be required to detect non-radiopaqueuroliths (7). Ultrasound scan reveals a clear hyperechoic area with acoustic shadows in the bladder stones (8). Radiography remains the basic imaging tool, covering the entire urinary tract, and also the complete length of the urethra to identify and locate the radiopaque stones (6). Surgical excision of the deleterious mineral masses along with medical management is the best option (9).In the instant case, efficacy of Laparotomy-Cystotomy under broad-spectrum antibiotic umbrella, conforming to the standard protocol, is evidenced on day 1 post-surgery.The patient appeared comfortable: eating, drinking, and performing other body functions normally. The skin sutures from the healed surgical wound were removed on day 14 post-surgery. Hill's S/D prescription food was recommended for life-long use to preempt with certainty recurrence of uroliths, and associated haematuria. Minnesota Urolith Center report on the chemical analysis of the referred cystouroliths, received on 21.11.2019 established 100% magnesium ammonium phosphate (struvite) mineral composition. These observations are consistent with a similar published case report (10). Further, the relevance of client education on home food management with optimized protein content is emphasized in an earlier communication (11).

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Successful Surgical Excision of A Ventrolateral Neck Region Malignant Melanoma Mass in A Senescent Female Dog

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Abstract

A Yorkshire female spayed dog (age 14years, body weight 5.6 kg) was presented in the clinic with the history of apigmented tumor growing on the right ventrolateral neck region, noticed by the owner for the first time in 2019.The annual health check and vaccinations schedules were up to date. However, the recommended surgical mass excision, and dental cleaning protocol were declined. In 2021, the in-house fine needle aspirate (FNA) sample microscopic examination ruled out mast cell tumor. The mass ruptured in early 2022,and on the health, check visit the patient was put on antibiotics regimen. Black pigment in the exudates was highly suggestive of melanoma. With the well-informed owner's formal consent, finally mass removal surgery was performed on July 7, 2022.Microscopic profile of the cutaneous lesion in referral histopathology report established malignant melanoma. Successful surgical excision is evidenced by the pet's uneventful recovery.

Introduction

A retrospective study of vulvovaginal melanocytic neoplasms in dogs (1) revealed that most of the masses including fibroma and leiomyosarcoma were of smooth muscle, or fibrous tissue origin. Of total 99 cases, 72 were classified as benign, 17 malignant, and the remaining 10 transmissible venereal tumors (TVTs). In the benign tumor cases, complete surgical excision, in tandem with ovariohysterectomy, was effective with no recurrence. Vulvovaginal tumors account for 2.4-3% of all neoplasms in dogs, the second most common after mammary gland tumors. Originating from the smooth muscle (leiomyoma) in the intact nulliparous female dogs: ranging in age from 2 to 18 years (av. 10.8 years), most of these masses are benign. Boxers are at increased genetic risk (2).

Admittedly, the borderline between benign and malignant melanocytic tumors is rather thin and subject to much controversy among the veterinary oncologists fraternity. Therefore, it is highly desirable to conform to the revised WHO tumors classification: the benign lesion designated as melanocytoma and the malignant growth, melanoma (3). Further, in clinical practice, melanocytic tumor diagnosis often poses a formidable challenge to the consultant pathologist because a wide range of neoplastic lesions (carcinoma, sarcoma, lymphoma) look alike under the microscope (4). The pathoclinical profile and therapeutic management of melanoma in dogs is reviewed in-depth (5). Accounting for up to 7% of all malignant tumors, melanomas develop in diverse locations (6). Metastasis in the lymph nodes is a frequently observed pathobioepisode (7). Surgical excision is the treatment of choice for the local cutaneous melanoma. The prognosis is favorable in tumors exhibiting benign histoarchitectural profile but remains guarded in the malignant growths with high chance (30-70%) of metastasis (8).

Occasionally, the field veterinarians globally face the highly challenging task of convincing, with utmost patience and perseverance, the owners of family dogs and cats on the prioritized health care problem. Companion animal welfare is the cherished goal, and client education on optimized health care is aimed to fulfill the mandate. This often-overlooked aspect in pet practice is highlighted in the present communication.

Case Description

A senescent (14 years, 6 kg body weight) spayed female Yorkshire was presented in the Milford Veterinary Clinic on July 7, 2022, for right ventrolateral neck tumor mass removal. The growth was first noticed on annual physical examination in May 2019. The suggested mass removal and referral diagnosis of the excision biopsy was constantly declined by the owner. Further, dental hygiene was very poor, and the recommended cleaning too was declined.

Year 2020: The persistent health issues had accentuated, but the owner was interested only in the vaccines update.

September 2021: Patient presented in the clinic with cough and bloody diarrhea was treated with antibiotics. The growth(1.5 cm x1.5 cm) was perceptibly enlarged. In-house FNA sample cytologyprofile ruled outmast cell tumor.

Differentials at this point of time: pigmented cyst, or melanoma. Since the cutaneous mass was progressively growing larger in size, tumor removal with referral histopathology, and concurrent dental cleaning in the patient under general anesthesia was again advised.

November 2021: The patient's scheduled annual physical examination and vaccination done. The growth measured 2cmx2cm **(Fig.1a, b).** With no further delay, removal of the prominent neck region growth and dental cleaning was strongly recommended to the owner, who appeared to be convinced.

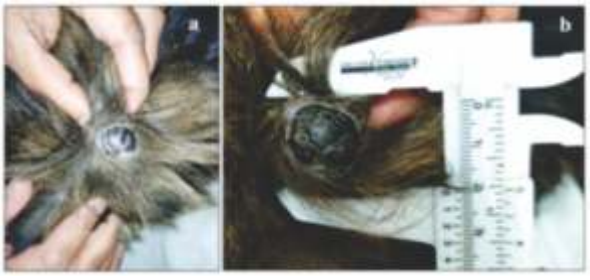

Fig.1 a. Macroscopic view b. Measurement with Vernier calipers.

January 2022: The owner was on the phone complaining about the skin lesion on the neck oozing. Message in reply: keep it sanitized till the scheduled appointment in the clinic two days later. The owner did not turn up with the pet.

February 2022: the patient was presented for coughing with no concerns for growth management, the main problem. Antibiotics regimen was initiated. Dog's neck, now oozing black exudates, was carefully bandaged.

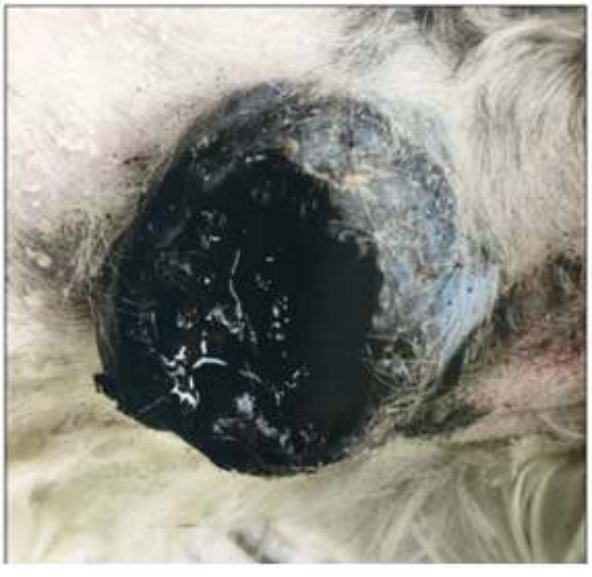

Fig. 2. Lacerated enlarged mass in the patient's neck.

May 2022: The growth with a lacerated centre and a volcano-like view had enlarged exponentially (6 cm x 6 cm) **(Fig. 2).** Growth removal, emphasized once more, was finally accepted. The affected area was shaved, and local antibiotic ointment applied followed by oral antibiotic course. After routine pre-surgery blood work surgical mass removal was scheduled in the morning hours of July 7, 2022.

Pre-surgery blood work

Table 1. Patient's hemogram*6.6.2022.

Parameter (Unit)	Result	Range	Status
TEC (1x106/µl)	5.41	5.65-8.87	L
Hematocrit (%)	39.2	37.3-61.7	N
Hemoglobin (g/dl)	13.5	13.1-20.5	N
MCV (fl)	72.5	61.6-73.5	N
MCH (g/dl)	25.0	21.2-25.9	N
MCHC (g/dl)	34.4	32.0-37.9	N
RDW (%)	15.7	13.6-21.7	N
Reticulocyte (1x103/µl)	44.4	10-110	N
TLC (1x103/µl)	11.44	5.1-16.8	N
Neutrophil (%)	61.7		
Lymphocyte (%)	26.7		
Eosinophil (%)	1.7		
Monocyte (%)	9.6		
Basophil (%)	0.3		
Neutrophil (1x103/µl)	7.06	2.95-11.6	N
Lymphocyte (1x103/µl)	3.05	1.05-5.10	N
Eosinophil (1x103/µl)	0.2	0.06-0.10	N
Monocyte (1x103/µl)	1.1	0.16-1.12	N
Basophil (1x103/µl)	0.03	0.00-1.10	N
Thrombocyte (1x103/µl)	351	148-484	N
MPV (fl)	12.1	8.7-13.2	N
PDW (%)	14.0	0.1-19.4	N
PCT (%)	0.42	0.14-0.46	N

***Procyte Dx Auto-cell counter N=Normal; L=Low; H=High**

Inference: Surgical mass removal is safe.

Mass Excision Surgery

I. The patient was sedated with acepromazine @ 0.025-0.2 mg/kg [10mg/ml] 0.02 ml and butorphanol tartrate @ 0.2 mg/kg (Torbugesic®, Zoetis US, Inc.) [10mg/ml] 0.05 ml, injected S/C. After 30 minutes sterilized IV catheter was carefully inserted and secured in situ. Anesthesia was induced with ketamine @ 2-10 mg/kg [50 mg/ml] 0.2

Table 2. Blood chemistry panel*6.6.2022

Parameter	Result	Range	Status
Glucose (mg/dl)	96	7-143	N
Creatinine (mg/dl)	2.0	0.5-1.8	H
BUN (mg/dl)	54	7-27	H
BUN/ Creatinine ratio	27		
Total protein (g/dl)	7.7	5.2-8.2	N
Albumin (g/dl)	2.9	2.2-3.9	N
Globulin (g/dl)	4.8	2.5-4.5	H
A/G ratio	0.6		
ALT (U/l)	106	10-125	N
ALKP (U/l)	131	23-212	N

***Catalyst Dx Autoanalyzer N=Normal; L=Low; H=High**

ml + midazolam @ 0.1-0.3 mg/kg [5mg/ml] 0.2 ml, injected IV. After intubation, the dog was transferred to isoflurane gas anesthesia, hooked to the auto monitoring systems for electrocardiogram (EKG), blood pressure, circulatory O2 levels, and pulse rate. The surgical site was shaved and sanitized.

II. Surgery was done by the attending pet physician (first author). Elliptical incision in the skin was made using sterilized #10 surgical blade. The mass was excised all round with a pair of Metzenbaum scissors. Bleeders were ligated with Monomend® (PRN Pharmacal)3-0 absorbable sutures **(Fig. 3).** Skin sutured with 3-0 Ethilon in the simple interrupted pattern. The second layer was juxtaposed in the simple continuous mattress pattern to reduce tension on the suture line. A neck bandage was applied. Smooth recovery from anesthesia was highly satisfying. Fluids infusion was stopped, and the IV catheter carefully withdrawn.

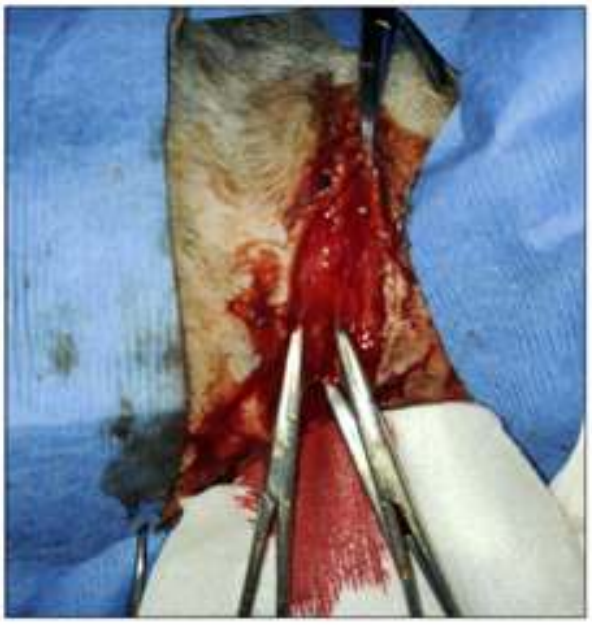

Fig. 3. Surgery in progress.

NSAID, Meloxicam (Metacam ® , Boehringer Ingelheim) @ 0.2 mg/kg, and broad spectrum ampicillin antibiotic, Polyflex ® Boehringer Ingelheim @ 30 mg/ kg were injected S/C. Take home antibiotic cefpodoxime proxetil @ 5-10 mg/kg (Simplicef ® , Zoetis US, Inc.) 100 mg tablet 1/2 OD x 10 days, and oral Metacam ® @ 0.1 mg/kg [1.5 mg/ml] 0.3 ml SID x 5 days PO with food were dispensed with advisory. Surgically excised biopsy histopathology referral was declined by the owner, but for intellectual curiosity the clinic paid the cost.

Following day, owner's phone call update: patient is doing very well. Two days later, recheck visit: anamnesis revealed that the companion animal is eating, drinking and moving about freely. Patient seen scratching on the bandage, but the suture line was intact. Skin sutures were removed on day 14 post-surgery, and the incision site, still healing naturally appeared in fine shape.

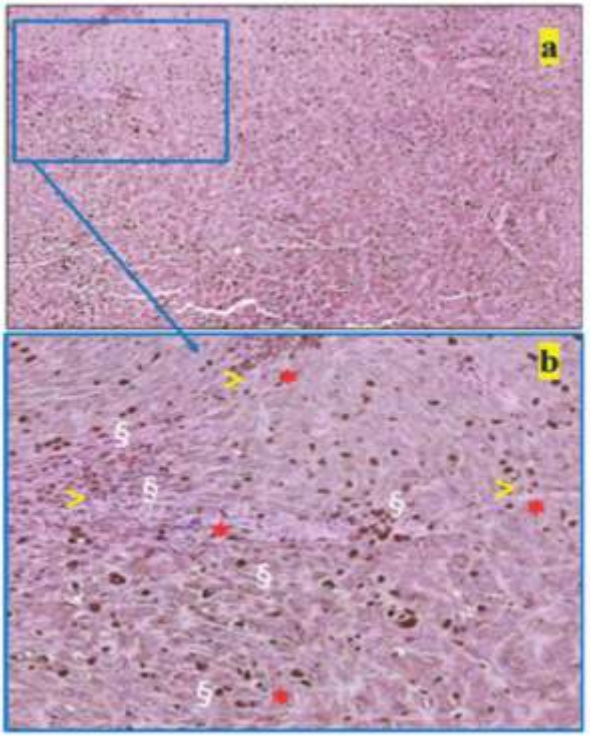

Fig. 4 a. (H&E, 400x).

Fig. 4 b. Inset enlarged view: Polyhedral and spindloid cells*proliferating in a disorganized pattern as solid sheets. Individual cells exhibit mild to moderate intensity brown cytoplasmic pigmentation and pleomorphic nuclei with variable-sized nucleoli>, Biopsy Type: Excisional.

Clinical History

Subcutaneous ulcerated black firm mass (6 cm x 6 cm)located in the right lateral/ventral neck.

Microscopic profile

Poorly demarcated and non-encapsulated proliferation of atypicalpolyhedral and spindloidcellsis characteristic. These cells are seen proliferating as solid sheets in a notably disorganized manner **(Fig. 4a).**

The individual cells exhibit mild to moderate quantity of brown cytoplasmic pigment, and moderately pleomorphic nuclei with the nucleoli varying in size, markedly **(Fig. 4b).**

Pathologist's interpretation

Malignant melanoma. Mitotic count: 9/10 HPF (400x).

Margins

Impacting the lateral and deep specimen margins. No vascular invasion.

Discussion

Cutaneous melanocytic tumors in the dog and cat are well-documented (9). Malignancy of melanocytic tumors in haired skin is best predicted by the extent of cytological atypia. Local recurrence or metastasis, based on the one year follow-up statistical study, occurred in 50% cases (10).In the instant case, the excision biopsy revealed cytological features of malignancy.However, complete surgical excision with safe margins allround was highly effective. The companion animal's signs of tangible reliefbear ample testimony to this contention.It is pertinent to recall that in an earlier published report from this clinic, curative surgery ofvulvar lip malignant spindle cell melanoma (Mitotic Index 5)in a8 year old Vizsladog resulted in uneventful early recovery (11).

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Virophages: Are They Future Antivirals?

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Importance

This article suggests alternate antiviral approaches in the control of novel emerging viral infections.

Summary

Virophages are viruses having the nucleic acid core either DNA or RNA, and are smaller in size, they require the co-infection of another virus for multiplication. The co-infecting viruses are typically giant viruses. Virophages completely depend on the viral machinery of the co-infecting giant virus for their replication and assembly. One of the characteristics features of these virophages is that they have a parasitic relationship with the co-infecting virus. Their dependence upon the giant virus for replication often results in the deactivation of the giant viruses. This phenomenon needs to be explored further and studies in depth for using these virophage as weapon for killing the novel viruses with potential infectious capacity.

Introduction

Viruses were considered to be small particles with the ability to pass through 0. 2-micron pore filters and remain invisible under a light microscope, in contrast to microbes (1). Virus is a sub cellular infective agent during the 20th century; viruses became increasingly established as small entities which typically contain a protein coat surrounding an RNA or DNA core of genetic material. It lacks all its own machinery for metabolism, reproduction and other activities, their growth and multiplication only occur in living cells using the host cell machinery system, and was consecutively described as obligate parasites of eukaryote, then bacteria and archaea (2), while some were discovered as causative agents of disease. Virophages are small dsDNA viruses recently discovered viruses in association with some giant viruses (GVs), and then found in metagenomics samples with an apparent broad world wide distribution. The interactions of virophages with the host giant viruses were of interest to a number of previous studies. The discovery of these novel agents opened a question on virus evolution and their composition. Most of the virophages are unable to propagate independently, it is suggested that virophages cannot be considered as bonafide viruses. Therefore, some researchers defined virophages as satellite viruses, provirophages, or gene transfer elements (3). In reality virophage uses transcriptional machinery of the giant viruses to replicate, that may resemble the nuclear replication of small dsDNA viruses. The virophages biology and its composition is of great interest, the nature, properties and life cycle of virophages needs to extensively be investigated or the betterment of scientific community.

Origin of Virophages

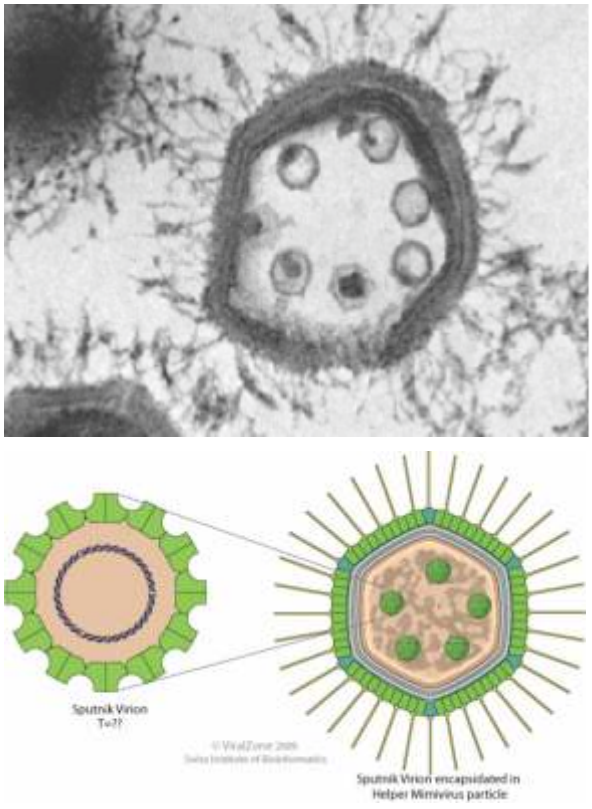
The science of virophages begins with the discovery of giant mimivirus, Acanthamoeba polyphaga Mimivirus (APMV) identified in 2003 and belongs to the Mimivirus genus, Mimiviridae family was originally isolated from a cooling tower in the United Kingdom. It is the largest known virus, with a capsid 750 nanometers in diameter and a double-stranded DNA genome 1.2 million base pairs in length. This giant virus replicates in amoeba such as Acanthamoeba sp; in this host they form large, cytoplasmic 'factories' where the DNA replicates and new virions are assembled. This new virus does not replicate in amoebae unless the cell is also infected with mimivirus or mamavirus (4). The virophage differs from satellite virus is that the latter do not interfere with the replication of helper viruses but virophage does that.

In 2008, the first virophage Sputnik was identified in an Acanthamoeba castellanii mamavirus (ACMV), a giant virus of the Mimivirus genus of Mimiviridae family. This giant virus was found inside the protozoan Acanthamoeba castellanii, in a Paris water-cooling tower and named as virophage, which stands for a 'virus eater'(4). Later, Sputnik virophage was demonstrated to infect the first giant virus. Currently, the virophages are classified to belong to the Lavidaviridae family (3).

Morphology of Virophage (Sputnik)

Three dimensional structural studies have only been carried out on Sputnik virophages, using cryo-electron microscopy to produce an electron density map of the icosahedral Sputnik virus at 3.5-Å resolution, sufficient to verify the identity of most amino acids in the capsid proteins and to establish the identity of the pentameric protein forming the fivefold vertices. EM reveled 74 nm diameter virion (sputnik) having hexagonal surface lattice with a T = 27 icosahedral symmetry capsids composed of 260 trimeric capsomers and 12 pentameric capsomers. The trimeric capsomers consist of three double “jelly-roll” major capsid proteins creating pseudohexameric capsomer symmetry. The pentameric capsomers consist of five single jelly-roll proteins. The release of the genome by displacing one or more of the pentameric capsomers may be the result of a low-pH environment. Pentameric capsomers have central cavities that could serve for DNA entry or exit, as in bacteriophages (5). Sputnik has double-stranded, circular DNA genome with the size of 18-kbp, highly AT-rich genome, which is predicted to encode 21 proteins ranging from 88 to 779 amino acids in size. Of these 21 proteins, 13 do not

have detectable homologues in current sequence databases. The other eight genes have homologues in viruses whose hosts are from all three domains of life, the Eukarya, Archaea, and Bacteria. The chimeric characteristics of the Sputnik genome implies that it is involved in lateral gene transfer between viruses.



(Sputnik found inside mamavirus Courtesy Nature, 2008)

Characteristics Features of Virophages

- Co infection of sputnik in Acanthamoeba castellanii mamavirus (ACMV) considerably affects the replicating cycle of mamavirus and results in 70% decrease in the yield of infective mamavirus.
- Sputnik multiplication inside mamavirus results in significant increase in production of morphological anomalies in mamavirus.
- The reduced yield of Acanthamoeba castellanii mamavirus results in threefold decrease in lysis of host cell Acanthamoeba spp (Protozoa that serves a host for ACMV). Therefore, the sputnik named it as virophage.
- The entire life cycle of sputnik occurs in viral factory of mimivirus no signals have been detected within the amoeba nucleus.

Major Breakthroughs in Virophage Research

In 2011, the second virophage existence was confirmed by identification of Mavirus, a virophage that infects a giant virus Cafateria roenbergensis (CroV) of the genus Cafateriavirus, family Mimiviridae. Mavirus was isolated for the first time from the flagellate Cafateria roenbergensis that populates the coastal waters of Gulf of Mexico in Texas. This virophage also has a spherical dsDNA genome, which probably encodes 20 proteins (6). The third virophage, Organic Lake Virophage (OLV) discovered in 2011 in the salty waters of Antarctica which preys on virus infecting phototrophic algae. OLV, like the Sputnik, also has a double stranded DNA genome that is circular in shape and 26421 bp in size and encodes 24 proteins which are 27–42% identical with the Sputnik proteins (7). The OLVs' effect on giant viruses infecting algae impacts their count and regulates organic matter in their aqueous environment (8). Sputnik 2, which was discovered in 2012, has circular, double stranded DNA genome and with a capsid that has icosahedral symmetry (7). It infects Lentille virus, a giant virus, genus Mimivirus, family Mimiviridae that was found in A. polyphaga eukaryote harvested from a contact lens fluid (9). In 2013 Virophage, Sputnik 3, PGV (Phaeocystis globosa virophage) was identified and five more new metagenomic sequences were identified in the same year they were defined as ALM and YSLV1-4 virophages. They all have circular double stranded DNA and icosahedral symmetry of the capsid. RNV (Rio Negro virophage) was identified in 2014 in the Negro River in the Amazon rainforest in Brazil.

Zamilon virophage was isolated in 2014 from the soil samples from Tunisia infects a Mont1 giant virus7. Zamilon has a 70–76% genetic identity with Sputnik, three new virophages, YSLV5, YSLV6 and YSLV7, were identified in 2015 as a metagenetic material in the Yellowstone Lake (US) (10). Their DNA was double stranded and spherical, and their capsids were probably icosahedral 7. A homologous to Zamilon strain of dsDNA discovered in 2015 was named Zamilon 2. RVP (Rumen virophage) was identified in a metagenetic material in 2015. Its linear genome is different from the genomes of the other virophages it is a 'hybrid virophage' – a combination of a virophage and large polinton, DNA transposon, i.e., giant virus transpoviron DNA (11). New metagenetic study in Asia revealed two novel virophages, the first was DSLV (Dishui Lake virophage) rom china with a circular double stranded DNA genome and showed a significant homology to all the virophages identified in Yellowstone Lake (YSLV 1-7) (7). Although it was assigned no giant virus host, the probable candidate may be Phycodnaviridae virus that infects (unspecified) algae. The second one is QLV (Qinghai Lake virophage) identified in 2016 from the metagenomic material from Qinghai Lake, Tibetan Mountains. Probably infects Phycodnaviridae of Algae (12). Nearly 20 virophages have been isolated and confirmed of which, 18 genomes are available in the GenBank database.

Interactions Between Virophages and Giant Viruses

There are two probable ways in which virophage - infected giant viruses enter amoebae and flagellates.

The first way is called IEM (independent entry mode) and is common for Mavirus virophage and its giant virus – CroV. Virophage and giant virus both independently enter a protozoan where they later both replicate (13). The other way, called PEM (paired entry mode), is thought to be used by Sputnik virophage and its giant virus – Mimivirus (APMV). In this way, the co-infection occurs when the giant virus and virophage are entangled and together enter the host organism – A. polyphaga. This way consists of two phases. First, Sputnik adheres to Mimivirus (APMV) and this complex successively enters the amoeba via phagocytosis. This entry stage was confirmed with electron microscope imaging (9).

Applications of Virophages

- Virophages are parasites of giant viruses within protists. They reduce giant virus production and increase host cell survival. They provide a defense system for protists against giant viruses in diverse environments.
- The virophage DNA genome contains genes related to those in viruses that infect eukaryotes, prokaryotes, and archaea. Virophages may therefore function to transfer genes among viruses.
- Virophages (OLV) multiplies in the phycodnaviruses and reduces the mortality of the host algal cell may be essential for maintaining stability of the microbial food web and algal bloom.
- It is well known that cell killing by viruses has a major impact on ocean ecology. By regulating virus-induced cell lysis, virophages might also have a major effect on aquatic ecology. This possibility makes me wonder if there are virophages of animal viruses that might regulate viral pathogenesis.

Virophage- Future Antiviral Perspectives

After Sputnik discovery nearly twenty virophages have been identified and all the virophages has the common thing that they do not harm the host but harm the virus infecting the host. These discoveries on virophages could potentially open up a new window for extensive research and there are possibilities in near future that the virophages can be used as an antiviral drug. This field of novel antiviral strategy requires extensive scientific research and field study on virophages around the globe. These novel viruses may also be tested invitro against major vial pathogens of humans and animals for their inhibitory effect. In near future the virophages can be used in the future to target more complex viral infections, like the global crises of HIV, Dengue and Zika infections etc.,

Conclusion

Virophages have been isolated or detected in various locations and in a broad range of habitats worldwide, including the deep ocean and inland. Humans, therefore, could be commonly exposed to virophages, although currently limited evidence exists of their presence in humans based on serology and metagenomics. the abundance of virophages in various and different environments, it has been suggested that they can play a role in regulating the dynamics of populations of giant viruses and their eukaryotic hosts, which may have a substantial influence on some ecosystems. The distribution of virophages, the consequences of their infection and the interactions with their giant viral hosts within eukaryotic cells deserves extensive further research.

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Potential Threat of Monkeypox: A Review

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Introduction

The monkeypox virus, which belongs to the *Poxviridae* family, *Chordopoxvirinae* subfamily, and *Orthopoxvirus* genus, causes monkeypox disease (1). The monkeypox virus was isolated and identified for the first time in 1958, when monkeys transferred from Singapore to a Danish research centre became ill (2). The first confirmed human case occurred in 1970, when the virus was recovered from a 9-month-old baby suspected of having smallpox in the Democratic Republic of Congo (3). Monkeypox has become endemic in the Democratic Republic of the Congo (DRC) and has spread to other African countries, primarily in Central and West Africa, since then. The first case of monkeypox outside of Africa were recorded in 2003 (4). Monkeypox disease is closely related to the variola virus (smallpox virus), and it causes a smallpox-like illness. According to historical statistics, smallpox immunisation with vaccinia virus (another orthopoxvirus) was 85 percent effective in preventing monkeypox (5). Following the elimination of smallpox in 1980, however, routine vaccination against the disease was no longer recommended (6). The frequency and severity of clinical signs and symptoms are significantly reduced by residual immunity from previous vaccinations. In unvaccinated people, case fatality rates (CFR) range from 0 to 11 percent (7). Immunocompromised people, such as those with untreated HIV infections, are more susceptible to disease and have a higher chance of death (8).

Etiology

Monkeypox belongs to the *Poxviridae* family, *chordopoxvirinae* subfamily, *orthopoxvirus* genus, and Monkeypox virus species. The monkeypox virus seems to be quite huge under electron microscopy (200-250 nanometers). Poxviruses are brick-shaped, having a linear double-stranded DNA genome encased in a lipoprotein envelope. The monkeypox virus is divided into two genetic clades: the central African (Congo Basin) clade and the west African clade. The Congo Basin clade was assumed to be more transmissible and to have caused more severe sickness in the past(9).

Host

Rope squirrels, tree squirrels, Gambian poached rats, dormice, various monkey species, and others have all been known to carry the monkeypox virus. People who live in or near forested regions may be exposed to infected animals in an indirect or low-level manner (9).

Transmission

Animal-to-Human (Zoonotic) Transmission - Direct contact with diseased animals' blood, body fluids, or cutaneous or mucosal sores. A possible risk factor is eating undercooked meat and other animal products from infected animals (9).

Human-to-Human Transmission -Close contact with respiratory secretions, infected person's skin lesions, or recently contaminated objects, transmission through the placenta from mother to foetus (which can cause congenital monkeypox), or close contact during and after birth.

Outbreak

Monkeypox is a global public health concern since it affects not only countries in West and Central Africa, but also the rest of the world. In the United States of America, the first monkeypox outbreak outside of Africa occurred in 2003, and it was connected to contact with infected pet prairie dogs. Gambian pouched rats and dormice had been smuggled into the nation from Ghana to house these pets.Over 70 cases of monkeypox were reported in the United States as a result of this outbreak. Travelers from Nigeria to Israel in September 2018, the United Kingdom in December 2019, May 2021 and May 2022, Singapore in May 2019, and the United States of America in July and November 2021 have all been reported to have monkeypox. In 2022 the first documented case of a person with travel ties to Nigeria, was confirmed on May 6, 2022, but it has been suggested that instances had already begun to spread around Europe in the preceding months (10).Cases began to be reported from a growing number of nations and areas beginning on May 18, primarily in Europe, but also in North and South America, Asia, North Africa, and Australia. As of June 2nd, 793 cases had been confirmed (11).

Clinical Sign and Symptoms

Monkeypox requires 6 to 13 days to incubate (from infection to beginning of symptoms), although it can take anywhere from 5 to 21 days. Monkeypox has a case fatality rate that has traditionally fluctuated from 0% to 11% in the general population, with a greater rate among small children. The case fatality ratio has been approximately 3–6% in recent years.

The infection can be divided into two periods

- Fever, acute headache, lymphadenopathy (swelling of the lymph nodes), back discomfort, myalgia (muscle aches), and intense asthenia characterise the invasion stage (which lasts between 0–5 days). When compared to other diseases that may appear identical at first, monkeypox has a specific feature: lymphadenopathy (chickenpox, measles, smallpox).
- The skin eruption usually starts 1–3 days after the fever appears. The rash appears to be focused more on the face and extremities than the trunk. The face (in 95% of cases), palms of the hands, and soles of the feet are all affected (in 75 percent of cases). Oral mucous membranes (in 70% of patients), genitalia (30%), and conjunctivae (20%), as well as the cornea, are also affected. The rash progresses from macules (flattened lesions) to papules (slightly raised firm lesions), vesicles (clear fluid-filled lesions), pustules (yellowish fluid-filled lesions), and crusts that dry up and flake off. The number of lesions might range from a few to thousands.

Diagnosis

Diagnosis of monkeypox is by culture, polymerase chain reaction (PCR), immunohistochemistry, or electron microscopy, depending on which tests are available.

Transmission Electron Microscopy - The sample exhibited numerous brick-shaped particles, characteristic of orthopox viruses.

Culture - Within24 hours of infection, cytopathic effect was observed in Vero cells, exhibiting typical monolayer separation and cell rounding (12).

PCR - PCR diagnosis was based on specific primers to discriminate between the West African (581 bp) and the Congo-Basin (832 bp) clades by product size (13).

Differential Diagnosis

Smallpox, generalized vaccinia, disseminated zoster, Chickenpox, Eczema herpeticum, Disseminated herpes simplex, Syphilis, Yaws, Scabies, Rickettsial pox, Measles, Bacterial skin infections, Drug-associated eruption (14).

Prevention and Control

Vaccination

Vaccination against smallpox was demonstrated through several observational studies to be about 85% effective in preventing monkeypox.At the present time, the original (first-generation) smallpox vaccines are no longer available to the public. A still newer vaccine based on a modified attenuated vaccinia virus (Ankara strain) was approved for the prevention of monkeypox in 2019. This is a two-dose vaccine for which availability remains limited. Smallpox and monkeypox vaccines are developed in formulations based on the vaccinia virus due to cross-protection afforded for the immune response to Orthopoxviruses.

Raising awareness of risk factors and educating people about the measures they can take to reduce exposure to the virus is the main prevention strategy for monkeypox.

1. Reducing the Risk of Human-to-Human Transmission - Surveillance and rapid identification of new cases is critical for outbreak containment.

2. Reducing the Risk of Zoonotic Transmission - Unprotected contact with wild animals, especially those that are sick or dead, including their meat, blood and other parts must be avoided.

3. Preventing Monkeypox Through Restrictions on Animal Trade - Captive animals that are potentially infected with monkeypox should be isolated from other animals and placed into immediate quarantine. Any animals that might have come into contact with an infected animal should be quarantined, handled with standard precautions and observed for monkeypox symptoms for 30 days.

Treatment

There is no proven, safe treatment for monkeypox virus infection. Treatment of monkeypox is supportive (15). Potentially useful drugs include

- The antiviral drug tecovirimat (approved by the US Food and Drug Administration [FDA] for the treatment of smallpox)
- The antiviral drugs cidofovir or brincidofovir (CMX001)

Conclusion

Monkeypox is a major health risk for those living in endemic areas such as the DRC and other African countries where the virus has been proved to circulate, but it is also a worldwide health security concern, as demonstrated by the 2003 outbreak in the United States. To prevent enhanced transmission efficiency or pathogenicity, appropriate and efficient interventions and active surveillance efforts are urgently needed. Monkey Pox is the most common orthopoxvirus in humans, at least in endemic areas and maybe worldwide. Monkeypox is no longer a rare illness, and therefore requires more care.

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Housing Management of Pet Birds

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Introduction

Pet birds are highly intelligent species, which require regular exercise and mental incitement, therefore it is ideal to provide good housing and management protocols depending on species like space, environmental enrichment, interaction with pet owners and other factors like temperature, ventilation, sight and sound etc should be considered. Proper housing ensures a stress and boredom free, safe and simulation of natural environment for birds. Hence good housing system will provide ample scope for pet birds to elicit their normal behaviour under captivity.

Space

Any enclosure should provide sufficient space according to their sex, size and age of the bird. For birds who are confined in cages should have regular access to flight aviary and opportunity to fly in free spaces when kept in indoors.

The following guidelines shall be considered,

1. The minimum width of a cage for a pair of birds should be three times of their combined wingspan (the wingspan being the length from the tip of one wing to the tip of the other wing, when both wings are stretched out).
2. The minimum length of a cage should permit at least two wing beats (the more the better) between perches. Perches should be placed far enough from the ends of the cage to allow the birds to turn around on the perches without scraping their tail feathers against the cage.
3. The minimum height of a cage should be three times the length from head to tip of tail of the largest bird to be confined in it and should be increased accordingly if more than one pair or more than one species is kept in the cage.
4. The cage should be constructed or positioned such that at least one perch is at standing shoulder height (for the sense of security of the birds).
5. The cage should provide room to fly between perches in an approximately horizontal plane and to fully extend his/her wings and to fly without damage to their wings or feathers on the cages.

Species-Specific Considerations

Small Birds (Parrotlets, Finches, Lovebirds, Parakeets, Canaries)

- Bar spacing should not exceed ½ inch (so birds do not poke their heads through)
- Cage housing should measure at least one square foot for small birds.

Parrotlets, Parakeets, Finches, Canaries

Medium-Sized Birds (Cockatiels, Small Conures, Quaker Parrot)

- Bar spacing should not exceed ¾ inch.
- Cage housing should measure at least one and a half square feet for medium birds.

Cockatiels Quaker Parrot

Large Birds (Macaws, Cockatoos, Amazons, Greys)

- Bar spacing can be from ¾ inch to 1½ inch.
- The largest birds do well in cages with six mm bars spaced further apart.
- They need roomy cages/aviaries in which their tails do not poke through the bars.
- Cages for singly-housed birds should be at least one and a half times the birds' natural wing span in all directions

- Ideally all birds should have cages/aviaries large enough to accommodate flight and/or be provided with ample out of cage time in a bird-safe room for exercise.


MacawsCockatoos

Qualities of Cage Materials

- Cages should be square or rectangular only, not circular or cylindrical.
- It should be sturdy, with bars that do not bend.
- Many large birds can open most locks, so padlocks are often recommended.
- Preferably made by stainless steel and use powder-coated paint.
- To allow outside-access to food and water dishes to replenish water and food. However, they should be equipped with openings that can be securely locked.
- Cages should not be made up of or have traces of Iron (which will rust), Wood (birds will simply chew through it and cannot be sanitized), Lead and Zinc (due to heavy metal poisoning) and plastic.
- New wire should be washed with a mild acidic solution such as vinegar followed by a rinse with water.
- Weathering the new cage for twelve months helps to reduce the risk. Ideally, leave new wire mesh to weather naturally before using it to construct the cage.

Means of Enrichment in Cages

Food Enrichment

- Weave or hang leafy greens and carrots from the cage bars or top of the cage. Ex. kale, mustard greens and dandelion greens.
- Wrap nuts or other treats (dry cereals, dried unsulfured fruits and dried hot peppers) in brown, white or colored paper and placed in various areas of the cage.
- Wrap portions of daily diet in paper cups or in brown, white or colored paper.
- Wrap treats or food in small paper board boxes and tie them with sisal, leather strips or cotton cord.
- Drill small holes in larger nuts like walnuts to make them easier to open, so that pet birds chew away the shell and get at the tasty treat inside.
- Cover bird's food dish with a piece of paper or card board. Start by placing the paper on top of the dish. Make a hole so that bird can see the food. As the bird becomes more proficient and removing the paper, you can increase the difficulty by folding the paper over the dish and eliminating the holes.
- Skewer fresh fruits (apples, oranges, grapes in a bunch, half or one quarter of a pomegranate) and vegetables (corn, chunks of red or yellow bell peppers, chunks of purple cabbage, broccoli and whole carrots) in whole or in part on stainless steel food skewers made for this purpose and hang them in the cage.
- Hang millet sprays.
- Utilize pre-made foraging toys and devices such as nut cages, foraging wheels and puzzle boxes to hold food and to provide additional variety and challenge.

Non-food enrichment items

- Wood is a natural favorite for many birds
- Wrap beads or other non-food items in paper or un-waxed paper cups.
- Use paper toys
- Stuff shredded paper or dried cornhusks into a paper bag along with nuts, dried fruits, beads, etc.
- Make paper balls with a surprise in the middle.
- Provide "finger-traps" (sold as party favors) with treats or beads hidden within them.
- Many birds like to play with beads. String them on short pieces of cotton rope, leather strips or sisal with knots in between. Attach to the cage bars or offer as foot toys. Be sure the size of the beads is safe and appropriate for your bird.
- Offer fresh branches from bird safe trees and include buds, leaves and fruits can provide for hours of foraging activity. Be sure the branches have not been sprayed with pesticides or taken from the roadside.
- For the heaviest chewers, provide untreated timber from local home center. One can then cut these into smaller pieces and drill them so they can be strung on short chain, rope or stainless steel toy holders. To increase interest, we can also drill holes in the wood to be stuffed with treats or beads.
- Purchase small or large wooden alphabet blocks made for children. Drill holes in them and hang them and can also offer them as foot toys or use as parts for larger toys.
- Large and small grapevine wreaths and willow wreaths can be found at many major craft stores during the winter season. Liven up the wreaths by attaching colorful beads, leather strips, nuts and

paper strips.

Cleanliness and Sanitation

Keeping a bird's environment clean is essential for their health. Because pet birds have very sensitive respiratory system, mould, fungus, bacteria, etc., can be extremely detrimental. Respiratory infections can be costly to treat and deadly to the birds.

In addition, captive birds are confined to relatively small living spaces, droppings often accumulate on cage parts and perches, and tend to contaminate food and water cups, resulting in bacterial proliferation and mold growth.

Below are some recommendations for proper cleaning and sanitation protocols.

Minimum Requirements

(I) Once a day

- Clean and disinfect food and water dishes
- Change cage papers
- Clean cage gates, bars, dirty perches and toys
- Change dry food
- Birds are quite messy and cages often contain bits of food, seed hulls, droppings, and feather dust, all of which need to be removed on a daily basis.

(ii) **Twice a day :** Change water (and re-clean water holder, if needed).

(iii) **Once a week:** Birds should be given a spray bath.

(iv) **Once a month:** Clean and disinfect cage and contents.

All cages, perches, food and water bowls, and other cage furnishings should be sterilized between occupants.

Minimum Cage Size for Pet Birds

Pet birds	In Pair (L x W x H)	Single Bird (L x W x H)
Amazon Parrots	4' x 3' x 4'	3'x 2'x 2'
Budgerigars	24" x 14" x 8"	*
Canaries	18" x 10" x 10"	*
Cockatiels	4' x 2' x 3'	26"x 20"x 20"
Cockatoos	4' x 4' x 3.5'	3'x 2'x 3.5'
Conures	4' x 4' x 4'	4'x 3'x 4'
Finches	2' x 2' x 2'	12"x 12"x 12"
Lovebirds	4' x 4' x 4'	*
Macaws	6' x 6' x 6'	3' x 2'x 3.5'
Mynah Birds	6' x 3' x 3'	6'x 3'x 3'

***- These birds prefer the company of other birds and should not be caged single (alone).**

General Points for Consideration

In addition, housing of pet birds should fulfil the following conditions

- It should be clean and hygienic – food and water containers should be located where they are least likely to be contaminated with faeces.
- Provide variety of different diameter perches with enough space for all birds. Perches should be rough and made up of natural, non-toxic wood to help prevent overgrown toenails.
- Sandpaper should be avoided as this may lead to footpad abrasions.
- Provide environmental enrichment for mental and physical stimulation.
- To afford an adequate number of feed and water dishes to meet the requirements of all birds.
- Sufficient nesting sites with suitable nesting material – if birds are breeding.
- To supply bathing water either through a sprinkler or in a container that is appropriate for the species.
- For aviary housing predators should not be able to gain entry to the aviary. This can be achieved by installing concrete barriers or galvanised steel or mesh (or a similar resistant material), buried to a depth of 300mm.
- It should be escape-proof.
- Enable simple structures to fly freely with clear lines of flight for pet birds and allow for easy cleaning.

Conclusion

Creating a spacious and safe housing environment for pet bird is vital for their welfare. It is important to consider space, environmental enrichment, safety from the outside environment, hygiene and the quality of cage material. Proper housing ensures a safe and stimulating environment so that birds can be free from stress and boredom. Finally, allowing as much space as possible and free flying opportunities will give the best chance for birds to engage in their normal behaviours.

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Asian Palm Civet: An Economic Importance

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Introduction

Asian palm civet (Paradoxurus hermaphroditus) also known as common palm civet belong to family viverrid. Native to South and Southeast Asia lives throughout the jungles of Asia. This animal is also called a toddy cat, but it's not a cat. The name comes from the civet's fondness for palm flower sap, which can be fermented into a liquor used in toddies. Asian palm civets are arboreal, so they spend most of their time in fruit trees and fig trees, preferring the tallest trees with very dense canopies and vines for seclusion and protection. Asian palm civets are classified as altricial, meaning the young need care from their parents after birth. Kopi luwak is a coffee that consists of partially digested coffee cherries, which were eaten and defecated by the Asian palm civet (Paradoxurus hermaphroditus). It is therefore also called civet coffee.

Evolution

The Philippine islands lie east of Borneo and are of volcanic origin. One of the Philippine islands, Palawan, was possibly connected to Borneo by a dry land corridor (1).Palawan and Borneo specimens are genetically close, so the Asian palm civet on Palawan Island might have dispersed from Borneo during the Pleistocene. It is possible that people later introduced Asian palm civet into other Philippines islands. Heaney et al. (2002)(2) considered the common palm civet to be a native species in the Philippines. However, human transport may also have been involved in the dispersal of the common palm civet to the Philippines.“Kopi” is the Indonesian word for coffee, and “Luwak” is the Indonesian name for the Asian Palm Civet. In different countries there are different names for this kind of coffee. Generally, it's also known as Civet Coffee, Coffee Alamid (on the Philippines), and Weasel Coffee (In Vietnam).

The story of Kopi Luwak begins in the late 1600's when the Dutch governor of India shipped the first seedling of Arabica coffee to the Dutch East Indies (now Indonesia). By 1711 the first exports began shipping to Europe, and Indonesia became the first place outside of Arabia & Ethiopia where coffee was widely cultivated.

The origin of kopi luwak is closely connected to the history of coffee production in Indonesia. Dutch colonialists established coffee plantations in Indonesia and imported beans from Yemen. In the 19th century, farmers in central Java started to brew and drink coffee from excreted beans collected in their plantations.

Fig:1. Asian Palm Civet

Fig 2. Asian Palm Civet eating coffee bean

Conservation

Asian palm civet (Paradoxurus hermaphrodites) protected under the Indian Wildlife Protection Act, 1972,is listed on CITES Appendix III. Since 2008, it is IUCN Red Listed as Least Concern as it accommodates to a broad range of habitats. It is widely distributed with large populations that in 2008 were thought unlikely to be declining In Indonesia, it is threatened by poaching and illegal wildlife trade; buyers use it for the increasing production of kopi luwak a form of coffee that involves ingestion and excretion of the beans by the animal.

Physical Appearance

Asian palm civets are small, weighing only about three kilograms with an average body length of 50 centimeters, and a tail that is 48 centimeters long, have longer and flatter skulls. Coat of civet is short, coarse, and are usually black or gray with black-tipped guard hairs all over, white patch of fur below

and above the eyes and on each side of the nose ,dark stripes down their back and the three rows of black spots freckled on each side of their body and covering their legs. However, these markings are less prominent in juveniles. Unlike other civets, Asian palm civet's tails do not have black rings. Rather, they are just tipped black on the very end. Another distinguishing factor that their neck hair grows backwards, whereas other members of the civet family have forward growing neck hair. However, the Asian palm civet doesn't have ringed tails like many other palm civets.

Habitat and Diet

The Asian palm civet prefers tropical Asian rainforests, but these animals are extremely adaptable and can flourish near human settlements as easily as in dense forest. Palm civets are opportunistic omnivores, and although most of their diet is fruit, they also eat insects, eggs and small reptiles.Palm civets have more specialized teeth for an omnivorous diet than other civets that mostly eat meat. The Asian palm civet in particular enjoys eating coffee cherries, and a luxury coffee made using the beans that pass through the civet's digestive system. It also feeds on palm flower sap, which when fermented becomes palm wine, a sweet liquor ("toddy"). Because of this habit, it is called the toddy cat. Asian palm civets typically live anywhere from 15 to 20 years. They live longer in captivity, living for as long as 24 years and 5 months (1).

Reproduction

Reproductive maturity of Asian palm civet (female) 11 to 12 months and (male) 9 to 11 months respectively. In March 2010, a pair of palm civets was observed when attempting to mate. The pair copulated on the tree branch for about five minutes. During that period, the male mounted the female 4-5 times. Despite being generally solitary, Asian palm civets come together in the same resting trees to continuously mate for a period of one to fifteen days (1,4).

Asian palm civets find mates using scent markings from their anal gland, average estrous cycle of about 82 days. Gestation period of two months. They typically have up to two litters per year. They go into resting trees to mate, give birth, and take care of young, spending the whole mating period in their tree of choice. Kittens are born with their eyes closed and fur covering their bodies. Palm civet babies are very small, weighing only about 80 grams at birth. At 11 days, their eyes open and by two months old are weaned. After about three months, these civets are considered full grown.

Economic Importance for Humans

One of the earliest uses that humans have used Asian palm civets for was their sweet-smelling musk. In the past it was used to treat such things as scabies, but today it is only used for perfume. To get civet oil, the scent gland must be scraped out with a special tool, which is a difficult task and if not done properly is painful for the civet. The musk can also be produced when the civet is harassed. Often, this industry is supported by trappers that go into the wild and capture wild civets to obtain their oil. People also use civets as rodent catchers, since they eat rats and mice (1, 4).

Asian palm civets are best known for aiding in the production of an expensive coffee, Kopi luwak, by passing coffee cherries through their digestive tract. As the cherries go through palm civet's digestive tracts, they get a unique “gamy” flavor and people extract these pits from the civet feces. This coffee is in high demand because of civet's tendencies to only pick the ripest coffee cherries. Due to it becoming a trendy drink, civets are being increasingly captured from the wild and fed coffee beans to mass-produce this blend. Many of these civets are housed in battery cage systems which have been criticized on animal welfare grounds .

India has started the production of this variety on a small scale in Karnataka's Coorg district. The process of producing this coffee involves having the civet cat ingest coffee beans. The cat's poop is then collected and processed. Also known as luwak coffee, civet coffee's market price is extremely high as it is considered more nutritious than other varieties. It also has a high cost of production because of the unusual method of producing it, as well as the processing and quality certification involved. Popular in the Gulf nations and Europe, civet coffee is sold at Rs 20,000–25,000 per kg.A startup, Coorg Consolidated Commodities (CCC), has decided to produce this coffee at a small scale in the country's largest coffee-producing state. CCC has also decided to open a café to serve this coffee locally.

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