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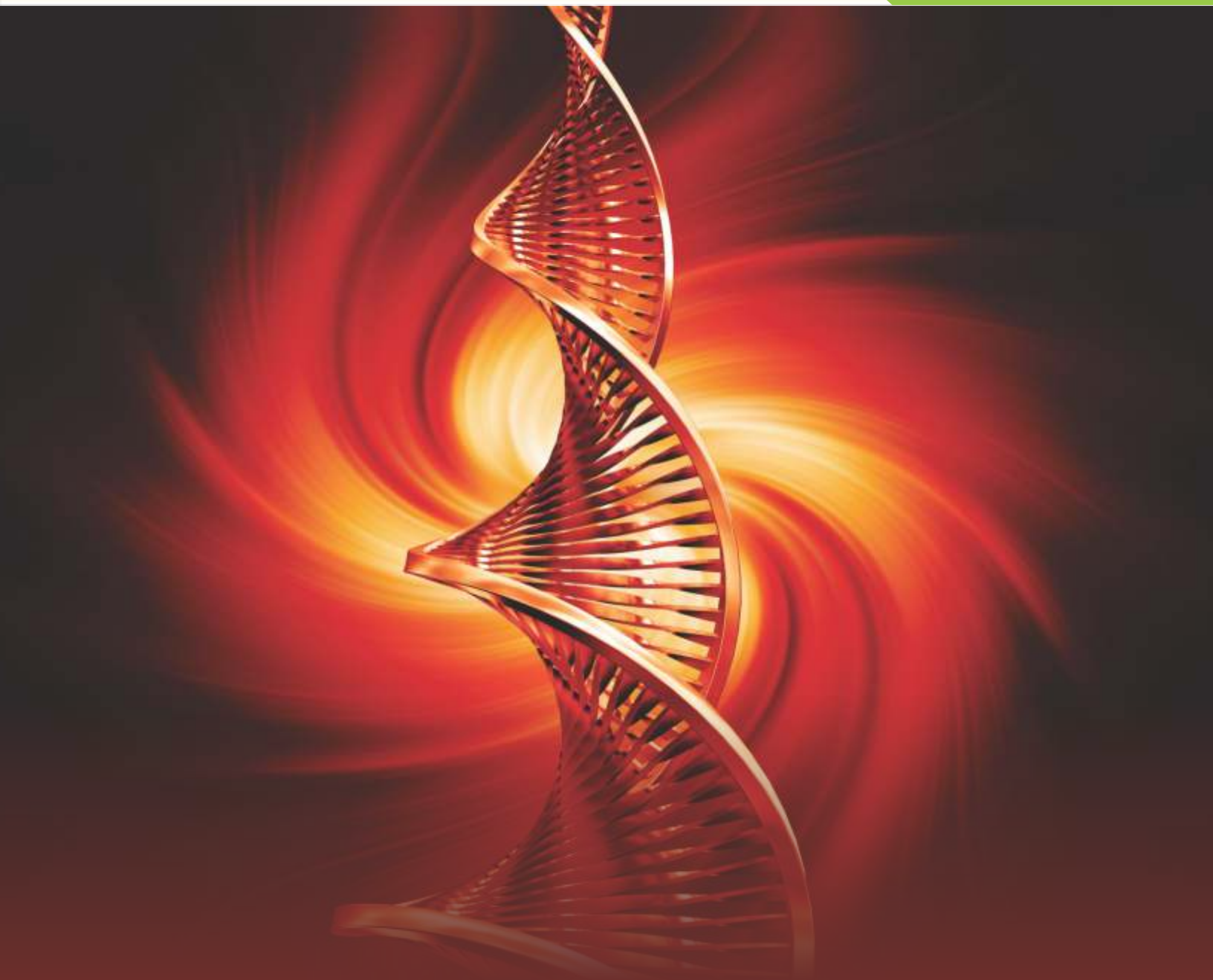

INDIAN IMMUNOLOGICALS LIMITED

Reader's Desk

It is very excellent magazine. I will suggest for adding articles on Jhone's disease and on Actinomycosis Fungal Mastitis with pictures.

Dr Vishnu Kumar Garg
Bhilwara, Rajasthan.

It is very excellent magazine with ultimate products, IIL products very sound with affordable prices. Request for articles on V.Prolapse & Repeat breeding cases.

Dr Manish Singh Charan
Chittorgarh, Pratapgarh, Uttar Pradesh

Diversified topics covered in this magazine viz animal health, animal breeding, animal nutrition etc. Articles covered are suitable for field vets & policy maker. It is highly recommended to go through each & every issue at this magazine.

Dr Santosh Kumar Sharma
Jaipur, Rajasthan

An excellent magazine with full technical information. Provide some articles on African swine fever, proper treatment of metritis, Pyometra in Bitch, Anaplasmosis and Trypnosomiasis in dogs.

Dr Shailendra Kr Thakur
Ranchi, Jharkhand

It is good published material for study the general disease and have depth key knowledge for support to the field veterinarian.

Dr Rajesh
Jaipur, Rajasthan

Good to see such informative magazine, should continue having such detailed information regarding diseases along with products and medicines details. Good for field veterinarians and dairy professionals.

Dr Dheeraj
Chandigarh, Punjab

Information booster, a lot of practical knowledge shared which helps directly in diagnosis & treatment of animals.

Dr Karnvir Singh Sodhi
Ludhiana, Punjab

Raksha Technical Review provided latest information & articles about veterinary issues with excellent design and layout. This magazine covers all the domestic species and the information with deep influence.

Dr Rakesh Kumar Tailor
Ajmer, Rajasthan

Contents

From the Editor's desk

Our world is now recovered from the devastation caused by COVID-19. For most part of last year has been managing the aftermath of pandemic and multiple disease outbreaks. We now experiencing reemergence of vaccine preventable diseases in humans and animals, primarily due to disruption in vaccination coverage during pandemic time. There are many emerging zoonotic diseases that are evolving. Amidst all the above situations, new technologies are proving to be safe and faster to scale up during emergency situations, i.e., viral vector-based vaccines, mRNA-based therapeutics and vaccines, etc. You will find an article about such a topic in the articles of interest section.

Tropical Deg Nala disease in dairy animals results from fungal Infection. The disease's name is derived from the first recorded endemic area near the Nala(rivulet), named Deg in pre-partition India. Deg Nala disease is a common infective disease in both cattle and buffalos. The disease history, epidemiology, etiology, pathogenesis, clinical profile, Diagnosis, and treatment were widely described in the article, *Deg Nala Disease in Buffaloes in India: An Overview* in the Large Animal Section.

Large Animal Section brings articles on *Successful Management of 3rd Degree Cervico Vaginal Prolapse in A Cow – A Case Report* and *Management of Abortion Followed by Retention of Fetal Membranes in A Buffalo.*

In the Grazers and Browsers Section, the article *Diagnosis and Therapeutic Management of Visceral Schistosomiasis in Sheep,* details the disease introduction, history, and pathological findings including treatment protocol.

In the Companion Animal Section, the article *Hepatic Mastocytoma, Splenic Lymphoma and Thigh Spindle Cell Sarcoma in a Senescent Male Dog* elaborates on the case description periodically along with the four-stage treatment schedule.

The articles *Critical Ethylene Glycol Toxicity in Dog: A Case Report* in which the owner opted for euthanasia for the pet and *Modified Robert Jones Bandaging for the Hindlimb in Male dogs* explain the technique of this bandaging for the hind limb in dogs with important instructions for owners.

C*utaneous Mast Cell Tumor in Dog: Treatment with intra-tumoral Injection of tigilanol tiglate* briefed on the treatment with an FDA-approved medicine which activates a protein that spreads throughout the treated tumor and disintegrates the tumor cells.

The article *Pathobiology of Canine and Feline Cirrhosis: An Overview* narrates common ailments in dogs and cats.

A Rare Case of Feline Trichoblastoma: Diagnosis and Treatment discusses a rare tumor case in feline; *Gunshot Injury in Cat's Right Elbow Joint: A Case Report* describes that gunshot injuries in animals and humans occur accidentally or intentionally all over the world; *Recent Updates on Feline Panleucopenia Virus with Respect to Its Therapeutic Management* focusses on a parvoviral infectious disease of kittens; and *Fatal Pneumoperitoneum in a Cat after Complete Recovery from Deep Facial Bite Wound: A Case Report,* are articles of interest for readers delight in the Companion Animal Section.

In the General Articles Section, the Articles *Gene Therapy in Humans and Livestock Species: Current Status and Future Perspectives;* and *Emerging Viral Zoonotic Diseases in India: An Overview* are articles of interest.

We hope the readers will enjoy reading the latest journal.

Dr Priyabrata Pattnaik
Dy Managing Director

Managing Director's Message

Dear Patrons,

Season's Greetings!!

2022 was yet another eventful year for IIL. We have obtained licenses for producing Measles - Rubella Vaccine and Tetanus-Diphtheria vaccine. Hepatitis-A vaccine license is expected anytime now. Other vaccine candidates in queue are Dengue and Infectious Bovine Rhinotracheitis (IBR). Our research collaboration with Aqua biologicals institutions e.g., Central Institute of Brackish Water Aquaculture (CIBA), Central Institute of Fish Education (CIFE) is progressing well.

Adapting to new technologies has always been the forte of IIL. From delivery of animal vaccines to the remotest places in the North-East using drones to creation of Virtual Medical Representatives to interact with the medical fraternity, IIL keeps pace with the changing ways of doing business in the era of digital economy.

IIL is committed towards the objectives of Environmental, Social and Governance. Our initiatives include gradual eradication of Rabies, supporting conservation of energy through supply of Bio-gas kits to rural households, providing nutrition supplements to school children, donating medical support infrastructure e.g., Kidney dialysis equipment, Oxygen generation units etc., facilitating rural street electrification and so on.

As the financial year is coming to an end shortly, we wish you all a great success ahead in chasing your aspirations and realizing the dreams.

Warm Regards
Dr K Anand Kumar

Deg Nala Disease in Buffalos in India: An Overview

Kumawat Dhawal, Kabita Roy, Shilpa Gajbhiye, Suman Kumar and Shruti Bhatt
Department of Veterinary Medicine, and Department of Pharmacology, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh.

Abstract

Tropical Deg Nala disease in dairy animals results from fungal infection: Aspergillus spp., Alternaria alternata, Mucor hiemalis, Fusarium spp., Cladosporium cladosporiodes, Penicillium notatum and their mycotoxins. Gangrenous lesions with the sloughed off epidermis in the extremities, namely the ear, tail, and the limbs are characteristic. With seasonal incidence, sporadic cases are reported mainly in winter, when paddy straw is used as the main sustenance roughage. In India, the disease occurs most often in Punjab, Haryana, Uttar Pradesh, Uttarakhand, Bihar, Bengal, Jharkhand and Chhattisgarh. On consumption, the moldy fodder/ straw releases mycotoxins into the intestinal lumen, which enter the liver parenchyma via the portal circulation, causing obstruction to normal blood flow in the ear, tail and feet leading to vasoconstriction and gangrenous lesions. The disease is clinically manifested as edema of the extremities, necrobiosis and sloughed off epidermis with febrile reaction, malaise and restricted body movements because of painful bleeding limbs. Diagnosis is based on the clinical signs, blood profile, microscopic examination and microcrobial culture of the exudates. Differential diagnosis from foot rot, chronic selenium toxicity and ergot poisoning is needed. Treatment protocol: anti-Deg Nala liquid @ 10 ml PO x 10 days, penta-sulphate mixture @ 60 g on day 1, and @ 30 g x 15 days with adequate quantum of linseed and molasses, antibiotics and supportive treatment. Effective preventive measures for the control of fungi and deleterious mycotoxins are strongly recommended.

Introduction

Characterized by edema of the extremities, extensive necrobiosis and sloughed-off epidermis, Deg Nala disease in buffalo, is often associated with pyrexia, malaise and restricted body movement because of the painful bleeding limbs (1,2). The disease name is derived from the first recorded endemic area near the 'Nala' (rivulet), named Deg in pre-partition India, now located within Pakistan (1). Seasonal incidence is characterized by sporadic cases reported in winter, when paddy straw constitutes the main roughage (2). Deg Nala disease is a common infective disease in both cattle and buffalos, fed continuously degraded moist paddy straw and stored while still damp, or of poor nutritive value in the ripening stage. In point of fact, use of such potentially fungus-infested roughage poses a serious health hazard to the dairy animals: infections that result in ongoing debilitation, leading to significant economic losses to the marginal dairy farmers as well as large peri-urban commericial units, resulting from decreased milk yield confounded with stunted growth, high morbidity and mortality. The biodiversity of moulds, mycotoxins, clinical signs and lesions make it very challenging to decipher the precise pathogenesis (3). On presentaion, virtually all the affected buffalos reveal gangrene of the tail with a shrivelled appearance, and cold-on-palpation. Invariably, one or both ears exhibit covert signs of dry gangrene. In some cases, the muzzle and the tip of the tongue too appear gangrenous and clearly ulcerated. One or more hooves may show lesions of varying intensity in different stages of biodegradation. Occasionally, the affected feet and legs appear swollen right up to the knee joint; hair denuded with covert signs of inflammation. Subsequently, the lesions extend to the coronet, fetlock, pastern, knee and the hock regions. In the clinically advanced cases, the lower aspects of the feet become gangrenous, occasionally the hooves shed off and bones exposed (4).

Epidemiology

Extensive surveys of Deg Nala disease outbreaks in post-independence India (1968-1978) in total 370 dairy herds from 136 villages in Haryana state revealed a noteworthy regional prevalence, targetting year-after-year, vulnerable herds in certain specifiic locations. Further, these outbreaks invariably occurred during the winter season, coinciding with the feeding of dry rice straw because of the dearth of green fodder. In mid-west Nepal, Deg Nala disease outbreaks (1998-1999) were recorded in five villages in Banke district, and reoccurrence was observed again for one decade in the specified districts, and winter rain was held responsible (5). Outbreaks in buffalos in a cluster of villages in Haryana and Bihar states of India (1994-1998) are on record (6). In the eastern Indian states of Bihar, Jharkhand, West Bengal, and Orissa the onset of the disease was noted specifically during the winter months, i.e. November to February (7). Thus, the environmental milieu appears to be predominantly involved in triggering the pathobioepisode. Notably, apart from the original

geographic location in Deg Nala, the disease is rampant, also in all those areas where buffalos are kept on sustenance rice straw ration during the post-monsoon season (2,8). Wide-spread disease, causing considerable economic losses, has been reported also from the rice growing areas in neighbouring Pakistan (9). Thus, it is well-established that incomplete drying of the degrained rice straw before stacking, low-lying premises, or proximity to the water channels are the major contributary factors in promoting Deg Nala disease in buffalos.

Etiology

1. Fungal infestation of feed and fodder. The fungi affecting wheat and rice straw include *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Mucor hiemalis, Fusarium avanaceum, Fusasrium fusarioides, Fusarium oxysporum Cladosporium cladosporiodes* and *Penicillium notatum.* Concerted attempts to isolate the fungus and extract the mycotoxin(s) from paddy straw by the Commomwealth Mycology Laboratory, UK led to identification of Fusarium species as the main pathogenic entity (10). A total of 12 *Fusarium spp.* were isolated and identified from the fungal affected paddy straw, collected from the Deg Nala disease prone areas including Haryana, Punjab and West Bengal (7, 11, 12).
2. Mycotoxins. In the moist condition, chances of fungal growth and rate of production of mycotoxins increase exponentially. Feeding of fungi-infested rice straw, contaminated with mycotoxins promotes disease onset. The typical signs and symptoms of mycotoxicosis, named Deg Nala disease in popular parlance were systematically documented following in-depth analysis (13).

Pathogenesis

Deg Nala disease results from prolonged ingestion of fungus-infested rice/ wheat straw as the main roughage fed to the dairy animals. During the period of drought and low temperature in winter season, if humidity is increased due to rainfall, storage of rice straw near the water-logged areas, flooded conditions or storage of rice straw immediately after harvesting, may lead to massive growth of fungal colonies (11). These pathogenic fungi produce their ill-effects on animals through secondary metabolites, mycotoxins. Some fungi are capable of elaborating more than one class of mycotoxin, and some mycotoxins are synthesized by more than one kind of fungi (14). Dairy cattle and buffalos get exposed to mycotoxins on daily consumption of fungus contaminated feed (15). Molds are thread-like cylindrical fungi capable of targeting crops at any stage of growth in the field to synthesize and release mycotoxins during the harvesting time, storage or processing (16). Different molds produce a variety of mycotoxins, classed as secondary metabolites (17). The mycotoxin disintegrates the intestinal mucosal connective tissue components: collagen and elastin with collagenase and elastinase, respectively. The deleterious mycotoxin reaches the liver via the portal circulation leading to extensive hepatocytosis. Subsequently, in the dependent parts of ear, tail, foot, the blood supply is obstructed and the tissue cells perish because of anoxia. In the peripheral tissues, the toxin promotes collagen and elastin biodegradation, resulting in vasoconstriction and skin lesions (18). Finally, the structural and functional integrity of the vital internal organs: liver, lungs, heart, and kidneys is significantly compromised.

Clinical Profile

The visble clinical signs in the affected buffalo include edematous swelling of the dependent parts and gangrenous ulceration of the tail, ear tips and muzzle **(Fig.1).** Eruption and crackling of skin, sloughing of tissues from the affected areas, alopecia, dried skin in different body parts **(Fig. 2),** restricted movements, and anorexia are also observed. In the later stages of the syndrome, hypothermia, emaciation, recumbence precede mortality.

Diagnosis

1. Tentative Diagnosis

(i) In-depth anamnesis from the owner/ animal attendents, history of feeding of mouldy straw for extended periods of time.

(ii) Clinical signs on presentation: emaciation, gangrenous lesions with the on-going characteristic sloughed off epidermis in the extremities: the ear, tail, and the limbs (19, 20).

Fig 1 a. Deg Nala patient debilitated and dehydrated, b. Cutaneous foot lesions, c. Tail gangrene in advanced Deg Nala.

Fig 2 a. Swelling of fetlock, b. Gangrenous cutaneous lesions, c. Sloughed off hoof in advanced Deg Nala

(iii) Patient's physical examination: hypothermia, bradycardia, dehydration, exanthema and icterus (21).

Note: Differential diagnosis from foot rot, chronic selenium toxicity and ergot poisoning, based on specialized laboratory test reports, is recommended.

2 **Hematology** Increased frequency of circulatory band neutrophils, giant platelets, hypo-albuminemia and hyperglobulinemia represent the major changes in the patient's blood profile (19). Marked neutrophilia concurrent with reduced erythrocyte count (TEC), haemoglobin (Hb) concentration and packed cell volume (PCV) % commonly observed. Microscopic examination of Giemsa-stained blood smear: dark blue discrete particles on the red cells are clearly discernible.

3. **Post-mortem Findings** Indurated blood vessels, excessive straw-coloured fluid in subcutaneous tissues and heart, and gangrenous skin lesions. Necrotic and degenerative changes in skeletal muscles, hemorrhages on serosal surfaces on the dorsal aspect of rumen and gastro-intestinal stasis.

4. **Histopathological Examination** Necrobiotic degradation with the loss of cellular architectural profile in the affected body parts (13).

5 **Microbial Culture** Representative samples of the affected animal's skin scrappings and rice straw in SDA medium (96 hr) and isolation of fungi on potato dextrose agar, yeast soluble starch agar, Czapek's Dox agar, Sabouraud's agar revealing the presence of typical fungal hyphae provide the corroborative evidence (9).

Highlights of Cutaneous Necrobiosis

1. Progessive dermal lesions in the lateral aspects of the trunk region, sloughed off skin areas leave an actively bleeding exposed surface.

2.. The oozing blood exudate undergoes continuous desiccation leading to scab formation.

3. Eextensive surface oedematous swelling involves the dewlap and limbs.

Treatment

Nutritional Support Fungus-infested straw should not be fed to the dairy animals. On-field observations: marked reduction in the incidence of disease after cessation of the moldy rice straw feeding (13).

Remedial Therapy The affected buffalo should be kept on sodium hydroxide treated rice straw: 1% solution (10 g/ l water), uniformly mixed with 20 kg straw. Penta-sulphate mixture (ferrous sulphate 166 g, copper sulphate 24 g, zinc sulphate 75 g, cobalt sulphate 15 g and magnesium sulphate 100 g) @ 60 g on day1, and @ 30 g OD for 15 days, mixed with linseed and molasses (4). Anti-Degnala liquid @ 10 ml PO for 10 days (5, 22). Mineral mixture and lactoline: mixed in straw for use.

Prophylaxis

1. In the rice growing tracts, feeding of alkali-treated rice straw (sodium hydroxide @ 1g/400ml water (4% solution), mixed in 20 kg dry rice straw.

2. The owners are advised to use copper and zinc micro-minerals: large animals @ 1-2 g x 10-15 days; and followers @ 0.5 g x 10-15 days, mixed uniformly in water and flour and and divided into 15 boluses of equal size.

3. Proper management for the crops and feed ingredients is the basic requirement for minimizing the intensity of mycotoxicosis in dairy animals (23). Hazardous mycotoxins in the contaminated feeds and environment include hepatotoxins, nephrotoxins, and neuromusculotoxins, potentially carcinogenic/ mutagenic. Aflatoxin is the most potent hepatocarcinogen-mutagen.

4. Mycotoxins contamination may be minimized with rigidly implemented three-tier protocol.

(I) Primary The crucial basic step includes the development of fungus-resistant varieties of fodder plants, inhibiting on-field infection, ensuring the recommended time-tested schedules. Dry plant seeds before and during storage. Store fodder at low temperature, if possible. Use fungicides and safe preservatives. Control insect infestation in the stored bulk grains with the approved insecticides.

(II) Secondary The pre-existing toxigenic fungal load, is eliminated, or further growth is strictly controlled with timely measures, including re-drying the affected commodities, removal of the contaminated seeds, inactivation/ detoxification of contaminated stock

(III) Tertiary If the products are heavily infested with toxic fungi, measures should be taken to block the further transmission of fungi/ hazardous toxins into daily feed, fodder and microenvironment. For example, peanut oil extracted from poor-grade peanut seeds invariably contains high levels of aflatoxins. The oil-soluble toxin needs to be totally eliminated during the oil refining process.

Validated practices, namely complete destruction of the contaminated products, or assured detoxification of mycotoxin are recommended.

Discussion

In the treatment of Deg Nala disease, administration of Tetracycline in the early stage is effective concurrent with antiseptic dressing of the cutaneous wounds, till complete healing (19). Oxytetracycline LA @ 1ml/ 10 kg body weight is recommended to preempt secondary bacterial infection.The lesions are washed with lukewarm water and dressed with nitroglycerin 2% ointment (4). Recovery occurs in 10-15 weeks time. Further, 2 g zinc sulphate OD x 10 days, administered orally is advised. Zinc effectively combats selenium toxicity, which may develop as a comorbidity (24). Regular use of concentrated (4%) sodium hydroxide solution treated rice straw during winter is beneficial, apparently by inhibiting the growth of pathogenic fungi, implicated in biosynthesis and release of deleterious mycotoxins.

Mycotoxin Binding Agents Result-oriented laboratory investigations on the microorganisms and enzymes were undertaken to detect potent mycotoxins binding entities in the gastrointestinal tract of the affected dairy animals (25). Yeast sludge is effectively utilized for mycotoxin absorption and detoxification (26). In this context it is pertinent to reiterate that an organic compound in yeast sludge, modified glucomannan is capable of adsorbing over 90% aflatoxins in 90 minutes time (27). Further, only 1% yeast sludge neutralizes high concentrations (100-200 ppb) of aflatoxins (28). These observations are very promising for future application in the dairy industry.

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Successful Management of 3rd Degree Cervico Vaginal Prolapse in A Cow - A Case Report

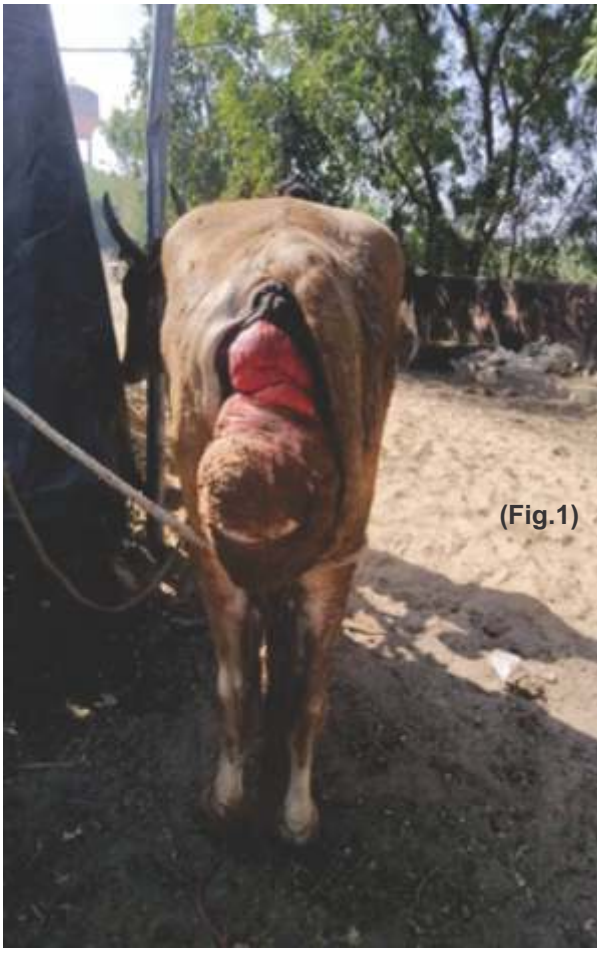
Chandra Shekher Sarswat, Adarsh Verma, Ajay Kumar, Ajay Kumar Bairwa, Anjana Singhal, Ankita Kumari and Anuj Kumar
Dept. of Veterinary Gynaecology & Obstetrics, PGI of Veterinary Education & Research, RAJUVAS, Jaipur, Rajasthan.

Abstract

The present clinical case reports successful management cervico vaginal prolapse in non-descript cow during extreme cold weather.

Introduction

Prolapse of vagina and cervix (CVP) is a disorder of ruminant normally in late gestation (1). Occasionally, it is observed after parturition and rarely does it occur unconnected with pregnancy and parturition (2). Subsequent to prolapse, the tissues appear almost normal, but within a few hours they become enlarged and edematous (3). The etiological factors may be attributed to the condition like higher estrogen secretion from placenta, heredity, mineral deficiency, increased intra-abdominal pressure, excessive relaxation, weakening and atony of the vaginal musculature, pelvic ligaments and vulvar sphincter muscles, bacterial or fungal infections, and ingestion of phyto-estrogens, hormonal imbalance, etc. (4). In managing cervico-vaginal prolapse various surgical or nonsurgical techniques (5) and medicines (6) have been practiced with varying degrees of result. The purpose of this case report is to explain the successful correction and management of post partum cervico vaginal prolapse in a cow during extreme cold environment.



(Fig.1)

Case History and Clinical Examination

A non-descript indigenous cow (>05 years age) was left abandoned in Gaushala due to the failure in repositioning of the cervico vaginal prolapsed mass. On clinical examination, it was noticed that the cow was in standing condition with large swollen and edematous hardened prolapsed mass in hanging position, felt extreme cool due to surrounding temperature less than 10°C since last night (first week of January 2023) **(Fig.1).** Signs of discomfort, restlessness, continuous straining was observed prominently in the suffered cow.

(Fig.2)

Treatment

The cow was restrained in lateral recumbency at sloppy area where the pelvic area was lifted upside and rest part downside in order to overcome the ruminal pressure and to control abdominal

straining. 2% lignocaine solution (7 ml) was administered for caudal epidural analgesia. The prolapsed mass was lifted upward above the level of ischial arch to release the retained urine and then clean with potassium permanganate solution to remove all sand with debris. The edema of mass was reduced by applying hypertonic sugar solution and ice packs. The hardened and highly cool about to frost (as surrounding temperature was less than 10°C) prolapsed mass was manually repositioned by initially gentle pushing the lateral walls and middle portion by fisted hands followed by elevating the mass with the palm of other hand. The repositioning of the cooled prolapsed mass failed due to hardened prolapsed mass and excessive straining by the cow. However repeated manual action by hands on the prolapsed mass increased the blood circulation which resulted into softening. After three to four attempts the softened prolapsed mass was repositioned within the pelvic cavity of the cow appropriately **(Fig.2).** Cow was administered with antibiotic and NSAIDs along with calcium therapy.

Discussion

Genital prolapse, including vaginal prolapse in ruminants, is considered as an emergency maternal disorder that needs immediate attention before any further complication (7). Various predisposing factors have been suggested for uterine prolapse in the cow, i.e., hypocalcaemia, prolonged dystocia, fetal traction, fetal oversize, retained fetal membranes, chronic disease and paresis (8).

Conclusion

Hence, it is concluded from the above clinical findings that hard prolapsed mass due to surrounding less temperature may be overcome by manipulation of the mass which resulted into softening of mass and easy repositioning within the pelvic cavity without any complications.

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Management of Abortion Followed by Retention of Fetal Membranes in A Buffalo

M Rajashri, P Vishal Kumar and K Ramchandra Reddy
Dept. of Veterinary Gynaecology and Obstetrics, College of Veterinary science,
P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, Telangana.

Abstract

The present case study reports successful therapeutic management of retained fetal membranes in a Buffalo.

Introduction

Failure of expulsion of placental membranes within 8 hrs after parturition in cattle and buffalo results in condition called as Retention of fetal membranes (RFM) which is one of the common maladies during puerperium in buffaloes (1). Retention of fetal membranes has adverse effects on reproduction (i.e. metritis, slower uterine involution and reduced conception rates) in cattle (2) and incurs economic losses in the herd due to decreased milk production, treatment cost and decreased market value of the animal (3). Clinical examination often show a close relation between an early start of follicular activity in the ovaries after parturition in an undisturbed puerperal period, while cows with retained fetal membranes have delays and problems with recommencement of normal ovarian activity (4). Kunbhar et al., (2011) (5) reported an incidence of retention of placenta fall in the range of 10-15%. The incidence of retention of fetal membranes is increased by abortion, premature birth, dystocia, hypocalcemia, twin birth, high environmental temperature, senility, induction of parturition, placentitis and nutritional disturbances (6).

Case History and Clinical Observation

A pleuriparous non-descript cow was presented to TVCC (Teaching Veterinary Clinical Complex), College of Veterinary Science, Rajendranagar, Hyderabad, Telangana with fetal membranes hanging from the vulva **(Fig.1).** The cow aborted 8 months fetus a day before, but the fetal membranes were not expelled even after 24 hours of abortion. Clinical examination revealed normal rectal temperature (101.6°F) but animal was anorexic, dull, depressed with elevated respiratory rate and heart beat. Pervaginal examination revealed apparent lochial discharges which filled the retained Placenta.

Treatment

Retained fetal membranes hanging from the vulva were removed by slow, gentle traction to remove the potential source of infection. Herbal uterine cleanser (Exapar liquid) was administered orally @ 100 ml for expulsion of remnants of placenta and early uterine involution along with Inj. Oxytocin 20IU (I/M). Intramuscular Antibiotic Inj. Enrofloxacin @ 5 mg/kg b.wt and Antihistamin @ 5mg/kg b.wt I/M were administered for consecutively for 3 days to combat bacterial infection.

Discussion

Postpartum metritis (PPM) is a common sequel of ROP, and the rationale behind antibiotics for ROP is to prevent or treat metritis and its subsequent negative effects on fertility (7). The herds with a history of selenium deficiency had a high incidence of RFM, and according to their suggestion supplementation of vitamin E and selenium can help to reduce placental retention (8,9).

Kunbhar et al. (2011) (5) concluded that administration of oxytocin and antibiotics helped in the expulsion of placenta and resumption of early postpartum estrus in buffaloes. Careful nutritional management particularly during the transition period along with creation of awareness among farmers and animal handlers regarding the risk factors of RP should be done to alleviate the incidence of RFM.

Fig. 1
Retained fetal membranes hanging from the vulva

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Diagnosis and Therapeutic Management of Visceral Schistosomiasis in Sheep

N Ramachandra, P J Shruthi, P Ushakiran and B Baby Manasa
Animal Disease Diagnostic Laboratory, Department of Animal Husbandry Anantapuram, AP

Abstract

The present communication reports diagnosis of Visceral Schistosomiasis in 3 sheep with symptoms of mucoid and blood tinged diarrhea, tenesmus and dehydration based on necropsy findings, identification of Schistosoma Sps. eggs in intestinal scrapings and histopathology findings. Successful therapeutic management in the affected flock was observed with Praziquantel and Anthiomaline drugs.

Introduction

Schistosomiasis (bilharzia) is a tropical, zoonotic and parasitic disease caused by trematodes (blood flukes) of the genus Schistosoma that has public health and veterinary importance. Schistosomes are unique among trematodes and any other flatworms as they are dioecious with distinct sexual dimorphism between male and female (1, 2 ,3).

Schistosomiasis is wide spread in tropical and sub-tropical regions of the world where climatic, ecological and hygienic conditions favor their transmission (4,5). It is an economically important disease of domesticated animals causing considerable morbidity in parts of the Middle East, South America, Southeast Asia and, particularly, in sub-Saharan Africa (6,7).

In many countries, *S. bovis* is one of the main species of veterinary and zoonotic importance. In West Africa, *S. curassoni* is responsible for schistosomiasis in small ruminants (goat and sheep) (4). Among various species *Schistosoma indicum* and *Schistosoma spindale* are the common causes of visceral schistosomiasis in cattle. Although most infections in endemic areas are subclinical, higher prevalence causes significant losses in terms of growth, productivity and increased susceptibility to other parasitic or bacterial diseases (7).

Schistosomiasis is a zoonotic disease with a complex transmission cycle involving aquatic snails as intermediate hosts and mammalian definitive hosts (domestic ruminants, horses, cats and humans) (4,). The pathology and clinical signs are due to irritation caused by the spined eggs (7,8). Infective larvae grow in an intermediate host (fresh-water snails) before penetrating the skin of the definitive human host. Mature adult worms reside in the mesenteric (*Schistosoma mansoni* and *Schistosoma japonicum*) or pelvic (*Schistosoma haematobium*) veins, where female worms lay eggs, which are secreted in stool or urine. Eggs trapped in the surrounding tissues and organs, such as the liver and bladder, cause inflammatory immune responses (including granulomas) that result in intestinal, hepato-splenic or urogenital disease (6).

The affected livestock exhibit typical clinical signs such as marked diarrhoea mixed with blood or mucous, dehydration, pallor of mucus membrane, marked weight loss, emaciation and decreased production (7, 8).

Diagnosis is based on clinical signs, detection of eggs in fecal samples and serology (for worm antigens in serum). At necropsy, macroscopic examination of the mesenteric veins for the presence of adult worms or microscopic examination of scrapings of the intestinal mucosa or of crushed liver tissue (both for eggs) helps in diagnosis. Morphology of eggs of different Schistosoma species vary. Eggs of *S. bovis, S. curassoni* and *S. mattheei* are spindle-shaped and that of *S. indicum* are oval shaped with a terminal spine. Very low fecal egg excretion is seen in chronic infections.

Schistosomiasis elimination will require a multifaceted approach, including treatment, snail control, information, education and communication, improved water, sanitation and hygiene, accurate diagnostics, and surveillance-response systems that are readily tailored to social-ecological settings.

Anthiomaline and Praziquantel are drugs of choice to treat Schistosomiasis. Praziquantel @ 20-30 mg/kg is highly effective to treat schistosomiasis in sheep. However, two treatments, 3–5 weeks apart may be required. Triclabendazole showed adulticidal effect at a dosage of 20 mg/kg against female schistosome worms.

There are reports of Schistosomiasis caused by *S. nasale, S. spindale* and *S. mansoni* from various parts of India and abroad (8, 9,10) but reports of *S. indicum* in sheep are meagre (11) among available literature. Hence, the present work, Diagnosis and

therapeutic management of Visceral Schistosomiasis caused by *S. indicum* in sheep has been communicated.

History and Pathological Findings

3 cases of Visceral Schistosomiasis were recorded in sheep presented for post mortem examination at Animal Disease Diagnostic Laboratory, Anantapuram from June 2022 to November 2022. The animals exhibited typical symptoms like dullness, mucoid and blood tinged diarrhea, colic, tenesmus for 7-10 days before death. On necropsy, the carcasses were emaciated, dehydrated with paleness of visible mucous membranes.

There was dilatation of right ventricle and degenerative changes in heart. Lungs were enlarged, brownish in color with marbling in anterior lobes and resembled maedi lung **(Fig 1).** Trachea and bronchi were congested. Liver was dark brownish in color, hard, enlarged with diffuse nodular lesions in parenchyma **(Fig 2).** On cut section nutmeg appearance of liver was noticed. Spleen was slightly enlarged. There was diffuse reddening and congestion of intestines in one case **(Fig 3)** with greatly enlarged mesenteric blood vessels which were occluded with adult Schistosoma flukes in lumen **(Fig 4).** Intestine mucosa thickened with necrotic and hemorrhagic lesions. Small nodules were seen protruded out from intestine in one case. Mesenteric lymph nodes were swollen and hemorrhagic.

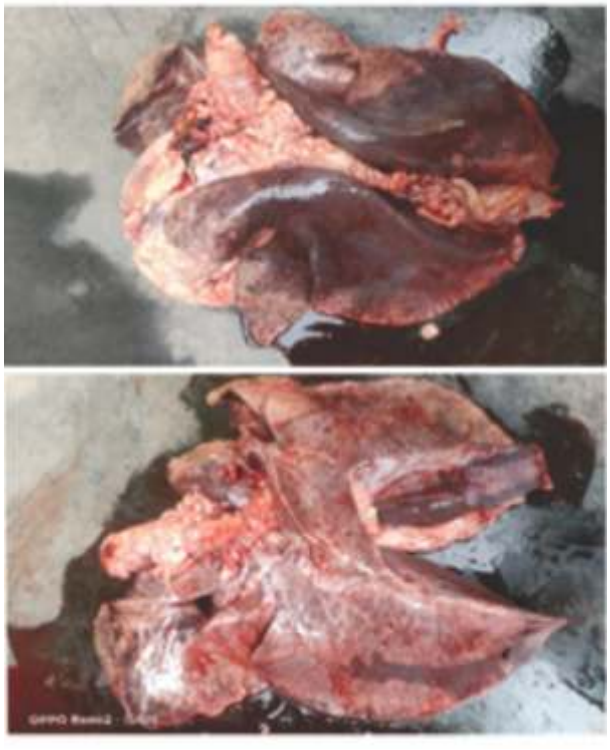

Fig 1 Enlarged, Brown colored lungs resembling Maedi Lungs

Fig 2 Brown colored, Nodular appearance of liver

Fig 3 Diffuse reddening of Intestines with greatly enlarged mesenteric blood vessel.

Fig 4 Greatly enlarged mesenteric blood vessels with adult flukes in lumen

Direct smear examination of intestinal scrapings revealed *S.indicum* eggs. The eggs were oval shaped with a well developed miracidium inside and a short terminal spine in all 3 cases **(Fig 5).** Gram staining of intestinal scrapings revealed *S.indicum* eggs and cut section of flukes **(Fig 6).**

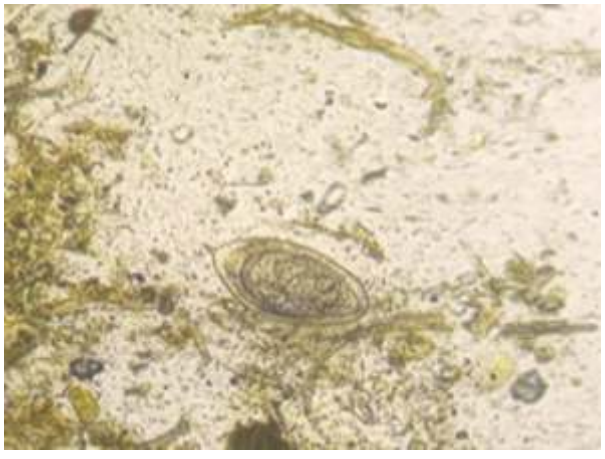

Fig 5 Schistosoma indicum egg with well developed miracidium inside and a short terminal spine.

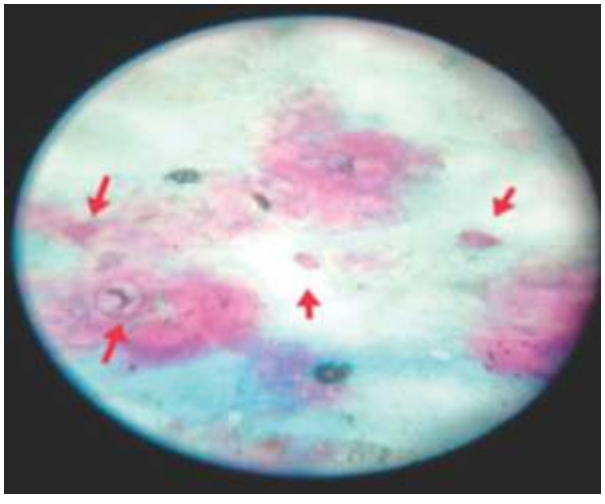

Fig 6 Intestinal scraping – Schistosoma eggs with a terminal spine – Grams staining

Tissue pieces of lung, liver & intestines were collected in 10% formalin and sent to Veterinary Biological Research Institute (VBRI), Vijayawada for histopathological examination.

Histopathology of lungs and liver revealed granulomatous reaction to ova. A granuloma consisting of macrophages, giant cells, lymphocytes, eosinophils and collagen fibers were seen surrounding eggs with spine. Congestion, edema, infiltration and fibrosis were observed in lungs **(Fig 7).** Granulomas with eggs and marked fibrosis with severe infiltration of cells were observed in liver **(Fig 8).** Cut section of parasite was noticed in mesenteric blood vessels.

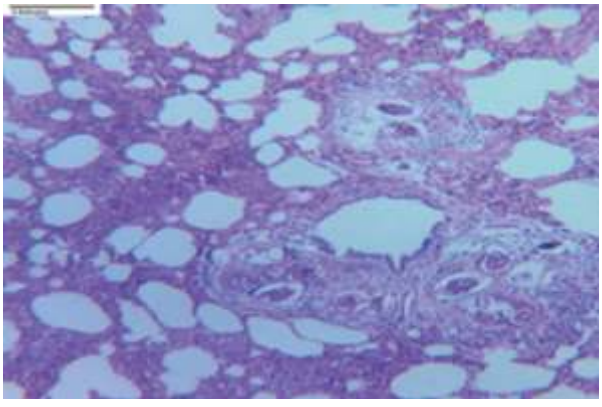

Fig 7 Lung – Granulomatous lesion surrounding Schistosoma eggs with spine

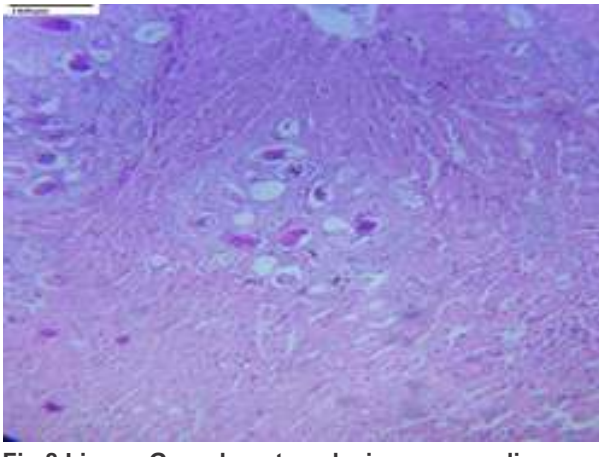

Fig 8 Liver – Granulomatous lesion surrounding Schistosoma eggs with spine

Treatment

The owners of 3 sheep flocks were advised to deworm their flocks with susp. Tapenil® (Praziquantel) @ 20-30mg/Kg BW for 1day followed by susp. Sharkoferol vet® for next 3days. Sheep with diarrhea were treated with Inj. Anthiomaline® @ 1ml/15Kg BW. The status of flock was enquired after 10 days and a significant control of diarrhea and improvement in the flock was recorded.

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Hepatic Mastocytoma, Splenic Lymphoma and Thigh Spindle Cell Sarcoma in a Senescent Male Dog

Sabita Rakshit, Dale Clark, Kabita Roy and I C Datta
Milford Veterinary Clinic, 110 Canal Street, Milford MI, USA and College of Veterinary Science, (NDVSU), Jabalpur, Madhya Pradesh.

Abstract

A 13-year-old neutered male Labrador mix breed dog was presented in the Milford Veterinary Clinic, Milford, USA on September 21, 2022, with the complaint of compromised mobility from right hind limb involvement. Anamnesis revealed that a subcutaneous growth in the right caudal thigh muscle, noticed recently, was getting enlarged progressively. In-house fine needle aspirate (FNA) cytology profile in tandem with the pathologist's corroborative report pointed to spindle cell sarcoma. Ultrasound-guided biopsies from the liver and spleen FNA slides were referred to IDEXX Lab. The pathologist's cytology report: hepatic mast cell tumor (mastocytoma), splenic lymphoma and appendicular spindle cell sarcoma. Complete mass removal surgery scheduled on October 18, 2022, was cancelled in view of the high risk associated with possible undetected abdominal tumor(s) against the backdrop of markedly elevated liver clinical enzymes concentrations, and a conspicuous round radiopaque patch in the abdominal survey radiographs. Long-term broad-spectrum antibiotic course was suggested, but in view of the poor prognosis the lady owner opted to get the aging dog with multiple malignancies euthanized for relief from suffering.

Introduction

Mast cell tumors (MCTs) or mastocytomas are common cutaneous tumors in dogs. Occasionally, MCTs may also appear in the subcutis, mucocutaneous structures, and the internal organs. Notably, nearly 50% of cutaneous mast cell tumors (CMCTs) are malignant (1). Prognosis is often poor in MCTs in the internal organs like the hepatic parenchyma in the instant case, and euthanasia / mortality is the end result of the pathobioepisode (2). Canine lymphoma (cL), a common neoplasia in dogs is comparable to non-Hodgkin lymphoma in human patients (3, 4, 5). Though the precise aetiology remains uncertain, environmental factors appear to be involved in the pathogenesis (6,7). Wide ranging clinical manifestations and subtypes (B and T cell) are characteristic. In pet practice globally generalized 'multicentric' peripheral lymphadenopathy with intermediate to large size B-cell line high-grade canine lymphoma is encountered in >75% cases (8). Occasionally, extra nodal lymphoma: mediastinal, hepatic, splenic, renal, cutaneous, ocular, central nervous system, pituitary is also encountered (9).

Soft tissue sarcomas comprise a diverse group of malignant mesenchymal tissue tumors like spindle cell sarcoma, fibrosarcoma, malignant fibrous histiosarcoma and nerve sheath tumor. Originating from the skin, subcutaneous tissue, or the palate, these tumors are often clinically manifested as raised fleshy masses, varying markedly in size, morphology, and anatomical location. Soft tissue sarcomas represent 15% of all skin and subcutaneous tumors in dogs (10,11,12). Recurrence is common after conservative surgical excision because of the high propensity of local invasion of the remaining neoplastic cells (13). This communication documents the rare combination of hepatic mastocytoma, splenic lymphoma, subcutaneous spindle cell sarcoma in the right caudal muscle area along with benign scattered lipomas (fatty tumor) all over the body with unrelated aetiopathogenesis in a senescent neutered male dog.

Case Description

March 17, 2022

A 13-year-old (36.8 kg) neutered male Labrador mix breed dog was presented in the Milford Veterinary Clinic for scheduled dental cleaning and health check. Eight subcutaneous masses of varying size and cytology evaluation of all FNA sample pointed to benign lipoma (fatty tumor). Dental radiographs were taken, and total dental cleaning was done under isoflurane+oxygen gas anesthesia.

September 21, 2022

The pet was presented in the clinic with compromised mobility from right hind limb involvement. A growth in the right caudal thigh muscle area was getting progressively enlarged during the past 2-3 weeks. Food and water intake normal, and no coughing, sneezing, or diarrhoea episodes were observed by the vigilant well-informed lady owner.

From the growth in the right caudal thigh muscle (3

cm x 2 cm) fine needle aspirate (FNA) samples were collected, and in-house cytology revealed clusters of spindle cells **(Fig.1)**. These samples were sent to IDEXX Lab for evaluation by the pathologist.

Pathologist's Report

The proliferation of not clearly differentiated spindle cells with mild inflammation may suggest reactive fibroplasias, or a neoplastic transformation: namely soft tissue sarcoma. Aggregates of small to moderate sized cells exhibit round to oval nuclei with basophilic cytoplasm of low to moderate intensity are visible. Lymphocytes and macrophages are scanty. No infectious agents are seen. In-house FNA cytology profile, in tandem with the pathologist's report, established spindle cell sarcoma. In perspective, the owner was advised to keep her pet on broad-spectrum long-term antibiotic therapy. If no positive response is observed, then surgical total mass removal will be scheduled in the clinic.

October 18, 2022

Caudal subcutaneous mass removal surgery was scheduled in the morning hours. Routine blood work was done.

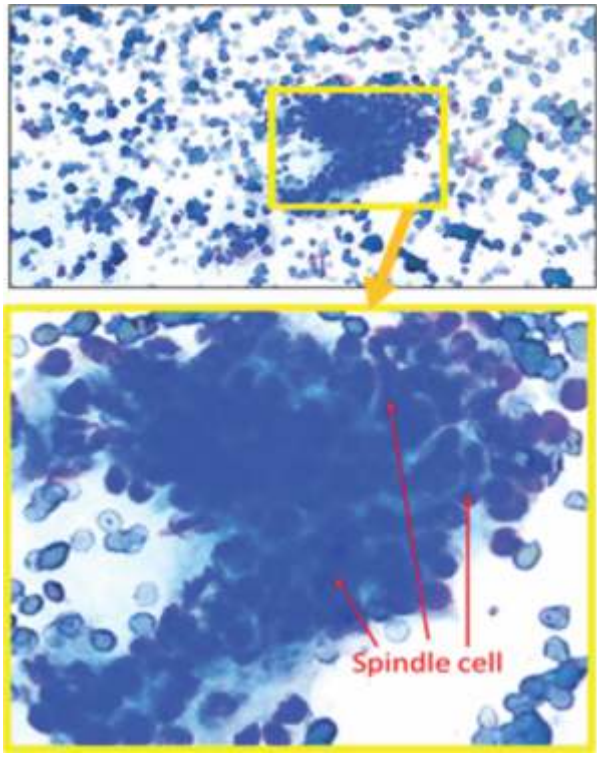

Fig 1. Thigh mass FNA cytology: Spindle cells.

The haemogram parameters **(Table 1)** were WNL but in the blood chemistry panel **(Table 2)** the liver clinical enzyme concentrations were markedly elevated, compared to the Feb. 1, 2022, values. Accordingly, for monitoring the vital internal organs, urgent abdominal survey radiography was arranged. The two- view radiograms revealed hepatomegaly. A roundish opaque area in the caudal portion of liver was clearly discernible **(Fig. 2).** In view of the high risk relating to possible unidentified abdominal tumor(s), the planned thigh mass removal surgery was cancelled.

November 3, 2022

Referral Ultrasound Report

Mild cystitis pattern subdued chronic renal changes with small left kidney cyst, hepatopathy exhibiting hepatoma-like right to caudate mass lesion, mild splenomegaly with intermittent echogenic non-disruptive nodules, apparently benign.

Table 1. Patient's Haemogram

Parameter (Units)	Value 18.10.22	Value 1.2.22	Reference Interval
TEC (1x106/µL)	6.36	6.96	5.65-8.87
Hematocrit (%)	43.2	46.9	37.3-61.7
Hb (g/dL)	15.2	16.2	13.1-20.5
MCV (fL)	67.9	67.4	61.6-73.5
MCH (pg)	23.9	23.3	21.2-25.9
MCHC (%)	35.2	34.5	32.0-37.9
RDW (%)	14.9	15.2	13.8-21.7
Platelet (1x103/µL)	52.8	29.2	10.0- 110.0
Reticulocyte Hb (pg)	25.1	24.3	22.3- 29.6
TLC(1x 103/µL)	8.8	6.58	5.05-16.76
Neutrophil (%)	62.2	54.4	
Lymphocyte (%)	20.6	26.1	
Monocyte (%)	8.0	11.2	
Eosinophil (%)	9.0	8.1	
Basophil (%)	0.2	0.2	
Neutrophil (1x103/µL)	5.48	3.58	2.95-11.64
Lymphocyte (1x103/µL)	1.81	1.72	1.05-5.10
Monocyte (1x103/µL)	0.7	0.74	0.16-1.12
Eosinophil (1x103/µL)	0.79	0.53	0.06-1.23
Basophil (1x103/µL)	0.02	0.01	0.00-0.10
Platelet (1x103/ µL)	341	294	148-484

Auto CBC Analyzer

Table 2. Blood Chemistry Panel

Parameter (Units)	Value 18.10.22	Value 1.2.22	Reference Interval
Glucose (mg/dL)	94	104	74-143
SDMA (µg/dL)	-	8	0-14
Creatinine (mg/dL)	0.6	1.0	0.5-1.8

BUN (mg/dL)	13	14	7-27
BUN/ Creatinine ratio	21	14	
Phosphorus (mg/dL)	-	3.1	2.5-6.8
Calcium (mg/dL)	-	10.1	7.9-12.0
Sodium (mmol/L)	-	154	144-160
Potassium (mmol/L)	-	3.9	3.5-5.8
Na: K Ratio	-	39	
Chloride (mmol/L)	-	115	109-122
Total protein (g/dL)	6.7	6.8	5.2-8.2
Albumin (g/dL)	3.7	3.5	2.3-4.0
Globulin (g/dL)	2.9	3.2	2.5-4.5
A/G ratio	1.3	1.1	
ALT (U/L)	159 (H)	142 (H)	10-125
ALP (U/L)	1581 (H)	521 (H)	23-212
GGT (U/L)	-	3	0-11
Bilirubin (mg/dL)	-	0.2	0.0-0.9
Cholesterol (mg/dL)	-	191	110-320
Amylase (U/L)	-	611	500-1500
Lipase (U/L)	-	825	200-1800
Total T4 (µg/dL)	-	0.9 (L)	1.0-4.0

Blood Chemistry Autoanalyzer H=High; L=Low

Ultrasound guided biopsy, FNA slides referred to IDEXX Lab (September 22, 2022).

Referral Biopsy-Cytology Report

Liver

Slides of low cell density and mild to moderate blood contamination reveal clusters of hepatocytes against a basophilic background with low numbers of mast cells. Inflammatory leukocytes are clearly discernible. Hepatocytes with distinct cell borders exhibit round to oval nuclei with finely stippled chromatin and a single small, prominent nucleolus, basophilic granular cytoplasm moderately abundant Mast cells with distinct cell borders show round to oval nuclei with finely stippled chromatin and indistinct nucleoli. The cytoplasm is moderately abundant, basophilic with high numbers of metachromatic granules **(Fig.2)**. Pathologist's interpretation: Mastocytosis.

Spleen

Slides reveal low to moderate cell density with hemodilution. Lymphocytes, primarily of intermediate size with mildly dispersed chromatin and expanded basophilic cytoplasm are seen in large numbers, admixed with red blood cell precursors and occasional megakaryocytes against a basophilic background with scattered splenic stroma particles. Fewer small and large lymphocytes, low numbers of plasma cells are also seen. No infectious organisms, or significant populations of cells displaying criteria of malignancy are observed **(Fig.4)**. Pathologist's interpretation: Expanded population of intermediate Lymphocytes.

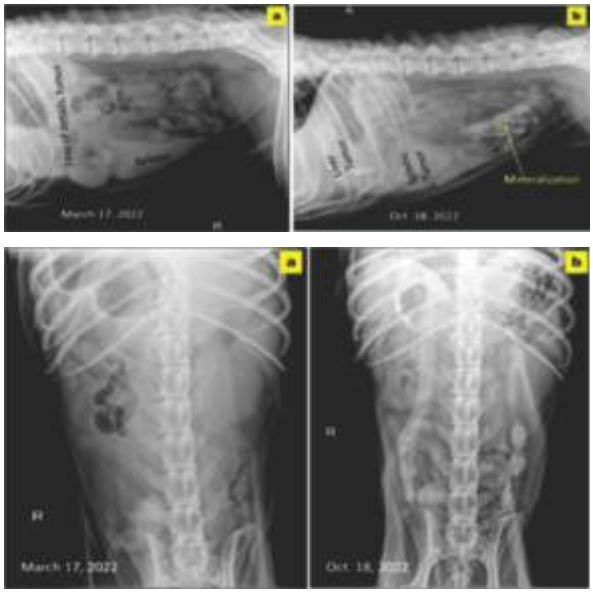

Fig. 2. Abdominal Survey Radiograph (R/L-above, V/D-below): mass in liver and spleen.

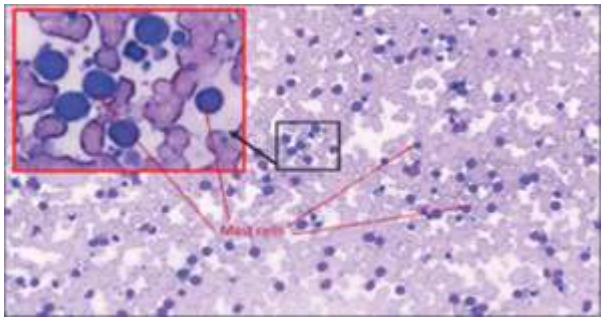

Fig. 3. Liver FNA Cytology: prominent Mast cells.

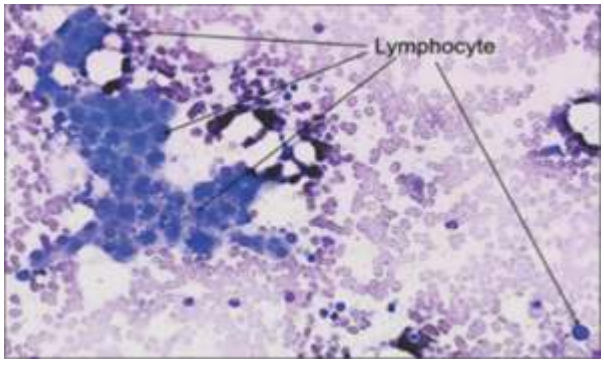

Fig. 4. Spleen FNA Cytology: Lymphocytes aggregates.

Discussion

In an earlier published report (10) we have described multicentric canine lymphoma with regional involvement of multiple mesenteric lymph nodes conforming to the WHO classification, grade

II in a female spayed dog. This communication documents a unique consortium of ongoing high-risk hepatic mast cell tumor and splenic lymphoma (both of uncertain time of onset and duration), noticed developing progressively; subcutaneous spindle cell sarcoma in caudal right thigh muscle area, and innocuous scattered belly surface lipomas in an aging companion dog. Judicious use of diagnostic imaging tool, radiography and evaluation of the status of the vital internal organs from the haemato-biochemical profile, combined with clinical judgment of the individual case, play a key role in successful medical/ surgical treatment.

In the instant case, the focus was on the complete surgical excision of a subcutaneous growth in the patient's right caudal thigh muscle region. However, increased pain perception on abdominal palpation raised serious clinical concerns relating to some undetected growth, possibly a tumor in some abdominal internal organ. Further, pre-anaesthesia blood chemistry panel revealed markedly enhanced circulatory titres of hepatic function test parameters: clinical enzymes, and urgent abdominal survey radiographs revealed a prominent round radiopaque patch, highly suggestive of tumor in the liver. Scheduled thigh mass removal surgery was cancelled forthwith. This decision is supported by the pathologist's cytology report on the referral ultrasound-guided biopsy FNA samples: hepatic mast cell tumor concomitant with splenic lymphoma. Further, suspicion of subcutaneous spindle cell sarcoma in the specified thigh region of the patient, based on the in-house FNA sample cytology profile was validated by the pathology expert's evidence-based report. In an earlier routine health check visit to the home clinic, physical examination revealed total eight cutaneous lipomas (fatty tumors) of varying size and location in the ventral abdomen. These lipomas are benign and usually do not need any treatment.

The field veterinarians globally need to be constantly updated with new developments in the result-oriented management of a plethora of diseases in the companion animals. In perspective, it is pertinent to put on record that the United States of America Food and Drug Administration (FDA) has recently approved the use of intratumor injection of Stelfonta® (tigilanol tiglate) from Virbac (14) in the cancer therapy of companion animals. The pharmacokinetic involves time-bound lysis of the specified cutaneous tumors, e.g., CMCTs which can be easily monitored under clinical surveillance.

The mandate includes proper case selection, pet owner's proper education and strict compliance of the four-stage treatment schedule.

Stage 1 oncomitant medications: prednisolone, or prednisone, H1 blocker (diphenylamine), and H2 blocker (famotidine) x 7 days, as per the specified guidelines.

Stage 2 Stelfonta intratumor injection: Properly calculated dose and well-distributed trajectories of the injection sites, consistent with the geometry of the mass.

Stage 3 Acute inflammatory response and tumor destruction (through haemorrhagic necrobiosis) in 4-7 days, and the open wound healing between 4-6 weeks.

Stage 4 Tumor site healing by second intention. Milford Veterinary Clinic is planning to provide this advanced anti-cancer medical treatment in the companion animals as per the protocol, for the cutaneous Mast Cell Tumors.

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Critical Ethylene Glycol Toxicity in Dog: A Case Report

Sabita Rakshit, Dale Clark, Kabita Roy and I C Datta
Milford Veterinary Clinic, 110 Canal Street, Milford MI, USA
College of Veterinary Science, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

Abstract

A neutered male Labrador Retriever was presented in the Milford Veterinary Clinic with the complaint of seizures, gasping, foaming in the mouth, recumbent, not able to walk and vomiting episodes. The owner, residing in his farm house, was not aware of ingestion of any non-edible hazardous item by his pet, who probably may have sneaked into the neighbor's farm and ingested something from the farm equipment, tractors and other vehicles. From the symptoms, this was a typical case of accidental poisoning with anti-freeze (ethylene glycol), and the top priority was controlling seizures for in-house laboratory diagnostics viz. blood work and thoraco-abdominal survey radiography. The CBC profile indicated heavy bacterial infection, and the blood chemistry panel pointed to kidney dysfunction, pancreatitis, tissue dehydration, and osmotic imbalance. Thoracic radiographs revealed signs of aspiration pneumonitis and abdominal images suggested renal hypertrophy and pancreatitis. Palliative treatment was initiated and referral to 24x7 Emergency Care was advised. However, because of poor prognosis the well-informed owner opted for euthanasia for this pet.

Introduction

Ethylene glycol (EG) toxicity in the companion dogs, resulting from accidental ingestion of products with as high as 95% of the chemical in antifreeze/ engine coolants is a multi-faceted systemic clinical syndrome (1-3). In the USA, EG toxicity is a common cause of poisoning in the companion animals; mortality rate 59-70% in dogs and 96-100% in cats (4,5), when antifreeze preparations are sprinkled on vehicles and several home appliances, toilet winterizing treatments (6). Experimental evidence has established the minimum toxic dose of purified EG in dogs 2.5 ml/kg, and the lethal dose 6.6 ml/kg body weight. Signs of EG toxicosis are manifested clinically when the circulatory titre exceeds 50 mg/dL (2).

On presentation in the clinic, history of ingestion of antifreeze EG preparation may be reported.

Stage 1 Clinical signs of toxicosis may include CNS depression, disorientation, ataxia, nausea, vomiting, diarrhea, polyuria and polydipsia, nystagmus, knuckling, seizures, muscle fasciculations, and coma (3). Notably, coma and/or seizures may supervene only with ingestion of large quantity of the toxic agent.

Stage 2 CNS symptoms subside and some dogs appear deceptively normal. However, with progressive metabolic acidosis, marked CNS depression, dehydration, tachypnea, tachycardia, muscle fasciculations, hypothermia, miosis, and coma and/ seizures may occur. Pulmonary edema and/or effusions may also be noticed (1-4, 7, 8).

Stage 3 Accentuated toxicosis is evidenced by oliguria, or anuria (renal failure), lethargy, nausea, anorexia, oral ulceration, painful distended abdomen, hypersalivation, vomiting, diarrhea, peritoneal/ pleural effusion, seizures and coma (9,10). Sudden death may occur at any stage (1-4). No gender or age predisposition is reported. Smaller breeds are potentially more vulnerable to EG toxicity (5).

Case Description

A 5-year-old, 25 kg body weight neutered male Labrador Retriever dog was presented in the Milford Veterinary Clinic on February 13, 2023 in the early morning hours with the complaint of seizure bouts, gasping, lameness, abdominal distension and vomiting episodes. The patient was foaming in the mouth, violent seizures and paddling. Anamnesis: The owner residing in his farm house was apprehensive of some toxic substance gulped by his companion animal in the neighbor's farm. Signalment: a typical case of.antifreeze (ethylene glycol) poisoning (1-4).

Diagnostics

I. Hemato-biochemical profile

Table 1. Patient's hemogram 13.2.2023.

Parameter (Units)	Result	Normal Range	Status
TEC (1x106/µl)	9.02	5.65-8.87	High
Hb(g/dl)	20.5	13.1-20.5	
HCT (%)	64.7	37.3-61.7	High
MCV (fl)	71.7	61.6-73.5	
MCH (pg)	22.7	21.2-25.9	
MCHC g/dl)	31.7	32.0-37.9	Low

RDW (%)	19.7	13.6-21.7	
Reticulocyte (1x103/µl)	165.1	10.0-110	High
Reticulocyte (%)	1.8		
Retic-Hb(pg)	26.4	22.3-29.6	
TLC (1x103/µl)	24.22	5.05-16.8	High
Neutrophil (%)	81.7		
Lymphocyte %)	10.1		
Eosinophil (%)	1.4		
Monocyte (%)	6.5		
Basophil (%)	0.3		
Neutrophil (1x103/µl)	19.77	2.95-11.6	High
Eosinophil(1x103/µl)	0.34	0.06-1.23	
Lymphocyte (1x103/µl)	2.45	1.05-5.10	
Monocyte (1x103/µl)	1.58	0.16-1.12	High
Basophil (1x103/µl)	0.08	0- 0.10	
Platelet (1x103/µl)	280	148-484	

Auto cell counter H=High L=Low

Table 2. Patient's blood chemistry profile 13.2.2023.

Parameter (Units)	Result	Normal Range	Status
Glucose (mg/dL)	169	70-143	High
SDMA (µg/dL)	38	0-14	High
Creatinine (mg/dL)	10.9	0.5-1.8	High
BUN (mg/dL)	45	7-27	High
BUN/Creatinine ratio	4		
Calcium(mg/dL)	7.0	7.9-12.0	Low
Phosphate(mg/dL)	11.4	2.5-6.8	High
Total protein mg/dL)	8.2	5.2-8.2	
Albumin (g/dL)	5.0	2.2-3.9	High
Globulin(g/dL)	3.2	2.5-4.5	
A/G ratio	1.6		
ALT (U/L)	66	10-125	
ALP ((U/L)	70	23-212	
GGT (U/L)	11	0-11	
Amylase (U/L)	>2500	500-1500	High
Lipase U/L)	4195	200-1600	High
Total bilirubin (mg/dL)	0.5	0-0.9	
Cholesterol (mg/dL)	186	110-320	
Na+ (mmol/L)	168	144-160	High
K+ (mmol/L)	3.7	3.5-5.8	
Cl- (mmol/L)	102	109-122	Low
TT4	0.5	1-4	Low

Analyzer: Catalyst

Diagnosis

The patient was sedated with Midazolam [5mg/ml]; the initial dose 0.5ml, injected IM to permit urgent in-house blood work, urinalysis. The patient started to seize violently and uncontrollably that a booster dose 1.5 ml IV was given, preceding thoraco-abdominal 2 view survey radiography.

Physical Examination

rectal temperature 100.9 0F, heart rate 110 beats/ minute, respiratory rate not determined because of gasping, capillary refill time (CRT) not recorded in

II. Radiography

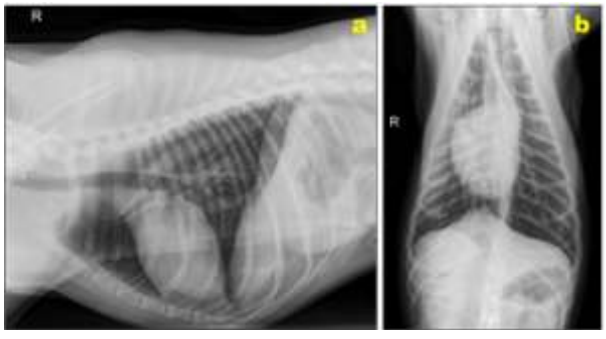

Fig.1a. RL view: Thoracic radiograph
Fig 1b. VD view: Inference: Aspiration Pneumonia

view of foaming and seizures, Visible mucous membranes red; patient apparently dehydrated. Lungs sounds muffled, body condition score (BCS) 3/5.

The in-house thoracic survey radiographs **(Fig. 1a, b)** revealed radiopacity in the lungs, suggesting aspiration pneumonia, supporting the clinical inference from the CBC profile and the physical findings. The abdominal survey radiographs **(Fig. 2a, b)** showed enlargement of the kidney tissue with ongoing renal dysfunction and failure. Subdued opacity in the cranial abdomen indicated pancreatitis. The enlarged kidneys are outlined in **Fig. 3a, b.**

Since the radiographs provided strong evidence supporting the clinical symptoms of antifreeze

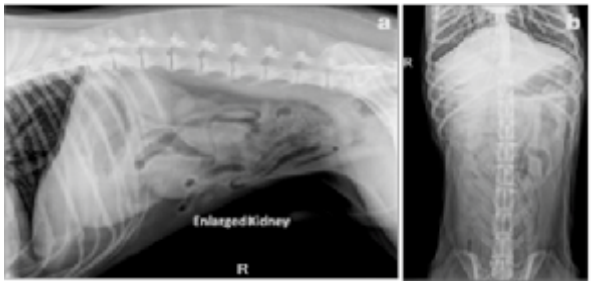

Fig. 2a. RL view: Abdominal radiograph
Fig 2b. VD Enlarged Kidneys

Fig. 3a &b. Abdominal radiograph: Kidneys outlined

(ethylene glycol) poisoning, urine samples for urinalysis were needed to confirm the presence of calcium carbonate crystals. Ultrasound-guided cystocentesis planned, but the bladder was empty most likely due to renal failure.

Treatment

In the clinic, palliative treatment was given: Isosmotic fluid infusion started with the IV catheter to control generalized tissue dehydration. Since the critical condition needed 24x7 care, the owner was advised to shift the patient to the Emergency Referral. However, in view of the high cost and poor prognosis he decided to get the pet euthanized, conforming to the norms.

Discussion

Critical appraisal of the carefully scanned published reports on the major clinical signs of antifreeze ethylene glycol chemical toxicity in dogs (1,2,3,4): CNS depression, lethargy, anorexia, disorientation, ataxia, suppressed righting reflexes, Muscle fasciculation, seizures, tachypnea, dyspnea, hypersalivation, nausea, vomiting, abdominal pain, renal dysfunction resulting oliguria/ anuria, hypothermia were fully present in the case under report.

Evaluation of the pathoclinical significance of the patient's complete blood count **(Table 1)** revealed marked hemoconcentration, evidenced by the high values of total erythrocyte count and packed cell volume, associated with generalized tissue dehydration. Increased reticulocyte count pointed towards anemia, attributable mainly to kidney failure, as kidneys are also a source of erythropoiesis, apart from the bone marrow. Leukocytosis resulting from neutrophilia and monocytosis is related to advanced microbial infection from aspiration pneumonia, in the immune-suppressed companion dog.

The blood chemistry panel **(Table 2)** is highly relevant in the context of the patient's multi-dimensional deranged intermediary metabolism in EG toxicity. Thus, hyperglycemia is evident, presumably because of impaired hepatic function. Renal function test parameters: notably increased values of symmetric dimethyl arginase (SDMA), a biomarker of impaired glomerular filtration rate (GFR) with concomitantly increased creatinine and blood urea nitrogen (BUN) values clearly indicate massive kidney damage. Cell water homeostasis is also adversely affected. This contention is corroborated by hypernatremia with borderline hypokalemia, and grossly increased serum albumin concentration. Hypochloremia points to increased anion gap from excessive vomiting and subsequent loss of chloride (stomach content has Hydrochloric acid). In the home clinic, only palliative treatment was given with IV infusion of balanced isosmotic solution and sedative to control the seizures. Remedial therapy in advanced **(stage 3)** EG toxicity mandated 24x7 Emergency Care. However, the well-informed owner opted for the pet's euthanasia.

Preventive Measures

1. Regularly check the vehicles, snow blowers and other appliances in the regions with sub-zero weather conditions in winter.
2. Prevent leakage of ethylene glycol products. Use sealed containers.
3. Ensure no entry of pets to car washing area.
4. Adopt ecofriendly antifreeze products that do not contain ethylene glycol.

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Modified Robert Jones Bandaging for the Hindlimb in Male Dogs

Nikita Gupta and Vandana Sangwan
Dept. of Vet. Surgery and Radiology, College of Vet. Science, GADVASU, Ludhiana, Punjab

Abstract

The article explains the technique of modified Robert Jones bandaging for the hind limb (femur, tibia or hip) in dogs. The proximal bones like hip and femur and sometimes tibia in heavy dogs require different type of bandaging. Bandaging of these bones is required as a first-aid for fractures and dislocations, as a post-operative protection for incision site, or for oedema and pain management. Since, femur fracture is the most common fracture encountered in dogs, its bandaging is of utmost importance. The bandaging of these bones is specialized and if proper care is not taken, may lead to complications of limb swelling, scrotal oedema, obstruction to faeces and urination.

Introduction

Femur fractures are most commonly encountered long bone fractures in dogs. In large breeds, the fracture is mostly comminuted or oblique/spiral and the sharp fragments usually pierce the muscles, leading to severe haemorrhage and limb oedema. While, in small dogs or puppies, they may pierce out of the skin due to less muscle mass. Initial heavy Modified Robert Jones bandaging (MRJB) of femur is very helpful in ceasing the internal bleeding and preventing oedema and pain. If oedema is already present, the bandage should be done up to the paw or up to the point of oedema, so that it can be resorbed (1).

Indications:

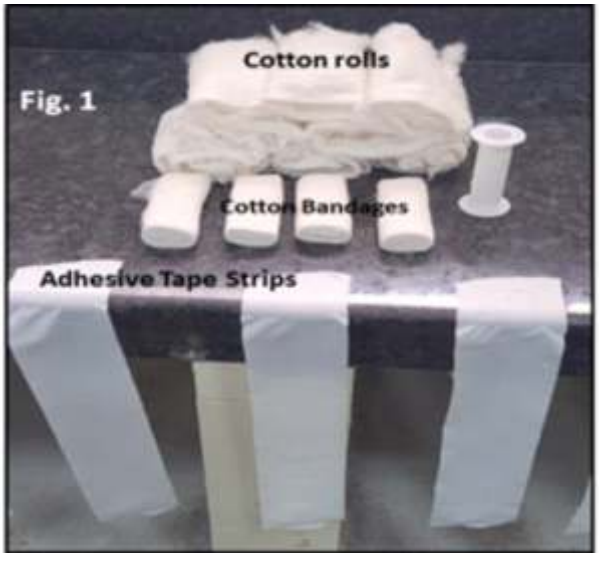
1. Femur fracture
2. Stifle surgery
3. Femur head ostectomy
4. Lacerations or growth removal in this region

Materials required

1. Cotton (100gms for ≤ 8 kgs dogs, 200gms for ≤ 20 kgs dogs)
2. Cotton Bandages (2" for toy breeds, 4" for ≤ 10 Kgs dog and 6" for > 10kgs dogs)
3. Adhesive Cloth tape (4 or 6 inches)
4. PVC (poly vinyl chloride) splint of length from stifle to hock may be applied medially to tibia if MRJB is done for tibia fracture as well. The splint should be little wider than the limb in tibia region and should be strong enough to not bend.

Preparations (Fig. 1)

1. Make 6/4/2 inch cotton rolls (5-6) as per the size of the dog and keep aside. The thickness of the roll should be half to one inch depending on the size of the dog.



2. Take out appropriate sized bandages out of the wrapper (usually 2 required) as mentioned above. If 2" bandages are not available, the 4" can be cut into halves with a scalpel blade.
3. Take out strips of adhesive tape (5-6 in numbers) with the open end of tape adhered to some slab or table. The length of the strip varies from 8" to 20" depending on the size of the dog. The length should be such that at least one roll is completed with one strip. For toy breeds, make longitudinal halves of the 4" strip if 2" tape is not available.

Restraint of Dog for Bandaging (Fig. 2)

1. The dog is restrained in the lateral recumbency with the affected limb kept upwards and while standing, the affected side is oriented towards the doctor. The owner is allowed to stand on the other side.
2. The dog is muzzled and the owner is allowed to hold the head of the dog.

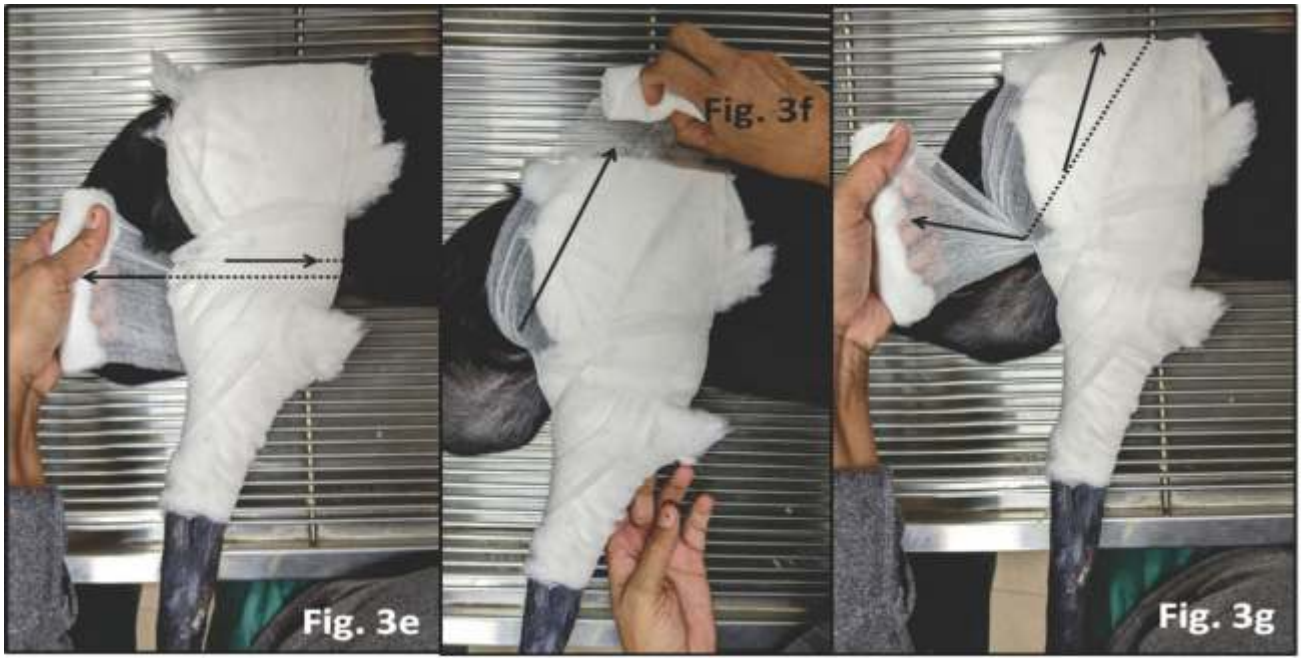
3. The doctor holds the fore and unaffected hind limb of the dog which is on his/her far side and pulls them simultaneously on its side to make the dog lie down on the table.
4. The owner will keep the head pressed with one hand (white star) and can also hold the lower or both forelimbs with the other hand. The elbow of the owner may be allowed to press on the dog scapula region at this point.
5. The affected hindlimb which faces upwards is kept free for bandaging at this point.
6. One person is required to hold the lower hindlimb from the paw which is to be pulled caudally. The same person also holds the affected hindlimb paw with other hand and pulls it straight at the level to body (Black arrow). The person doing bandaging will stand on the right of this person.
7. If required, the chest of the dog can be lightly pressed.

Technique of Bandaging

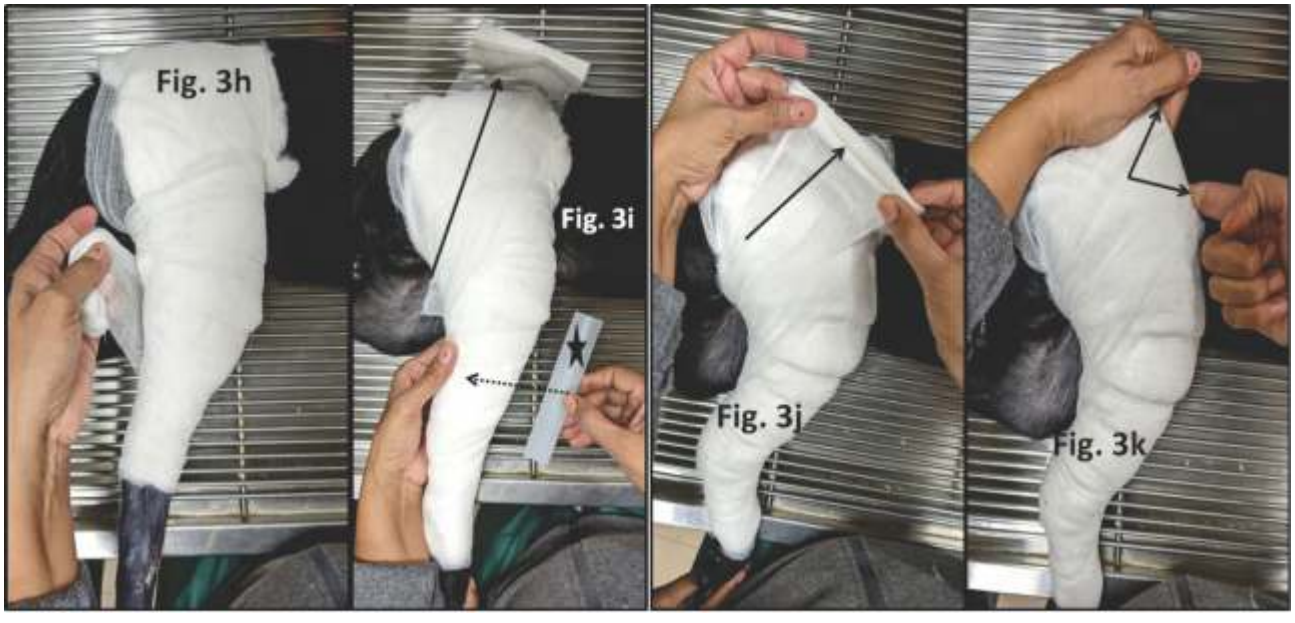
The premade cotton rolls are wrapped around the part to be bandaged in an oblique overlapping manner as shown in Fig. 3a. For, hip joint, bandaging till proximal tibia may be done and for distal femur, bandaging may include hock. The rolls should be wrapped from down to up or vice-versa on the limb.

The bandaging of the limb is started from the lowermost part (black star) and the bandage is unrolled over the region distal to stifle, which is then taken over the hip (Fig. 3c, black arrow) and is again brought in front (Fig. 3c, black dotted arrow) and then taken to the caudal aspect of the stifle joint region from underneath the affected limb (Fig. 3d, black dotted and bold arrow).

The first layer of bandage can be applied lightly and is just to keep the cotton in place. In males the bandage cannot be rolled from the front as it will press the prepuce. Never take back the bandage over the hip directly. Always take one or two rounds on the stifle or distal femur region and then take it over the body. This will prevent tourniquet on the scrotum and hence, scrotal oedema. While wrapping the bandage, always apply pressure on the proximal aspect of the bandage and the distal should be left free, this will help in preventing the tourniquet effect due to bandaging, therefore preventing limb oedema.



The bandage is rolled over the stifle or distally at least once (Fig. 3e) before taking is again over the hip (Fig. 3f). This process may be repeated to make a clean pressure on the affected part and compressing all the cotton with each wrap (Fig. 3g).



The splint may be incorporated in the bandage at this stage, if required for tibia support (medially, Black star, Fig. 3i). The last part of the bandage is embedded underneath the upper cranial or caudal region of hip (Fig. 3j, black arrow and Fig. 3k).

The first adhesive tape strip is pasted from the caudal aspect i.e dorsal to the hip, coming ventrally and cranially below the stifle (Black arrow, Fig. 3l). The caudal hair may be included in it. The second strip is pasted from the cranial aspect to the ventral aspect of stifle and underneath (Fig. 3m). The 3rd strip is pasted obliquely from caudal to cranial and under the limb at the level of stifle and coming back to the front (Fig. 3n). In similar fashion whole bandage is wrapped in adhesive tape.

IMPORTANT INSTRUCTIONS FOR OWNERS

1. Keep bandage clean and dry.
2. The limbs can be covered with plastic coverings while going out for walks to prevent it from getting soiled. These coverings should be removed within half an hour to prevent excessive accumulation of moisture.
3. The bandaged limb should be observed for any swelling. In case there is swelling on the toes, owner is advised to press the toes frequently to reduce the swelling. Alternatively, an inch of bandage can be cut from most distal site to slightly loosen the bandage, which will eventually reduce the swelling. If swelling is not yet resolved, bandage has to be reapplied.
4. Evaluate the bandage for slippage. Observe patient for any discomfort or pain, indicated by excessive licking or chewing the bandage.
5. Thin skinned dogs like Greyhound are more prone to ischaemic necrosis of the limb due to tight bandaging and may be seen as change in colouration of the limb and other gangrenous changes (2).

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Cutaneous Mast Cell Tumor in Dog: Treatment with Intratumoral Injection of tigilanol tiglate

Sabita Rakshit, Dale Clark, Kabita Roy and I C Datta
Milford Veterinary Clinic, 110 Canal Street, Milford, MI, USA College of Veterinary Science (NDVSU), Jabalpur, Madhya Pradesh

Abstract

A male neutered Pitbull dog (6-year old, 30 kg body weight) was presented in the Milford Veterinary Clinic for scheduled vaccinations. The attending physician was informed by the owner, the patient's history of licking the groin area often, and drinking excessive quantities of water. Nearly a week ago a bump on the tummy was also noticed. In the instant case, use of Stelfonta® (tigilanol tiglate) was recommended. Skeptical owner was educated but he refrained from the treatment. Food Drug Administration (FDA) has approved the use of Stelfonta directly at the Cutaneous Mast Cell Tumor (CMCT) site subcutaneously. It activates a protein that spreads throughout the treated tumor, which disintegrates the tumor cells.

Introduction

Cutaneous Mast Cell tumors (CMCTs) often appearing as raised, erythromatous, alopecic masses are the common malignant skin neoplasms in dogs, globally. These may also develop in various benign or invasive clinical presentations in different body parts. Though there is no established genetic predisposition, the over represented breeds include Boxer, Bulldog, Golden Retriever, Labrador Retriever, and Shar Pei (1, 2). In perspective, better understanding of the various treatment options viz. surgical excision (3, 4, 5), radiation therapy, or a combination of both will enable the pet practitioner to educate and guide the owner on the most appropriate treatment option of the individual cases (6, 7, 8, 9, 10, 11). Successful medical management of non-resectable mast cell tumors with combination adjuvant therapies is evidenced by high quality of life and long survival time in the family dog (12, 13, 14, 15, 16).

Case Description

A six years old, 30 kg body weight, neutered Pitbull dog was presented in the Milford Veterinary Clinic for vaccines update. Anamnesis revealed that the pet was seen licking the groin area and had signs of polydipsia. A bump was noticed on the belly about a week ago. A single dark red and ulcerated lick granuloma on the skin surface in the groin area was exhibited. No prior history of allergies or dermatitis.

Physical Examination

Rectal temperature 102.3^{0}F, heart rate 96 beats/ minute, respiratory rate, pant, capillary refill time (CRT) <2 seconds, visible mucous membranes pale pink, body condition score (BCS) 3/5. A raisin-sized growth was noticed on the left lateral preputial area ~0.5cm × 0.5 cm, dark red in color **(Fig. 1).** Fine needle aspirate (FNA) cytology revealed dark blue roundish and oval shaped cells, densely pigmented suggestive of mast cell tumor **(Fig. 2).**

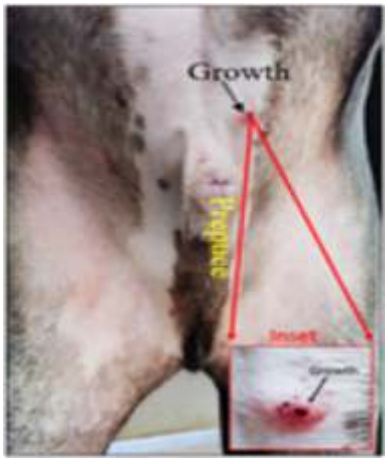

Fig. 1: Growth near the preputial area

I. Hematobiochemical profile

Table 1. Patient's hemogram

Parameter (Units)	Result	Reference Interval	Status
TEC (1x106/ µL)	8.52	5.65-8.87	N
Haemoglobin (g/dL)	19.8	13.1-20.5	N
HCT (%)	58.4	37.3-61.7	N
MCV (fL)	68.5	61.6-73.5	N
MCH (pg)	23.2	21.2-25.9	N
MCHC (g/ dL)	33.9	32.0-37.9	N
RDW (%)	18.9	13.6-21.7	N
Reticulocyte(1x 103/µL)	34.1	10-110	N
Reticulocyte (%)	0.4		N
Reticulocyte-Hb (pg)	24.2	22.3-29.6	N

TLC (1x 103/ µL)	7.02	5.1-16.8	N
Neutrophil (1x 103µL)	4.96	2.95-11.6	N
Lymphocyte (1x 103µL)	1.57	1.05-5.10	N
Monocyte (1x 103µL)	0.22	0.16-1.12	N
Eosinophil (1x 103µL)	0.26	0.06-1.23	N
Basophil (1x 103µL)	0.01	0-0.1	N
Thrombocyte (1x 103µL)	231	148-484	N

***Auto cell counter N= Normal value**

Table 2. Patient's Blood Chemistry Profile

Parameter (Units)	Result	Reference Interval	Status
Glucose (mg/ dL)	110	70-103	N
SDMA (µg/ dL)	12	0-14	N
Creatinine (mg/ dL)	1.1	0.5-1.8	N
BUN (mg/ dL)	9	7-27	N
BUN/Creatinine ratio	9		
Calcium (mg/ dL)	10.1	7.9-12.0	N
Phosphate (mg/ dL)	3.5	2.5-6.8	N
Total protein (g/dL)	6.7	5.2-8.2	N
Albumin (g/ dL)	3.5	2.2-3.9	N
Globulin (g/ dL)	3.2	2.5-4.5	N
A/G ratio	1.1		
ALT (U/L)	40	10-125	N
ALP (U/L)	60	23-212	N
GGT (U/L)	0	0-11	N
Amylase (U/L)	621	500-1500	N
Lipase (U/L)	2226	200-1800	H
Total bilirubin (mg/ dL)	0.2	0-0.9	N
Cholesterol (mg/ dL)	274	110-320	N
Na+ mmol/L)	153	144-160	N
K+ (mmol/ L)	4.5	3.5-5.8	N
Cl- (mmol/L)	111	109-122	N
TT4	1.3	1.0-4.0	N

****Analyzer Catalyst N= Normal value**

This was corroborated by the report on a parallel sample referred to the regional IDEXX Laboratory.

Canine Influenza, Bordetella, DA2PP (distemper, adenovirus, parvo and parainfluenza), Leptospirosis vaccines were given. Heartworm, and tick panel (Ehrlichiosis, Anaplasmosis and Lyme disease) tested negative.

II. Coprology The fecal sample tested negative for endoparasites, cysts or ova but positive for the Giardia antigen. Panacur was used to clear up the infection.

III. FNA Cytology:

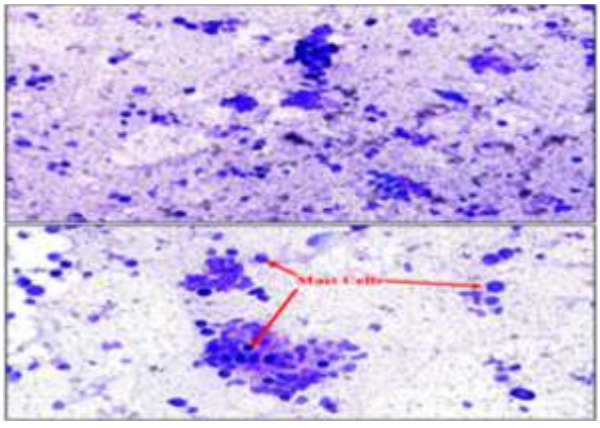

Fig. 2: FNA Cytology: Mast cell tumour

Discussion

Critical appraisal of the pertinent published reports revealed that Mast Cell Tumors (MCTs) are the most common skin tumors in dogs, globally. These are usually present as growths of varying size on or under the skin (1,2). Complete surgical mass removal may be very challenging when the tumors are located in certain body parts like the leg. Most importantly, if the mass is not removed completely with safe margins all round, the left over malignant cells may multiply and spread rapidly. On November 16, 2020, the United States Food and Drug Administration (FDA) approved Stelfonta® (tigilanol tiglate) QBiotics Group Ltd., and Virbac, as a prescription intratumoral injectable drug for treatment of dogs with non-metastatic cutaneous MCTs, and subcutaneously only in the specified areas in the leg. This novel treatment option is a good news for the pet practitioners on the judicious use of tigilanol tiglate with all mandatory biosafety precautions and client education. Tigilanol tiglate is a biologically active pharmaceutical compound extracted from the seed of the native Australian blushwood (Fontainea prosper) tree found in the North Queensland rainforest. Tigilanol tiglate was discovered and developed in Australia and has been extensively researched over many years. Stelfonta, injected directly into the tumor mass, activates a protein that spreads and disintegrates the tumor cells. The Veterinarian administering Stelfonta has to undergo safety training because of risks involved of accidentally self-injection. The drug is given with a corticosteroid and H1 and H2 receptor blocking agent to decrease the risk of severe adverse reactions, including death, from mast cell degranulation. Wound formation can happen at the injection site, including cellulitis and tissue sloughing, but most wounds heal gradually and the tumor shrinks. Tumor destruction and healing typically happens between 4-6 weeks. This is a highly innovative additional treatment option. In

the case under report, the well-informed owner is still skeptical to go forward with the novel treatment and prefers to wait and watch at this point of time.

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Pathobiology of Canine and Feline Cirrhosis: An Overview

Rashmi Choudhary, Supriya Shukla, Danveer S. Yadav, Nidhi S. Choudhary and Jyotsana Shakkarpude
College of Veterinary Science and A.H., Mhow, Madhya Pradesh

Abstract

Long-term exposure of liver with the damaging agents causes progressive damage, parenchymal alterations and vascular architectural distortion which eventually results in liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma which is the end-stage of most chronic liver diseases. In dogs, chronic liver disease tends to progress to an eventual outcome of severe fibrosis or cirrhosis while in cats progressive disease rarely results in the same type of end stage changes. The parenchyma is usually affected secondarily to systemic illnesses in cats while the biliary system is the prime target for infectious agents (eg, bacteria and flukes) and non-infectious conditions (e.g., neoplasia and cysts). In a fibrotic liver the total collagen content is 3- to 10- fold higher than normal. It leads to shunting of the portal and arterial blood supply directly into the hepatic outflow (central veins) compromising exchange between hepatic sinusoids and the adjacent liver parenchymal hepatocytes. Many types of cells, cytokines and miRNAs are involved in the initiation and progression of liver fibrosis. The main complications of cirrhosis are portal hypertension, acquired portal systemic shunting, ascites, hepatic encephalopathy, gastrointestinal ulceration, bacterial infections and renal failure. Hepatocyte derived serum microRNA biomarkers hold promise for distinguishing among several hepatobiliary diseases in dogs.

Introduction

The liver shows an unique regenerative response to injuries produced by physical or toxic agents which induce tissue damage. Long-term exposure of liver with the damaging agents causes progressive damage, parenchymal alterations and vascular architectural distortion which eventually results in liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma which is the end-stage of most chronic liver diseases (1). Chronic hepatitis is recognized and well-documented liver disorder in canines. Cirrhosis is the end stage of chronic hepatitis and is defined as a diffuse process characterized by fibrosis of the liver and the conversion of normal liver architecture into structurally abnormal nodules, micro- or macronodular (2). It is considered irreversible, although the point at which this happens is not well-defined. In dogs, chronic liver disease tends to progress to an eventual outcome of severe fibrosis or cirrhosis while in cats progressive disease rarely results in the same type of end stage changes (3).

Cirrhosis is an irreversible change and usually idiopathic in origin. It is a regularly diagnosed condition in dogs and is less frequently encountered in cats (4). It can occur in dogs of any age but is most common in middle-aged or older dogs (range 7 months to 16 years) i.e., specific age and gender predispositions exist for individual breeds. Some breeds such as cocker spaniels, Doberman pinschers, and Labrador retrievers are more commonly affected (5). Diseases of the biliary tree and gallbladder are more common in cats than diseases of the liver parenchyma. The parenchyma is usually affected secondarily to systemic illnesses in cats while the biliary system is the prime target for infectious agents (eg, bacteria and flukes) and non-infectious conditions (e.g., neoplasia and cysts) (6).

Cirrhosis is much less common in cats and diffuse hepatic fibrosis usually represents chronic biliary disease. The feline liver constitutes approximately 3 to 4% of total body weight. Parenchymal liver diseases of feline include lipidosis, amyloidosis and feline infectious peritonitis. In these diseases the liver parenchyma is not the primary target but is affected as part of the systemic disease. Inflammatory bile duct disease is amongst the most common hepatopathies in cats. Biliary cirrhosis in cats is associated with hepatomegaly in which the liver surface become firm and variable nodular appearance. Histology reveals marked portal fibrosis and bile duct hyperplasia. It is felt to be a sequela to chronic inflammatory changes in the intrahepatic bile ducts. Biliary cirrhosis is misidentified in cats with ductal plate malformations (a form of polycystic liver disease). Chronic cholangitis in cats can be induced by liver fluke infestation.

Chronic cholangitis associated with liver fluke infestation is regularly observed in cats in endemic areas. Infections are caused by members of the family Opisthorchiidae. These liver flukes require snails and fresh water fish as intermediary hosts. Cats become infected by eating raw fresh water fish in which metacercariae are encysted. Young liver flukes migrate from the small intestines to the liver

via the bile ducts and cause inflammation of the common bile duct and the large extra- and intrahepatic bile ducts. This chronic inflammation results in malformation and irregular dilatation of these ducts and can be fatal if it is left untreated due to a number of complications including liver cancer (7). It is a rare disease because it occurs in outdoor and feral cats that have hunting habit that make them more susceptible to contracting the fluke. Feline cholangitis may be accompanied by pancreatitis, inflammatory bowel disease, and cholecystitis. Lymphocytic cholangitis is one of the most common inflammatory hepatic diseases in cats that affects the biliary tree and progresses slowly into pronounced fibrosis in the portal areas. Chronic inflammation in the bile ducts causes dilatations and strictures and may eventually lead to fibrosis and cirrhosis.

Two morphological categories of cirrhotic liver can be distinguished i.e., micronodular cirrhosis with nodules less than 3mm in size (the size of a normal lobule) and nodules are of same size and macronodular cirrhosis with nodules greater than 3mm in size (up to several cm) and nodules are of different sizes. Micronodular cirrhosis develops from regular and diffuse alterations and fibrosis of the acini whereas macronodular cirrhosis develops from irregularly distributed larger areas of necrosis with secondary collapse and scarring and the development of portal-central vascular connections. The World Health Organization (WHO) classification also defines a mixed category in which the nodules are both larger and smaller than 3 mm. Mixed cirrhosis is often found in primary biliary cirrhosis and primary sclerosing cholangitis. Incomplete septal cirrhosis is a highly regressed form of cirrhosis often associated with portal hypertension but normal hepatocellular function. The additional pathogenetic element in cirrhosis is the regenerative hyperplasia of hepatocytes.

This process is usually viewed as an attempt to restore parenchymal integrity but it also contributes to the nodularity and overall architectural disorganization. Two major morphologic types of cirrhosis are recognized, termed micronodular cirrhosis and macronodular cirrhosis. In micronodular cirrhosis, regenerative hepatocyte nodules are scarcely larger than the size of a normal lobular unit of parenchyma, and are separated by thin connective tissue septa. However, the nodules show none of the landmarks observed in the normal liver, such as portal tracts or central veins. Macronodular cirrhosis is characterized by the presence of large irregular nodules that may contain portal tracts and efferent vessels. This form of cirrhosis may result from multilobular necrosis and formation of scars surrounding an area of parenchyma larger than a single lobule. In addition, micronodular cirrhosis can progress to the macronodular form through persistent regeneration and expansion of existing nodules.

In a fibrotic liver, the total collagen content is 3- to 10- fold higher than normal (8). It leads to shunting of the portal and arterial blood supply directly into the hepatic outflow (central veins) compromising exchange between hepatic sinusoids and the adjacent liver parenchyma i.e., hepatocytes. The hepatic sinusoids are lined by fenestrated endothelia which rest on a sheet of permeable connective tissue (the space of Disse) which contains hepatic stellate cells (HSC) and some mononuclear cells. The other side of the space of Disse is lined by hepatocytes which execute most of the known liver functions. In cirrhosis the space of Disse is filled with scar tissue and endothelial fenestrations are lost, a process termed sinusoidal capillarization.

Histologically, cirrhosis is characterized by vascularized fibrotic septa that link portal tracts with each other and with central veins leading to hepatocyte islands that are surrounded by fibrotic septa and which are devoid of a central vein . The major clinical consequences of cirrhosis are impaired hepatocyte (liver) function, an increased intrahepatic resistance (portal hypertension) and the development of hepatocellular carcinoma (HCC). The general circulatory abnormalities in cirrhosis (splanchnic vasodilation, vasoconstriction and hypoperfusion of kidneys, water and salt retention, increased cardiac output) are intimately linked to the hepatic vascular alterations and the resulting portal hypertension.

Some animals may have compensated cirrhotic disease and show no or minor clinical signs while other animals show manifestations of liver failure e.g. hyperbilirubinemia, coagulopathies, edema due to hypoalbuminemia, ascites and hepatoencephalopathy. Splenic dysregulation may occur during liver cirrhosis and frequently accompanied by multiple complications including splenomegaly and hypersplenism following the development of portal hypertension. Portal congestion is widely considered the initial cause of splenomegaly during liver cirrhosis (9).

Causes

- **Chronic Hepatitis (most common cause in dogs):**

  Canine chronic hepatitis (CCH) is a progressive inflammatory disease of unknown etiology. Several infectious and noninfectious causes have been suggested for chronic liver disease such as canine adenovirus type 1 (CAV-1), Leptospira interrogans var. grippotyphosa, canine acidophil cell hepatitis virus, copper

accumulation, drug administration, alpha-1-antitrypsin anomaly, and autoimmunity. CCH has been classified histomorphologically as chronic active hepatitis, chronic progressive hepatitis, and lobular dissecting hepatitis (10). Lobular dissecting hepatitis is a distinct type of chronic hepatitis that typically occurs in young dogs at an average age of 2 years. It has been reported in a number of dog breeds including the Standard Poodle, Rottweiler, German Shepherd, Golden Retriever, and American Cocker Spaniel. This disease has a rapid clinical course and a poor prognosis with a short survival time (11) Lobular dissecting hepatitis is histologically characterized by diffuse infiltration of inflammatory cells and dissection of the lobular parenchyma with reticulin fibers (type III collagen) surrounding single or small groups of hepatocytes with accumulation of abnormal ECM components mainly laminin and fibronectin (12).

- **Exposure to certain drugs:**

  Acetaminophen is well known as a dose-dependent hepatotoxin in dogs and cats. Toxic metabolites of acetaminophen cause oxidative injury to erythrocytes and hepatocytes resulting in methemoglobinemia and hepatic necrosis. Cats are uniquely susceptible to acetaminophen toxicosis because they are less efficient in converting acetaminophen to alternative nontoxic intermediates. Clinical signs in cats may develop after administration of as little as 162.5 mg (1/2 tablet).

  Phenobarbital has been associated with chronic hepatic disease and cirrhosis in dogs. Sulfonamide induced hepatopathies in dogs is associated with lack of genes that express the N-acetylation enzymes, so they cannot detoxify sulfonamides via this major metabolic pathway. Toxicity is thought to be due to P-450 oxidation of sulfonamides to the toxic intermediates, hydroxylamine and nitroso-SMX.

  Lomustine (CCNU) is an oral nitrosourea alkylating agent used for chemotherapy of brain tumors, mast cell tumors, and lymphoma in dogs. An idiosyncratic hepatic reaction has been described in dogs treated with this drug.

- **Mycotoxin contamination in pet food:**

  After ingestion, aflatoxins are absorbed into the circulatory system, then metabolized in the liver by microsomal mixed-function oxidases and cytosolic enzymes. The toxicity of aflatoxins is a result of the formation of the reactive aflatoxin B1 8,9-epoxide which binds covalently to cellular macromolecules such as DNA, RNA, and protein enzymes resulting in damage to liver cells. Binding to these macromolecules results in adduct formation and is thought to ultimately result in hepatocellular damage leading to impaired liver function, bile duct proliferation, bile stasis and liver fibrosis (13).

- Extrahepatic or common bile duct obstruction for more than 6 weeks in dogs or cats results in persistent peribiliary fibrosis, connective tissue bridging between portal tracts, remodelling of the liver with biliary cirrhosis, portal hypertension, and formation of acquired portosystemic shunts (14).

- Copper-storage hepatopathy: The disruption of copper homeostasis can lead to the accumulation of copper and ROS production. Excess ROS promote oxidative stress and consequently induce hepatic necrosis, inflammation and fibrosis (15). It is best characterized in Bedlington Terriers which have a mutation (deletion of exon 2) of the COMMD1 copper transporter gene. Failure to excrete copper into bile leads to chronic hepatitis and eventually cirrhosis and liver failure. Affected dogs develop high liver copper concentrations by 1 yr of age (normal: <400 mcg/g dry liver or 400 ppm) which progressively increase during the first 6 yr of life (values may be > 12,000 ppm) (16).

- Chronic cholangitis associated with liver fluke infestation in cats.

- Right sided heart failure or obstruction of the cranial vena cava leads to increased central venous pressure and passive venous hepatic congestion. Liver perfusion is impaired and ischemia and necrosis occur. Chronically, this can lead to centrilobular fibrosis.

- Liver cirrhosis has many other causes like inherited diseases such as hemochromatosis and Wilson's disease, primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis.

Risk factors

- Breed predisposition (Bedlington Terriers, Chihuahuas, Cocker Spaniels, Doberman Pinschers, Labrador Retrievers, Maltese, Skye Terriers, Springer Spaniels, Standard Poodles, West Highland White Terriers)

- Excessive intake of copper from the diet; accumulation of copper or iron in the liver

- Extrahepatic bile duct obstruction

- Exposure to hepatotoxins (e.g Phenobarbital, NSAID)

Clinico-Hemato-Biochemical Changes

Liver cirrhosis causes clinical and haemo-biochemical alterations in pet animals. Clinically affected animals shows inappetence, halitosis, hematochezia, abdominal distension, weight loss, polyuria, polydipsia, melena, icterus, anemia and jaundice. Hematological changes includes significantly lower levels of hemoglobin, lymphocytes, packed cell volume, mean corpuscular volume, mean corpuscular Hb (MCH) and platelet count while significantly higher levels of total leukocyte count, neutrophils and MCH concentration. Their blood smear examination reveals neutrophilic leukocytosis with the left shift. Biochemical alterations includes significantly lower levels of Glucose, total protein, albumin, A/G ratio and fibrinogen while significantly higher levels of creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, prothrombin time, and APTT(activated partial thromboplastin time).

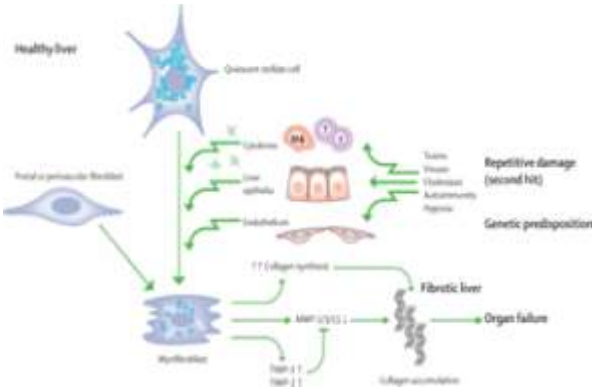
Pathophysiology

Many types of cells, cytokines and miRNAs are involved in the initiation and progression of liver fibrosis. Multiple cell types contribute to the pathogenesis of liver cirrhosis are hepatic parenchymal cells (i.e., hepatocytes) and non parenchymal cells. The walls of hepatic sinusoids are lined by three different non parenchymal cells i.e. liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs) and a multifunctional cell-type known as hepatic stellate cells (HSCs). Quiescent hepatic stellate cells (qHSCs) store vitamin A in lipid droplets but after activation these cells proliferate and progressively lose vitamin A storage and start the deposition of extracellular matrix (ECM) in the injured liver. Damaged hepatocytes release reactive oxygen species (ROS) and fibrogenic mediators which induces activation of HSCs and stimulate the fibrogenic actions of myofibroblasts (17). Upon activation HSCs undergoes trans differentiation and proliferate, acquiring a contractile and fibrogenic myofibroblast-like phenotype during liver injury (18). Activation of hepatic stellate cells (HSCs) is a pivotal event in fibrosis and a major contributor to extracellular matrix (ECM) deposition. Hyaluronic acid (HA) is a main component of the ECM. The HA concentration were found to be significantly increased in advanced liver fibrosis/cirrhosis ($P < 0.001$) and congenital portosystemic shunt ($P < 0.001$) compared to healthy dogs (19). Apoptosis of hepatocytes is a common event in liver injury and contributes to tissue inflammation, fibrogenesis and development of cirrhosis. Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells which mediates the exchange of plasma, nutrients, lipids and lipoproteins between hepatic sinusoids and adjacent hepatocytes through a filtration system that consists of fenestrae or non-diaphragmed pores. However, in pathological conditions their structural and functional features markedly change (20). Liver fibrosis is associated with decreased LSEC fenestration and appearance of an organized basal lamina in the space of Disse, a process called capillarization, which precedes the onset of liver fibrosis (21). Defenestration and capillarization of liver sinusoidal endothelial cells are major contributing factors to hepatic dysfunction in liver cirrhosis. Another key factor for the initiation and progression of fibrosis are resident macrophages i.e. kupffer cells that localize within the lumen of the liver sinusoids, accounting for about 30% of all sinusoidal cells (22). The extracellular matrix in the subendothelial space of Disse mainly consists of collagen type IV, laminin, and proteoglycans that are progressively replaced during fibrosis by collagen type I and III. This excess deposition disrupts the normal architecture of the hepatic lobule

After being exposed to nutrients and gut-derived bacterial products (called pathogen-associated molecular patterns (PAMPs), they sense and remove pathogens and dangerous molecules via pattern-recognition receptors (PRRs). The toll-like receptors (TLRs) are a class of PRRs which recognize gut microbiota-derived bacterial products such as LPS and peptidoglycans. Kupffer cell-derived IL-12 and IL-18 activate hepatic natural killer (NK) cells to increase the synthesis and release of antimicrobial IFN-γ . KCs can be induced into an activated state in which they secrete a wide variety of proinflammatory cytokines such as IL-6, IL-10, IL-13, TNF-α and TGF-β (23). These pro-inflammatory cytokines can induce collagen synthesis in the stellate cells of the liver with subsequent fibrosis and cirrhosis

Extracellular Matrix (ECM) Deposition

In a normal liver collagens IV and VI are present in the space of Disse but after fibrogenesis HSCs begin to proliferate, contract and deposit large amounts of collagen fibers mostly type I III and V. These fibers are usually surrounding around fibroblasts and HSCs in the liver along with nonfibrillary collagens (types IV and VI), glycosaminoglycans and proteoglycans (e.g, fibronectin, laminin and fibromodulin). During ECM accumulation cross-linking of matrix proteins occurs to render the ECM more resistant to degradation. Progressive deposition triggers increased density and stiffness of the ECM that may contribute to the loss of endothelial fenestrations of LSECs and activation of HSCs (24) that further contributes to liver fibrosis (25).



Role of Growth Factors and Cytokines in the Development of Hepatic Fibrosis

Platelet-Derived Growth Factor (PDGF) is the most potent factor that induces proliferation of HSCs among all polypeptide growth factors. It is released mainly by platelets but also by sinusoidal endothelial cells, activated liver resident macrophages and myofibroblasts. PDGF family has four members viz. PDGF-A, -B, -C and -D. PDGF and its receptors are markedly over expressed in fibrous tissues and its activity increases with the degree of liver fibrosis (26). A variety of factors such as viruses, chemicals, or mechanical damage to hepatocytes can induce KCs to synthesize and release PDGF. Upon binding to its specific receptor on the membrane of HSCs PDGF activates corresponding signal molecules and transcription factors leading to the activation of its downstream target genes and activation of HSCs. PDGF has been shown to upregulate the expression of MMP-2, MMP-9 and TIMP-1 and inhibit the activity of collagenase thereby reducing ECM degradation (27). PDGF-D can activate HSCs and exerts mitogenic and fibrogenic effects and plays an important role in matrix remodeling in liver fibrosis (28).

TGF-β is mainly synthesized by HSCs/myofibroblasts, KCs, LSECs, and hepatocytes in the liver. The TGF-β1 family is composed of six members, and among them, TGF-β1 has been shown to play a key role in the initiation and maintenance of liver fibrosis. TGF-β induces epithelial mesenchymal transition (EMT) in hepatocytes and it is responsible for activation of hepatic stellate cells (HSC) to myofibroblasts (MFB) phenotype. MFBs are the principal source of extracellular matrix protein (ECM) accumulation and prominent mediators of fibrogenesis.

TGF-β also mediates an epithelial-mesenchymal transition (EMT) process in hepatocytes that may contribute directly or indirectly to increase the MFB population. MFB are characterized by the expression of α-Smooth Muscle Actin, loss of retinoids and lipid droplets and de novo expression of receptors for mitogenic, fibrogenic and chemotactic factors leading an increase in proliferation and survival enhanced synthesis of matrix proteins (predominantly fibrillar collagens) and inhibitors of matrix degradation TIMPs and secretion of pro-inflammatory cytokines and chemokines. This provokes the progressive scar formation and the development of liver fibrosis.The expression level of TGF-β1 is increased in fibrotic liver and reaches a maximum at cirrhosis. TGF-β1 induces expression of the matrix-producing genes by causing upregulation of collagen types I and III, and TIMP-1, and downregulation of MMPs leading to excessive deposition of collagenous fibers and promoting the development of liver fibrosis (29). TGF-β1 also seems to be an important mediator of lysyl oxidase expression. Lysyl oxidases are copper-dependent amine oxidases that are important for cross-linking of ECM proteins and further activation of myofibroblast precursor cells (30). In addition, TGF-β1 has been shown to inhibit DNA synthesis and induces apoptosis of hepatocytes. TGF-β1-induced apoptosis is thought to be responsible for tissue loss and decrease in liver size seen in cirrhosis (31).

Connective tissue growth factor (CTGF) is another fibrogenic signal for HSCs. CTGF is involved in promoting the adhesion of HSCs to the ECM. 4 Patients with advanced disease showed higher serum concentrations of CTGF, and these were linked to stage of fibrosis. CTGF mRNA expression was shown to be upregulated in dogs with chronic hepatitis.

Endothelin-1 is a vasoactive peptide produced by endothelial cells and by activated HSCs in cirrhotic liver. ET-1 acts through 2 receptors: ET-1 receptor type A and ET-1 receptor type B, which can be found on quiescent and activated HSCs respectively. This promoted proliferation, contraction, and the maintenance of the activated state.

TNF-α is mainly produced by monocyte, macrophage, HSCs, and KCs. It has proinflammatory activities and cytotoxic effects in these cells. In the process of liver fibrosis TNF-α plays an important role in the activation of HSCs and synthesis of ECM (32).

Ils (Inter Leukins) have a complicated role in immune response, inflammation, and liver fibrogenesis.

Pro-fibrogenic ILs: KCs and SECs can rapidly produce ILs in response to liver tissue damage. IL-1 can directly activate HSCs and stimulate them to produce MMP-9, MMP-13 and TIMP-1 resulting in liver fibrogenesis. Another profibrotic cytokine is IL-17 whose expression level increases with degree of liver fibrosis indicating that IL-17 may be involved in disease progression and chronicity. Studies in mice have shown that IL-17 induces liver fibrosis through

multiple mechanisms, including upregulation of TNF-α, TGF-β1, and collagen 1α which is dependent on signal transducer and activator of transcription (STAT)3 signaling pathway, and promotion of myofibroblast-like change of HSCs (33).

miRNAs play a key role in various hepatic pathologies including hepatitis, cirrhosis and hepatoma (34) and may play pro- and antifibrogenic roles depending on cellular context and the nature of the stimuli. miR-21 has an important role in the pathogenesis and progression of hepatic fibrosis. miR-21 can downregulate TGF-β expression and suppress HSC activation. TGF-β1 induces expression of miR-181a and miR-181b and the latter can promote HSC proliferation by regulating p27 and the cell cycle. Elevation of serum level of miR-181b is suggested as a potential diagnostic biomarker for cirrhosis. miR-214-5p can increase expression of fibrosis-related genes (such as MMP-2, MMP-9, α-SMA, and TGF-β1) in LX-2 cells, and therefore, it may play crucial roles in HSC activation and progression of liver fibrosis 35).

Complications

The main complications of cirrhosis are portal hypertension, acquired portal systemic shunting, ascites, hepatic encephalopathy, gastrointestinal ulceration, bacterial infections, bleeding disorders and renal failure. The frequency of these complications is difficult to determine based on the stage of disease.

1. Portal Hypertension (PH)

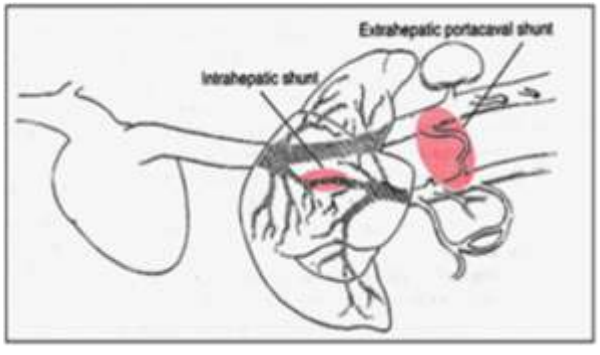
Portal hypertension is an abnormally high pressure in the portal circulation. The normal blood pressure in the portal vein is low, 0 to 5 mmHg. Portal hypertension can be caused by an increased delivery of blood to the portal system or by an increased resistance to the passage of portal blood. An increased delivery of blood occurs in animals with arteriovenous shunts in the splanchnic circulation usually in the liver causing the direct connection of the arterial blood pressure with the portal system. This is a rare condition, usually visible with ultrasonography as a pulsating bunch of vessels within 1 liver lobe (36). Usually, portal hypertension is caused by an increased resistance to the portal blood stream. It is the most important non-neoplastic complication of chronic liver disease leading to high morbidity and mortality. The primary factor involved in the development of portal hypertension is due to deregulation in the phenotype of all hepatic cell types. Portal hypertension can be because of prehepatic, intrahepatic (presinusoidal, sinusoidal or postsinusoidal) and posthepatic causes.

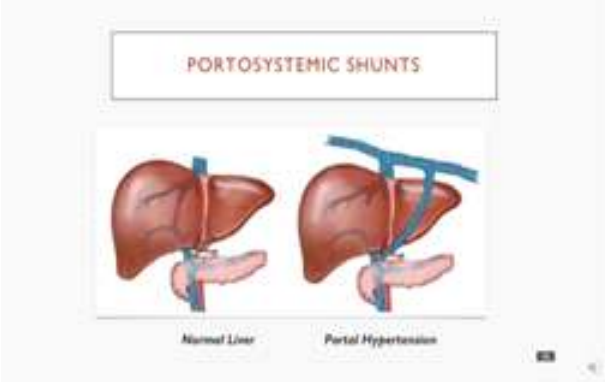
Prehepatic PH is because of increased resistance in the extrahepatic portal vein and is associated with mural or intraluminal obstruction (e.g congenital atresia or fibrosis, thrombosis, neoplasia) or extraluminal compression. Hepatic arteriovenous fistulas cause prehepatic PH as arterial blood floods the portal venous system (37).

Intrahepatic PH is because of increased resistance in the microscopic portal vein tributaries, sinusoids, or small hepatic veins. Presinusoidal intrahepatic PH occurs because of increased resistance in the terminal intrahepatic portal vein tributaries. Fibropolycystic liver disorders, such as congenital hepatic fibrosis and Caroli's disease also can cause presinusoidal PH in dogs and cats. These developmental disorders because of the persistence of embryonic duct structures are referred to as ductal plate malformations. They lead to varying degrees of bile duct pathology and portal fibrosis. A rare cause of prehepatic PH occurs in dogs with trematode infestations (Schistosoma japonicum and Heterobilharzia americana). Increased resistance to flow is because of granulomatous inflammation in the portal veins triggered by trematode eggs.

Sinusoidal intrahepatic PH most often is the result of fibrotic hepatopathies. In these disorders, SEC's lose their fenestrae and acquire a collagenous basement membrane (capillarization of the sinusoids) increasing the Intrahepatic venous resistance. In addition to this lobular fibrosis and the presence of regenerative nodules contribute to the obliteration and distortion of the sinusoids further increasing the Intrahepatic venous resistance leading to PH Postsinudoidal intrahepatic PH is associated with veno- occlusive disease caused by damage to the sinusoidal endothelium and hepatocytes in the centrilobular region resulting in obliteration of the small terminal hepatic veins and central veins by fibrosis.

Posthepatic PH is associated with obstruction of the larger hepatic veins, the posthepatic caudal vena cava, or right atrium. Obstruction at the right atrium because of right heart failure, pericardial disease, or pulmonary hypertension is a common reason for posthepatic PH in small animals.





2. Circulatory changes

Increases in the intrahepatic vascular resistance (IHVR) occur because of mechanical (fibrosis in the sinusoids, microthrombi in hepatic veins, and regenerative nodule formation) and dynamic (failure of sinusoidal relaxation) obstructive processes within the liver. At the same time, the splanchnic circulation undergoes progressive vasodilatation, leading to increases in portal blood flow (PBF) that further increase portal vein pressure. Systemic compensation for the splanchnic arterial vasodilation creates a hyperdynamic circulation marked by increased cardiac output (CO) and decreased peripheral vascular resistance (PVR). Progressive splanchnic vasodilatation leads to failure of this circulatory response and development of systemic vasodilatation and hypotension. This decrease in effective circulatory blood volume leads to activation of the renin-angiotensin- aldosterone-sympathetic system (RAAS) and the non osmotic release of antidiuretic hormone (ADH). This leads to avid renal sodium and water retention creating a volume overload and the development of ascites (36).

3. Acquired Portosystemic Shunts

The portal venous system is essential for the maintenance of the liver mass and function in mammals. The portal system collects blood from major abdominal organs (i.e., gastrointestinal tract, pancreas, spleen) delivering nutrients, bacteria and toxins from the intestine to the liver. In addition, the portal blood carries approximately from one-half to two-thirds of the oxygen supply to the liver and specific hepatotrophic factors. The portal blood is detoxified by the hepatocytes and then delivered into the systemic circulation via the hepatic veins and caudal vena cava. Blood coming from the digestive system is shunted from the portal circulation, around the liver effectively bypassing it.

Acquired shunts are classified based on anatomic location into portal precaval shunts which connect the portal vein with the caudal vena cava either directly or by way of the azygous vein, whereas portal postcaval shunts connect the portal vasculature to the cranial vena cava. A common site for precaval multiple acquired portosystemic shunts is between the portal vein and submucosal esophageal vessels. These shunts are calledesophageal varices and are prone to rupture and bleeding but are rarely of clinical relevance in dogs and cats (38). Varices in the dog most commonly occur in the left renal area and root of the mesentery. Most multiple acquired portosystemic shunts in small animals are portal postcaval shunts. In dogs large velar- omental shunts arise from the splenic vein which anastomose with embryonic connections to the left gonadal veins and empty into the left renal vein and ultimately the caudal vena cava. In dogs, smaller portal collateral vessels develop and are classified by their location and drainage patterns as left gastric, gastrophrenic, omental, gallbladder, abdominal wall duodenal, and colic varices. Cats tend to develop shunts between the left gastric veins and phrenicoabdominal veins and from the left colic vein to the left gonadal vein (36).

The implication of PSS is that toxins such as ammonia which would typically be removed by the liver can accumulate in the systemic circulation leading to clinical signs including stunted growth and neurological symptoms (Cullen et al., 2006).

4. Ascites

Once the portal hypertension develops, the increased venous resistance and increase in portal venous hydrostatic pressure drives fluid out of the vasculature and into the interstitial space. Additionally, ascites may develop as a result of hypoalbuminemia secondary to severe liver disease. Albumin is a plasma protein normally produced by the liver, and significant hepatic dysfunction may result in a clinically important decrease in albumin concentration. Albumin is the most important blood protein responsible for maintenance of normal plasma colloid oncotic pressure. If serum albumin levels decrease to less than approximately 1.6g/dL, plasma colloid oncotic pressure will become too low to maintain intravascular volume and fluid will enter the interstitial space. Animals with cirrhosis will often develop both portal hypertension and hypoalbuminemia concurrently, further increasing the risk of development of ascites (39).

Ascites is the consequence of a combination of splanchnic arterial vasodilation, decreased cardiac output, and activation of the Renin Angiotensin System which leads to sodium and water retention. In addition, high sinusoidal pressure drives fluid into the interstitial space. Ascites has been shown to be a negative prognostic indicator in dogs with chronic hepatitis.

5. Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a major complication that is closely related to the progression of end-stage liver disease. HE is often associated with acquired portosystemic shunting in dogs and cats. In the presence of acquired PSS, toxins normally filtered by the liver are transported from the gastrointestinal (GI) tract to the rest of the systemic circulation, including the central nervous system (CNS). While many toxins are involved in this process, ammonia is thought to be one of the most important. Ammonia is a breakdown product of ingested proteins and is normally absorbed by the GI tract transported to the liver and metabolized into urea. If acquired PSS are present, ammonia remains intact and is transported across the blood–brain barrier resulting in neuronal dysfunction and alterations in neurotransmission. HE may result in increased activation of neuronal gamma- aminobutyric acid (GABA)/ benzodiazepine receptors, which are responsible for neuronal suppression (8). Ammonia plays a central role in the pathogenesis of HE in dogs and cats, but other factors such as manganese and endogenous benzodiazepines may also contribute. Canine HE is often attributable to portosystemic shunting, either due to congenital portosystemic shunts (CPSS) or the formation of acquired portosystemic collateral vessels (APSC) due to portal hypertension. HE in cats is usually associated with CPSS or arginine deficiency secondary to feline hepatic lipidosis. Systemic inflammation and infection may enhance the pathological effects of ammonia in HE as well as induce cytokine mediated production of reactive oxygen species in the brain resulting in increased cerebral oedema.

Ammonia also causes astrocyte swelling mainly through excessive glutamine production which acts as an osmolyte. This results in cerebral oedema, increased intracranial pressure and eventually death (40). Severe ptyalism can be present particularly in cats and seizures may occur. Cats with PSS are more likely to have seizures than dogs. Seizures alone in the absence of other clinical signs of HE are never due to HE. Cats may also present with golden or copper colored irises secondary to decreased hepatic metabolism (41). Liver diseases are associated with elevated blood manganese levels and manganese accumulations within the brain (42). Dogs with CPSS have significantly increased blood manganese levels compared with healthy dogs and dogs with non hepatic illnesses.

6. Spontaneous bacterial peritonitis

Bacterial infection is a reversible complication of cirrhosis and accounts for major morbidity and mortality in man. There is little reference to an increased incidence of infection in animals with cirrhosis. It is likely that secondary infections may go unrecognized because of the complexity of other problems that go in hand with cirrhosis. Once infection develops the excessive response of pro-inflammatory cytokines on a pre-existing hemodynamic dysfunction further predispose the development of serious complications such as shock, acute-on-chronic liver failure, renal failure and death. Hyperdynamic circulation characterized by hypotension, low systemic vascular resistance, high cardiac output and a reduced sensitivity to vasoconstrictors are features of cirrhosis. These cardiovascular changes might be the result of increased synthesis of a vasodilator. Nitric oxide derived from vascular endothelium is a potent vasodilator that plays a key role in the homeostasis of blood pressure strikingly released in cirrhotic patients with sepsis and is a key driver of circulatory dysfunction. Cirrhotic patients showed significant increases in serum nitrite/nitrate which was significantly correlated with endotoxemia (43). There is also presence of increased gut permeability, reduced gut motility and altered gut flora, all of which leads to increased bacterial translocation. This increased bacterial translocation and consequent endotoxemia leads to increased blood stream bacterial infections that cause systemic inflammatory response syndrome, sepsis, multiorgan failure and death (44).

Once portal hypertension (PHT) is established the intestinal barrier functions are altered causing the passage of substances that are normally kept in the intestinal lumen. The disruption of the intestinal barrier at any level leads to an increase in intestinal permeability. Thus harmful substances such as MAMPs and PAMPs (LPS, microbial DNA, peptidoglycans and lipopeptides), metabolic products and whole bacteria massively reach local mesenteric lymph nodes that are unable to provide an adequate clearance. Hence, a variable amount of detrimental products is delivered to the liver through the mesenteric and portal circulation. This triggers a systemic inflammatory response developing from the liver. Kupffer cells play a pivotal role in orchestrating this mechanism. Indeed, the interaction between pathogen-associated molecular patterns on invading bacteria and TLRs (found on the baso lateral surface of enterocytes) activate intracellular molecular pathways resulting in the activation of the nuclear transcription factor (NF-κB) which activates the transcription of the mRNA encoding

proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12, IL-18), chemokines, vasoactive factors [nitric oxide (NO)] and reactive oxygen species (ROS). This local inflammatory storm leads to the recruitment of systemic leukocytes such as neutrophils, CD4+ T cells and monocytes that perpetuate liver inflammation. Net result of this process is the induction of hepatocyte apoptosis and necrosis. Both inflammatory cytokines and cell death cause the activation and proliferation of hepatic stellate cells (HSC) and the development of fibrosis under the stimulation of transforming growth factor-β (TGF-β) (45).

7. Coagulopathies

Impaired coagulation is a major hematologic manifestation of cirrhosis. Most of the clotting factors -I, II, V, VII, VIII, IX, and X are synthesized in the liver. Factors VII and VIII have the shortest half-lives (about 6 hours) and are the first to decrease with liver disease. Decrease in the activity of any or all of these factors slows coagulation, and abnormal bleeding poses a serious threat to the cirrhotic animal. Generally, bleeding from coagulation factor deficiency due to liver damage signifies a grave prognosis. Disseminated intravascular coagulation (DIC) often occurs with liver disease, the combination of a release of thromboplastic substances from the diseased liver and impaired hepatic clearance of activated coagulation factors may precipitate DIC. Thrombocytopenia is most frequent with cirrhosis in dogs and occurs less frequently in cats with hepatobiliary disease. Adequate platelet counts are typically seen in cats with the most common hepatopathies, hepatic lipidosis and cholangitis, unless complications like DIC are present (46).

8. Gastrointestinal Signs

Vomiting and diarrhea are frequent signs of liver disease in the dog. Of clinical importance are gastritis and gastric ulceration, which occur secondary to cirrhosis. Several theories have been proposed to explain the frequent occurence of these signs First, the negative nitrogen balance and low albumin levels in these animals result in an altered gastric mucous barrier and reduced cell turnover. Ulceration is further promoted by reduced gastric mucosal blood flow which often occurs with portal hypertension. Some dogs with cirrhosis have elevated serum gastrin levels and increased gastric acid production. The secretion of excessive quantities of hydrochloric acid sets the stage for gastritis and ulcer formation. In experimental animals with portal caval anastomosis, there is a sharp increase in gastric acid secretion. This may be due to the fact that elevated circulating bile acids are known to stimulate gastrin secretion or that histamine, a by-product of dietary histidine, which is normally degraded in its initial passage through the liver, bypasses the liver via collateral shunts and stimulates gastric parietal cells to secrete acid.

Diagnosis

The gold standard diagnosis of cirrhosis is liver biopsy with histopathology that identifies the presence and extent of fibrosis. However, liver biopsy is expensive and associated with a risk of hemorrhage and other complications (e.g post biopsy pain, peritonitis, shock or complications related to general anesthesia). In small animal medicine the following liver biopsy techniques are used: ultrasound-guided percutaneous needle biopsy, laparoscopic biopsy and surgical biopsy during laparotomy. Because many lesions (including fibrosis) are heterogeneously distributed throughout the hepatic parenchyma, liver biopsy is susceptible to sampling error. Sonographic findings (abdominal ultrasound) - include identification of multiple acquired portosystemic shunts, a small liver with irregular margins, presence of ascites, and a hyperechoic echotexture. But ultimately, biopsy and histopathology are required to obtain a definitive diagnosis of cirrhosis. Hepatic function testing can be performed by measuring pre- and postprandial bile acid concentrations or blood ammonia levels. Both tests may be elevated in the face of significant liver dysfunction or aPSS secondary to cirrhosis. Patients with cirrhosis will often have a known history of elevated liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT). Prothrombin time (PT) and partial thromboplastin time (PTT) may be increased, and antithrombin, fibrinogen, and protein C levels are decreased in dogs and cats with cirrhosis due to poor production of coagulation factors and/or decreased recycling of vitamin K. Vitamin K deficiency may be especially important in cats with severe liver disease.

Urinalysis is often normal in patients with cirrhosis, but may demonstrate dilute urine as a result of decreased BUN concentration. Urea is a major component of the renal medullary concentration gradient and when decreased may lead to the development of dilute urine. Bilirubinuria may be observed and is always significant in the cat and if 2+ or greater is also significant in the dog. Urate crystals may be observed in as many as 40–70% of dogs with canine PSS and secondary urinary tract infections are possible as a result of dilute urine.

Another innovative approach is to perform gene expression analysis on hepatic fine needle aspirates. Investigators showed upregulation of collagen and other fibrosis-related genes in livers of

dogs with CH. The upregulation in gene expression for PDGF, TGFb-1, TIMP-1, MMP2, and collagen type I and III, for example, showed a significant positive correlation with the severity of fibrosis.

Serum Biomarkers: Serum hyaluronic acid concentration is increased in dogs with hepatic disease especially cirrhosis and therefore holds some promise as a biomarker (47). Serum concentrations of TGFb-1, the 7S fragment of type IV collagen and procollagen type III N-terminal peptide also have been found to be increased in dogs with hepatic fibrosis. Recently, an index for the assessment of hepatic fibrosis was developed for use in dogs. This combines patient age, sex and several biochemical variables in a proprietary algorithm to create a fibrosis score. In 1 study, this index had a negative predictive value for the diagnosis of moderate fibrosis of 90–100% and distinguished dogs with clinically relevant fibrosis with a positive predictive value of 90–100%. MicroRNAs are small noncoding RNAs that have a distinct expression profile depending on the liver disease. Liver concentrations of hepatocyte derived microRNAs seem to correlate with serum concentrations. A recent study evaluated whether serum miRNA biomarkers hold promise for distinguishing among several hepatobiliary diseases in dogs. Two miRNAs were found to be increased in hepatobiliary disease: miR-200c in the hepatocellular carcinoma group (6 dogs) and miR-126 in the CH group (6 dogs) (48). Measurement of microRNAs in serum potentially could be used to assess hepatic fibrosis in dogs. However, further studies with greater sample sizes are needed to evaluate the sensitivity and specificity of these markers.

Conclusion

Owing to decreased functional parenchymal reserve and altered hepatic blood flow, cirrhosis is associated with the life-threatening complications of liver failure including hepatic encephalopathy, coagulation disorders and bacterial infections and complications of portal hypertension such as ascites, variceal rupture and hepatorenal syndrome. Liver cirrhosis causes clinical and hemo-biochemical alterations which require special consideration when treating the diseased animals. In addition to this the cirrhotic liver is a precancerous state and thus requires the systematic screening of pets for hepatocellular carcinoma.

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A Rare Case of Feline Trichoblastoma: Diagnosis and Treatment

Sabita Rakshit, Dale Clark, Kabita Roy and I C Datta
Milford Veterinary Clinic, 110 Canal Street, Milford, USA and College of Veterinary Science, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh.

Abstract

A 10-year-old neutered male Domestic Long Hair (DLH) cat was presented in the clinic on January 4, 2022, for treatment of a lump on the left lateral aspect of tail near the base, exhibiting intermittent bleeding for nearly six months. Physical examination: all vitals within the normal limits (WNL). Cytology of the Fine Needle Aspirate (FNA) from the lesion indicated abscess. Treatment comprising broad-spectrum antibiotics and pain medication was initiated. However, since the growth was progressively getting enlarged and turgid, surgical complete mass removal was planned and executed successfully on June 7, 2022. Excision biopsy referral histopathology report: benign trichoblastoma.

Introduction

Trichoblastoma, a rare cutaneous neoplasm, originates in the trichoblastic epithelium in the hair follicles (1). In a retrospective study of 680 cases of tumor in the European Short hair cats in central Italy, some skin and soft tissue neoplasms were found associated with prolonged exposure to UV radiation from the sun (2). In a comprehensive retrospective study of more than 9000 feline cutaneous tumors in the United Kingdom, on histopathological profile 55.9% were rated malignant. The most common incidence included basal cell tumor, fibrosarcoma, squamous cell carcinoma and mast cell tumor (3). The present communication reports on the successful complete surgical excision of trichoblastoma near the base of the tail in a house cat.

Case History

A 10-year-old 5.1 kg neutered male Domestic Long Hair (DLH) cat was presented in the Milford Veterinary Clinic on January 4, 2022, with a prominent lump on the tail. Physical examination: rectal temperature 101°F, heart rate 140 beats/minute, respiration rate 32/minute, Capillary refill time (CRT) <2 seconds, visible mucous membranes pale pink, and body condition score (BCS) 3/5. A large, moderately firm mass (5.5cm x 6cm) on the left lateral aspect of tail base **(Fig. 4),** was present for six months, oozing foul smelling exudates **(Fig.1).** The haemato-biochemical panel **(Tables 1, 2)** showed all parameters within normal limits (WNL).

Fig 1. Growth near the base of the tail

Diagnostics

I. Haematobiochemical profile

Table 1. Patient's haemogram on 7.6.2022.

Parameter (Units)	Result	Reference interval	Status
TEC (1x 06/µl)	8.87	6.54-12.2	Normal
Hemoglobin (g/dl)	12.2	9.8-16.2	Normal
Hematocrit (%)	34.9	30.3-52.3	Normal
MCV (fl)	39.3	35.9-53.1	Normal
MCH (g/dl)	13.8	11.8-17.3	Normal
MCHC (%)	35	28.1-35.8	Normal
TLC (1x 103/µl)	11.28	2.87-17.0	Normal
Reticulocyte (%)	0.2		
Neutrophil (%)	74.7		
Lymphocyte (%)	15.2		
Eosinophil (%)	5.1		
Monocyte (%)	4.8		
Basophil (%)	0.2		
Neutrophil (1x103/µl)	8.42	2.9-17	Normal
Lymphocyte (%)	1.72	0.92-6.88	Normal
Eosinophil (%)	0.58	0.17-1.57	Normal
Monocyte (%)	0.54	0.05-0.67	Normal
Basophil (%)	0.02	0.01-0.26	Normal
Platelets (1x 103/µl)	334	151-600	Normal

Catalyst One CBC Analyzer, IDEXX, USA

Table 2. Blood chemistry panel on 7.6.2022.

Parameter (Units)	Result	Reference	Status
Glucose (mg/dl)	91	74-159	Normal
Creatinine (mg/dl)	2.6	0.8-2-4	High
BUN (mg/dl)	39	16-36	High
BUN: Creatinine ratio	15		
Total protein (g/dl)	8.0	5.7-8.9	Normal
Albumin (g/dl)	2.7	2.3-3.4	Normal
Globulin (g/dl)	5.3	2.8-5.1	High
A/G ratio	0.5		
ALT (U/l)	23	12-130	Normal
ALKP (U/l)	41	14-111	Normal

Catalyst One Chemistry Analyzer, IDEXX, USA.

Treatment

To combat suspected microbial infection in the patient's tail growth (FNA cytology), Polyflex® [200mg/ml] @ 30mg/kg, 0.8 ml and pain medication Onsior® [20mg/ml] @ 2 mg/kg. 0.5 ml were injected S/C on January 4, 2022. Take home medications with advisory: antibiotic Simplicef® [100mg] ½ tab PO, SID x 10 days, and oral Onsior [10mg/ml] @ 1 tablet x 3 days. Animax® ointment was advised to be applied on the ulcerated lump. After two days, the owner messaged having difficulty in administering the oral pills. On January 7, 2022, the patient was presented in the clinic for injectable antibiotic, Convenia®, a slow-release antibiotic (potency10-14 days) + Buprenex for pain relief. On physical recheck, the lump appeared firm in consistency. Surgical removal of the mass was advised, but the owner wanted to wait. On April 2, 2022, the owner informed that the growth was enlarged and formally agreed for the mass removal. Surgery was scheduled in the morning hours on June 7, 2022. Pre-anesthetic blood work was done, mainly to monitor the hepatic and renal function test parameters. The tentative diagnosis of a cyst was a misconception.

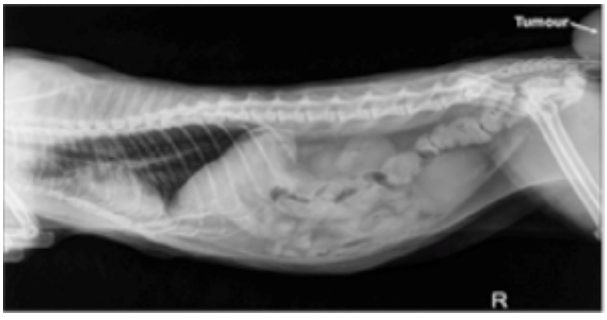

Fig 2. R/L Radiograph. A large tumor on the base of the tail. No growth in the body cavities.

The R/L survey radiograph **(Fig. 2)** revealed no evidence of tumor spread. Abdomen: no radiopaque patches of clinical concern were noticed. With assured biosafety, the patient was prepared for surgery **(Fig. 3):** pre-medicated with Torbugesic® 0.1 ml + Acepromazine 0.05 ml, injected S/C. After I/V catheterization on the left cephalic vein, fluids infusion started LRS. On induction with Ketamine + Midazolam: 0.2 ml each, injected I/V, the patient was intubated, and transferred to Isoflurane + O2 gas anesthesia. The surgical site was prepared aseptically **(Fig. 1).** An elliptical incision around the growth was made with #10 scalpel blade, and the mass excised bluntly with Metzenbaum scissors.

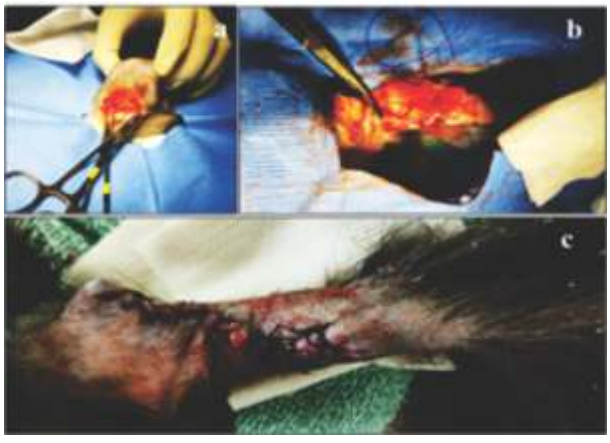

Fig 3. Mass removal surgery in progress.

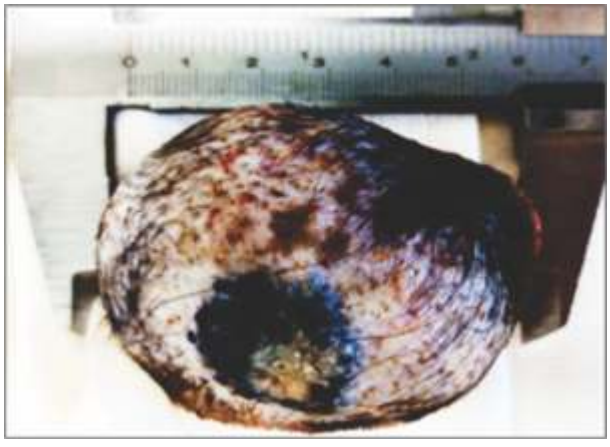

Fig 4. Surgically excised mass (5.5 cm x 6 cm).

Bleeder was securely ligated with 3-0 Monomend absorbable sutures. The subcutaneous tissues were closed with 3-0 absorbable sutures in the continuous pattern, and skin closed with 3-0 non-absorbable sutures in the interrupted cruciate pattern. Surgery was highly challenging as the tight skin edge was difficult to suture back after mass removal **(Fig. 3c).** Since the suture line was visibly under high tension, a mattress pattern layer was superimposed for effective reinforcement. Before discharge, Convenia and buprenex injections were given. Day 7 post-surgery: the patient on recheck

visit appeared in fine shape; surgical wound healing normally. Day 14 post-surgery: skin sutures removed completely. A residual small raw surface area is possibly licked by the cat. Therefore, constant use of e-collar was advised for strict compliance.

Histopathology Profile

Processed sections from the excised mass lesion demonstrate regionally extensive multilobular and multilocular cystic epithelial neoplasm, tumor cells comprising small basaloid epithelial cells, and in some areas exhibiting a variable degree of cytoplasmic keratinization, or squamous differentiation **(Fig. 5).** Tumor cells are seen in lobules and nests, and also line small to large cystic spaces. Multi-focal areas of tumor degeneration and modest necrosis with resultant pseudo cystic cavitation are visible. Supporting trabecular collagenous and fibroblast stroma with a moderate frequency of scattered, or clustered intratumorally, interstitial, heavily pigmented well-differentiated melanocytes are also seen. Solid sheets of lobules and nests of basaloid epithelial cells, interspersed with small basaloid/ cuboidal tumor cells, form well-defined, small calibre tortuous and branching tubule-ductular structures. The mitosis count (0-1/HPF) is distinctly low. The neoplastic focus: narrowly extirpated in the sections.

Microscopic interpretation

Complex histoarchitecture of the growth is consistent with benign, solid and cystic trichoblastoma, moderately pigmented. Surgical excision is narrowly complete.

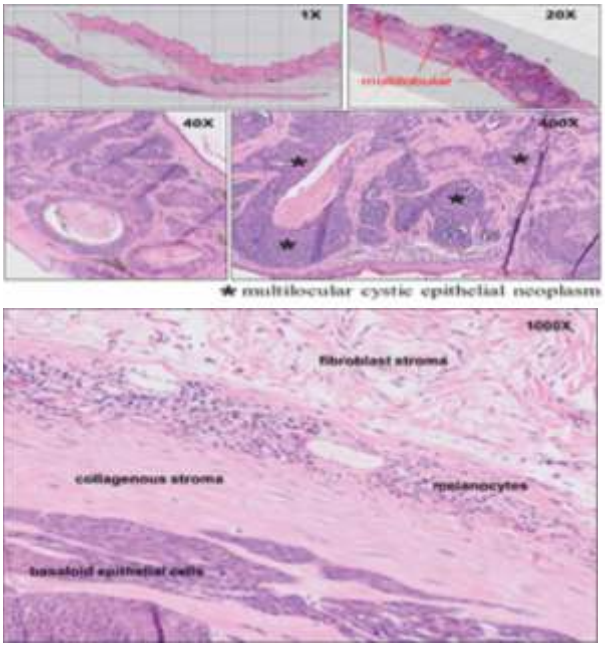

Fig 5. Regionally extensive multi-lobular, multi-locular cystic epithelioid neoplasm with occasional small basaloid epithelial cells (HE, 1000x).

Discussion

Trichoblastoma (basal cell tumor) is a benign neoplasm of follicular epithelial germ cell (trichoblastic) origin in dogs and cats (4). Middle-aged and older animals are typically affected. In cats, the cranial half of the trunk is the most commonly (but not exclusively) affected anatomical region. These lesions are occasionally melanin-pigmented and/or cystic. With complete excision, local recurrence is not anticipated. Improved cosmetics is highly satisfying to the pet loving families.

In the case under report, advanced modifier was used for in-depth histopathological evaluation of the excised trichoblastoma biopsy. In some areas, the neoplastic epithelial cells appear organized in small tubule-ductular pattern, reminiscent of other adnexal elements, e.g., apocrine ductular epithelial elements. Histological tumor profile is consistent with innocuous, clinically benign bio-entity. In perspective, complete surgical extirpation is expected to be curative. As the surgical mass removal appears narrowly complete in the sections, monitoring of the excision site and surrounding tissues for evidence of local recurrence is advised by the pathologist.

Infrared thermography for recording temperature differentials between the malignant and benign skin and soft tissue tumors in tandem with histopathology may contribute significantly to improved diagnosis and prognostic evaluation in feline oncology patients (5).

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Gunshot Injury in Cat's Right Elbow Joint: A Case Report

Sabita Rakshit, Dale Clark, Kabita Roy and I.C. Datta
Milford Veterinary Clinic, 110 Canal Street, Milford, MI, USA, College of Veterinary Science, NDVSU, Jabalpur, Madhya Pradesh

Abstract

A young intact indoor-out door male Domestic Short Hair (DSH) cat was presented in the clinic on October 4, 2022 for checking the right leg limping, and neuter surgery prerequisites. This stray cat visited the owner's house frequently and the kind-hearted gentleman decided to feed and ultimately adopt him. Owner mentioned that the patient, possibly got bitten by another animal, was limping thereafter. Physical examination: all vitals within the normal limits (WLN). Right elbow region, visibly swollen, scabby was extremely painful on palpation. Survey radiographs revealed the olecranon process of the elbow ulnar bone shattered, and a pellet entrapped in the biodegraded mass. The referral orthopaedic DVM, on thorough recheck of the patient with the submitted radiograph images, strongly recommended no surgical intervention in the instant case, and suggested long-term palliative treatment to promote the natural healing process by second intention. Recovery is fine, and the caring owner is highly satisfied.

Introduction

Gunshot injuries, in animals and humans, occur accidentally or intentionally all over the world. In cats and dogs, such episodes are encountered occasionally by the veterinarians for treatment and forensic jurisprudence (1,2,3). In a retrospective study, gunshot wounds in cats and dogs were evaluated in respect of age, gender, breed, gunshot type, nature and extent of lesions, time of incidence, and treatments given (4). The majority of patients were male dogs 73/99 and cats ¾ (all of mixed breed). These cats were targeted with high velocity air propelled bullets/ low velocity projectiles; no metallic objects were observed. Another retrospective study (5) revealed that gunshots resulted in soft tissue, orthopaedic, ophthalmic and neurological lesions. Medical treatment regimes were followed in most cases, and surgical intervention was rare. In this communication, we document a high impact gunshot wound in a recently adopted stray young male cat with a pellet lodged deep inside the fractured right elbow joint bones, not amenable to surgical intervention, according to the orthopedic DVM. Long-term palliative treatment was suggested by the specialist to promote the natural healing process.

Case History

An intact male stray cat (nearly 9 months of age) was sneaking into the present owner's house premises off and on and the kind hearted gentleman was offering food for the past few months. One day, the animal came to the door with a non-weight bearing posture on the right forelimb. Close monitoring revealed a bruised lesion, presumably a bite wound inflicted by another small wild animal like a racoon, or another feral cat. On humanitarian grounds, the owner decided to adopt and take care of the animal. The Domestic Short Hair (DSH) indoor-outdoor intact male cat was presented to Milford Veterinary Clinic on October 4, 2022 for treatment of the limping forelimb and neuter surgery prerequisites. Physical examination: rectal temperature 101.2^0 F, Heart rate 140 beats/minute, Respiration rate 32/minute, visible mucous membranes pink, and body condition score (BCS) 3/5. Right elbow area very painful on palpation, non-weight bearing and scabby, possibly from localized blood loss and infection. In-house radiographic imaging was advised to locate anticipated osteolysis, osteomyelitis, or fracture. The owner agreed to get the vaccines protocol updated on the stray cat, blood work and radiography of the affected limb to monitor the status. Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV) and Heartworm (HW) triple combo test was negative. Rabies and Feline Viral Rhinotracheitis, Calici and Panleukopenia (FVRCP) vaccines were given.

Diagnostics and Palliative Treatment

In-house right forelimb survey radiographs **(Fig. 1a & b)** revealed the olecranon process of ulnar bones in the elbow joint shattered, and pellet lodged inside the biodegraded mass. The owner was briefed on the significance of the radiograph images and advised to seek help from any of the referred orthopaedic DVMs of his choice for this highly challenging bone surgery. The patient's haemogram **(Table 1)** revealed increased total leukocyte count with eosinophilia, indicating microbial infection and hypersensitivity, possibly

resulting from parasitic infestation from consuming the prey. SDMA and Phosphorus were high **(Table 2)** from bone osteolytic and osteoblastic changes. Long-term antibiotic, Convenia® @ 8mg/kg, and pain medication, Buprenorphine @ 0.01 mg/kg were injected S/C. Take home medicament; oral Buprenex twice a day (BID) x 3 days was dispensed.

The owner informed that he had consulted the orthopaedic specialist, who strongly advised to leave the patient undisturbed for slow natural healing ultimately leading to fusion of the fractured elbow joint. Since too many small broken fragments, are clearly visible, fixing is not possible. The other option is right limb amputation. The owner followed the expert's advice and opted for the natural healing course.

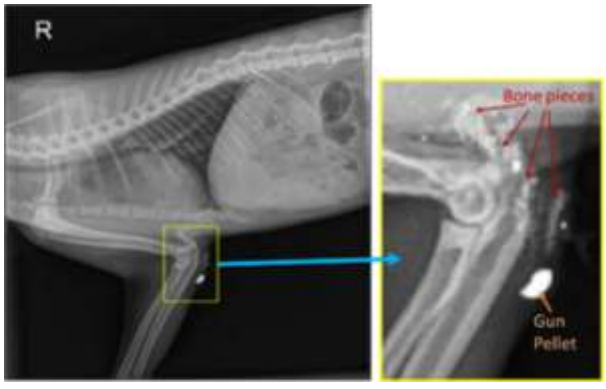

Fig. 1 a. R/L view: The olecranon process of ulnar bone shattered in many fragments.

Fig. 1b. R/L oblique view: pellet lodged in the tissue.

Haematobiochemical profile

Table 1. Patient's haemogram on 4.10.2022.

Parameter (Units)	Result	Range	Status
TEC (1x 06/µl)	7.78	6.54-12.2	Normal
Hemoglobin (g/dl)	11.8	9.8-16.2	Normal
Hematocrit (%)	38.2	30.3-52.3	Normal
MCV (fl)	49.1	35.9-53.1	
MCH (g/dl)	15.2	11.8-17.3	
MCHC (%)	30.9	28.1-35.8	
TLC (1x 103/µl)	17.73	2.87-17.0	
Neutrophil (1x103/µl)	8.03	2.9-17	
Lymphocyte (1x103/µl)	6.58	0.92-6.88	Normal
Eosinophil (1x103/µl)	2.11	0.17-1.57	Normal
Monocyte (1x103/µl)	0.64	0.05-0.67	Normal
Basophil (1x103/µl)	0	0.01-0.26	High
Platelets (1x 103/µl)	179	151-600	Normal

Table 2. Blood chemistry panel on 4.10.2022.

Parameter (Units)	Result	Range	Status
Glucose (mg/dL)	86	74-159	Normal
SDMA (µg/dL)	21	0-14	High
Creatinine ((mg/dL)	0.8	0.8-2-4	Normal
BUN (mg/dL)	27	16-36	Normal
Total protein (mg/dL)		5.7-8.9	Normal
Phosphorus (mg/dL)	8.6	3.1-7.5	High
Calcium (mg/dL)	10.2	7.8-11.3	Normal
Total Protein	7.4	5.7-8.9	Normal
Albumin (g/dl)	3.4	2.3-3.4	Normal
Globulin (g/dl)	4.0	2.8-5.1	Normal
A/G ratio	0.9		
ALT (U/L)	99	12-130	Normal
ALKP (U/L)	27	14-111	Normal
GGT (U/L)	0	0-4	Normal
TBil (mg/dL)	0.7	0.0-0.9	Normal
Chol (mg/dL)	106	65-225	Normal
Amylase (U/L)	892	500-1500	Normal
Lipase (U/L)	703	100-1400	Normal
Sodium (mmol/L)	157	150-165	Normal
Potassium (mmol/L)	4.7	3.5-5.8	Normal
Na/K	33		Normal
Chloride (mmol/L)	120	112-129	Normal
Osm Calc (mmol/kg)	316		
TT4 (µg/dL)	2.4	0.8-4.7	Normal

Catalyst One: CBC and Chemistry Analyzer, IDEXX, USA

Neuter surgery

November 7, 2022

In the OT, the feline patient was pre-medicated with butorphanol (Torbugesic®) and acepromazine. Following induction with ketamine and midazolam, the patient was transferred to isoflurane gas anaesthesia. The surgical site was properly

prepared and a linear incision was made with a sterile scalpel blade over each testicle on the scrotum, the testicle gently pushed out from the incision, tunica layers cut, identified the spermatic cord and vascular bundle and tied to each other, and the testicles removed. Skin incision healed by second intention. Polyflex® given concurrent with the pain medication, Buprenorphine. Take home oral Buprenex BID x 3 days. Recovery is highly satisfactory.

Discussion

In recent years, the incidence of gunshot violence in humans is rising steadily in different parts of the world, presumably because of multi-dimensional socio-economic issues. Sometimes, the innocent animals are victimized because of either recreation or revenge. However, many benevolent people come forward to adopt the run away or abandoned pets and seek timely treatment in veterinary clinic, despite financial constraints. The case under report is a good example. In many cases of gunshot injuries in cats, the precise time of shooting remains obscure. In the absence of accurate case history, these injuries may be mistaken as simple bite wounds, or vehicular accidents. Knowledge of the weapon employed will help the veterinarian in evaluating the tissue damage, and the line of treatment. A retrospective study established that in cats, most of the gunshot injuries were caused by low-velocity projectiles, rarely resulting in fatal injuries. Review of gunshot injuries in cats and dogs in urban and rural communities in a referral veterinary practice, Cedar Rapids, Iowa, USA corroborated the marked preference for low-velocity, low-kinetic energy firearms, and the limb wounds were most numerous during low visibility hours, or hunting excursions (6). The high percentage of metal projectiles in cats in urban, semi-urban and rural areas emphasizes the judicious use of survey radiography, preceding the increasingly popular modern diagnostic tool, MRI to avoid the possibility of deleterious metal migration from magnetic effects (4). Radiographic diagnosis was certainly very helpful in the instant case.

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Recent Updates on Feline Panleucopenia Virus with Respect to Its Therapeutic Management

Leibaknganbi Maibam, Suvendu Kumar Behera and Pradyumna Chakraborty
Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry,
Central Agricultural University, Selesih, Mizoram.

Abstract

Feline panleukopenia is a highly contagious and often lethal disease of cats and other Felidae typically characterized by depression, anorexia, high fever, vomiting, diarrhea, and consequent severe dehydration. To improve the health condition of the infected kitten intensive fluid therapy, glucose and potassium supplementation, antimicrobial, anthelmintic, antiemetic therapy and sometimes immunotherapy should be given to the infected animal. The disease is one of forgotten diseases of felines because of its sporadic occurrence, however, recent outbreaks in several parts of the country have compelled many small animal practitioners to update their knowledge with respect to management of the disease and hence the genesis of this manuscript. The authors have taken utmost care to make the manuscript subtle for the common readers at the same time informative for the pet practitioners.

Introduction

Feline Panleukopenia (FPV) is a parvoviral infectious disease of kittens typically characterized by depression, anorexia, high fever, vomiting, diarrhea, and consequent severe dehydration. Adult cats are much less often affected. Diagnosis is usually based on clinical signs, severe neutropenia, lymphopenia, and fecal viral antigen or PCR testing. Treatment includes intensive fluid therapy, glucose and potassium supplementation, antimicrobial, anthelmintic, and antiemetic therapy, and sometimes immunotherapy (1).

Therapeutic Management

1. Fluid

Intravenous (IV) fluid replacement and maintenance with a balanced isotonic crystalloid solution (e.g., lactated Ringer's solution with calculated potassium supplementation) is the foundation of therapy. 5% glucose should be added if hypoglycemia is suspected or proved. In addition to crystalloid infusion, transfusion of fresh-frozen plasma helps support plasma oncotic pressure and provides clotting factors to severely ill, hypoproteinemic kittens. It also provides some anti-FPV antibodies. Whole blood is preferable for the occasional cat that is severely anemic. The amount of fluids needed is about 3 to 4.4 % of cat weight per day, if cat is not eating. A 3 kg cat will get 90 to 130 ml fluids per 24 hours. Sub-cutaneous (SC) fluids should be given slowly in sick animals. Limit yourself to 125 ml in an adult at one time and scale down for kittens (2).

2. Antibiotics

As the gut barrier often is destroyed in FPV-infected cats, intestinal bacteria can invade the blood stream via translocation. Bacteremia can result, facilitated by the existing neutropenia, and leading to sepsis in these immunocompromised patients (3). Prevention of sepsis is essential, and a broad-spectrum antibiotic with a proven efficacy against gram-negative and anaerobic bacteria is recommended. Examples are ampicillin (10-20 mg/kg IV or SC q8h) or amoxicillin/clavulanic acid, fluoroquinolones or cephalosporins. Antibiotics should be administered parenterally (preferentially intravenously if indicated).

3. Anti- Emetics

Anti-emetics such as maropitant (1 mg/kg SC q24h) or metoclopramide (0.2-0.4 mg/kg SC q 6-8 hrs or 1-2 mg/kg IV over 24 hrs as a constant rate infusion should be given. Ondansetron is an option for severe intractable vomiting. Oral intake of water and food should only be restricted if vomiting persists, but feeding should be continued as long as possible, and restarted as soon as possible. Beneficial effects of early enteral nutrition have been reported in CPV-infected dogs and more data on supportive care are available for dogs (4) that can also be adapted for use in cats. A highly digestible diet is preferred, but if the cat does not accept it, any diet is better than no food intake at all.

4. Vitamin Supplements

Vitamin supplements, particularly of the B vitamin complex, can be given to prevent development of thiamine deficiency, which occurs occasionally in cats with FPV.

5. Miscellaneous

I. Feline recombinant interferon-omega (rFeIFN) is effective in the treatment of CPV-induced enteritis in dogs (5) and also inhibits replication of FPV in cell culture. The dose recommended is

1 mass unit/kg, SC, every 24 hours for 5 consecutive days, and three separate 5-day treatments must be performed at day 0, day 14, and day 60.

II. Anthelmintic: It is important to realize, that many cats with panleukopenia also have parasite infestation, especially those originating from shelter environments and therefore, faecal examinations and appropriate anthelmintic treatment (e.g., fenbendazole 50 mg/kg, per orally every 24 hours for 2–5 days, milbemycin-praziquantel, imidacloprid-moxidectin) is an important consideration as intestinal parasitism is a common comorbidity (6).

III. Neupogen (filgrastim) dose and schedule: Neupogen, a human granulocyte colony-stimulating factor (G-CSF) , is used to treat low neutrophil count. Recommended dose is 1 to 5 microgram per kg SC, once/twice a day for two to three days, then a day off and then again on the fourth or fifth day. This works well most of the time (2).

IV. Feline immune sera: It can be prepared in veterinary practice. The blood type of donor and recipient should match. The minimum amount required for protection is unknown, but the dose recommended for recipient cats is 2 to 4 ml of serum per kilogram body weight. Attention must be paid to sterility during collection, storage and administration. Jugular vein puncture is preferred, and the area over the jugular vein should be shaved and disinfected. Blood should be collected (at least twice the volume of serum required) into sterile tubes without additives and allowed to clot. Serum is then removed and can be stored at -20° C in single dose aliquots, as IgG is very stable, and can be kept for up to a year if frozen promptly after collection (7). Usually, sera are given subcutaneously, but intraperitoneal injection might be more feasible in kittens. If an immediate effect is needed, intravenous administration of plasma (instead of serum) should be used (8).

Prevention

I. **Vaccination** primary vaccination at 4-6 weeks of age in kittens, and boosters every 2 weeks until at least 20 weeks of age (intervals of 3-4 weeks for boosters may be appropriate for kittens in low-risk foster home environments). Cats should be revaccinated annually or triennially (1). Some of the commercially available vaccines in India are FELIGEN® CRP (Virbac, India), Nobivac® Tricat Trio (MSD Animal Health) etc.

II. **Sanitation** (1:30 dilution of household bleach) is the root of a healthy animal shelter environment and a key component in maintaining the health of the animals housed within.

Conclusion

Acute cases require vigorous fluid therapy and supportive nursing care in the isolation unit. Electrolyte disturbances (e.g., hypokalemia), hypoglycemia, hypoproteinemia, anemia, and opportunistic secondary infections often develop in severely affected cats. Anticipation of these possibilities, close monitoring, and prompt intervention are likely to improve outcome. The immunity produced by the panleukopenia vaccine is generally strong. Hence, it can only be prevented by giving proper vaccination.

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Fatal Pneumoperitoneum in a Cat after Complete Recovery from Deep Facial Bite Wound: A Case Report

Dale Clark, Sabita Rakshit, Kabita Roy and I C Datta
Milford Veterinary Clinic, 110 Canal Street, MI, USA and College of Veterinary Science and Animal Husbandry (NDVSU), Jabalpur, MP

Abstract

A 15 year old, male neutered Domestic Medium Hair (DMH) cat was presented in the Milford Veterinary Clinic, Milford, Michigan, USA on November29, 2021 for health check with a visible lump on the left cheek, extending into the neck region. The owner informed that the indoor-only cat with no apparent health issues was not examined by any veterinarian from 2016 onwards. Anamnesis revealed that the cat had stopped eating. Physical examination: rectal temperature normal, dehydrated, capillary refill time (CRT) >2 seconds, visible mucous membranes appeared pale, suggesting anemic state. The body condition was normal, weight 14 lbs. Differentials at this point: bite wound abscess from the companion younger cat, tumor, ear infection, anemia, renal dysfunction and tooth root abscess. Tension pneumo-peritoneum, life-threatening exponential accumulation of gas in the peritoneal cavity, may culminate in high risk respiratory failure, resulting from hypoxemia/ apnea. The case under report bears testimony to the above contention. In such biomedical emergency judicious surgical intervention: exploratory/ curative intent, and referral histopathology of representative biopsy sample is strongly recommended.

Introduction

Gastric ulcer, representing necrobiotic discontinuity in the mucosal layer of stomach, is attributed mainly to a quantum increase in hydrochloric acid concentration in gastric juice, or alterations in the gastric mucosal barrier (1,2). Gastrinoma (tumor of the pancreatic islet cells, promoting excessive gastrin secretion), impaired renal/hepatic function, and mast cell tumors are also implicated in the pathogenesis, acute or chronic. Mast cell tumors produce histamine, chemical mediator for accelerated acid release from the gastric parietal cells (3-5). Bioprotective gastric mucosal barrier is hampered/ disrupted by a plethora of disease conditions: inflammatory bowel disease (IBD), accidentally ingested foreign bodies, hepatic disease, traumatic insult to brain and spinal cord neurons, metabolic or respiratory acidosis, and hypoxemia1. Hypoproteinemia and cachexia may inhibit bioefficacy of the mucosal barrier with compromised cell turn over, and the natural healing process. Shock: septic, neurogenic, or hypovolemic may contribute to ulcerative biodegradation through impaired mucosal blood circulation (1,2). Gastric neoplasia: lymphoma, leiomyosarcoma, and pathogenic infections, e.g. helicobacteriosis are also associated with gastric ulceration (6,7).

Clinical signs on presentation include vomiting, hematemesis (bright red fresh/ ground coffee colored accumulated blood), weight loss, occasional melena, diarrhea, hyper-ventilation (panting), generalized tissue dehydration, increased abdominal pain perception, lethargy, and pale visible mucous membranes (8-10). No genetic, gender, or age predisposition is documented in the published case reports, globally. Notably, the clinical signs of blood loss in the gastrointestinal tract, such hematemesis and melena may be obscured by hypovolemia, sepsis, and/ or anemia (11). Gastro-duodenal (GU) ulcers may aggravate and lead to wall perforation (progressive/ acute) and life-threatening pneumoperitoneum. Air may enter the peritoneum from different routes and create a surgical emergency situation. The bioepisode may also result from penetrating trauma, passage of air from the pleural space, or ruptured abdominal internal organ, such as gall bladder, urinary bladder promoting infection. Iatrogenic pneumoperitonitis is associated with laparoscopy or abdominocentesis.

Retrospective study of spontaneous gastro-intestinal perforation in companion cats indicates lymphoma a frequent etiological factor (12), and the microscopic profile of ulcer peripheral tissue biopsies, harvested through exploratory/ curative intent surgery, is highly recommended. Clinical experiences suggest that meloxicam (Mobic, Boehringer Ingelheim) and/ or injudicious prednisolone administration may escalate pre-existing gastric lesions. Thus, the current trend for selective use of cyclooxygenase-2 (Cox-2) non-steroid anti-inflammatory drugs (NSAIDs) in human medicine may be hazardous in feline practice (13).

Case History

On November 29, 2021

A 15 years old male neutered indoors-only Domestic Medium Hair (DMH) cat was brought to the Milford Veterinary Clinic (MVC) having totally stopped eating and a visible lump on the left side of face, extending down to the neck. The patient had not been taken to any veterinarian for routine health checks since 2016. Physical examination: rectal temperature normal, signs of dehydration, capillary refill time (CRT) >2 seconds, visible mucous membranes pale, body weight 14 lbs., body condition score (BCS) being normal. Differentials: facial bite wound abscess, tumor, ear infection, anemia, renal dysfunction, and tooth root abscess.

Ear Cytology

No mites but yeast detected: left ear too numerous to count (TNTC), and right ear moderate. In-house survey radiographs **(Fig.1 a,b,c)** no evidence of malignancy in any internal organ, engorged gas in the stomach, diffuse radiopaque patches in the lung fields, with marked congestion indicating respiratory infection. Auscultation hampered by the patient purring. Referral hemogram **(Table 1)** revealed total RBC count subnormal, leukocytosis. Blood chemistry profile: serum SDMA and phosphate high values. Tentative diagnosis: abscess, from the facial bite wound, and early renal dysfunction. With the owner's formal consent, the shaved left side cheek area in the patient revealed penetrating teeth marks, presumably inflicted by the younger companion cat. Lancing was done and nearly 25 ml fetid pus was drained out. After flushing the abscess channel with diluted chlorhexidine solution, a sterile drain tube was inserted and fixed securely. Combination antibiotics: ampicillin injectable suspension (Polyflex®, Boehringer) @ 30mg/kg, cefovecin (Convenia®, Zoetis) @ 8mg/kg, Robenacoxib (Onsior®, Elanco) @ 0.91mg/lb. and vitamin B12 were administered subcutaneously, and the high calorie nutraceuticals preparation (Nutri-Cal, Vetoquinol) orally. The affected left ear was treated with gentamicin-clotrimazole otic ointment (Otomax®, Merck Animal Health). The patient was hospitalized. In watery diarrhea the fecal smear revealed TNTC Clostridium spp. and yeast. The fecal sample referred to IDEXX for ova test was negative. Matted fur in the hind quarters was shaved, and the facial abscess crater cleaned on the following day. Patient appeared relieved.

Dec. 1 Combination antibiotic Animax® (Dechra Veterinary Products), and mupirocin® ointments applied on the cleaned wound periphery, and the patient discharged in the evening with advisory on home medications: antibiotic oral drops, amoxicillin clavulanate (Clavamox®, Pfizer), and mupirocin ointment topical application after warm compress.

Dec. 7 Patient's health status improved with no coughing, sneezing, vomiting or diarrhea. Advised continued Clavamox and mupirocin home treatment, and patient's recheck after one week.

Dec. 14 Patient recovering fine, appetite improved, and the abscess wound healing well. The drain tube was removed, and dead skin gently scrapped off.

Dec. 15 Patient's health deteriorated exponentially with acute respiratory distress. The survey radiographs **(Fig.2 a,b,c)** indicated hyperacute (tension) pneumoperitoneum, and the hemogram accentuated anemia (PCV% 10).

Voluminous gas, trapped in the abdomen, was tapped out. Patient was hospitalized again.

Dec. 17 Patient passed away in the morning. Necropsy revealed stomach ulcer, with pooling of blood.

Hematobiochemical profile

Table 1. Patient's sequential hemogram

Parameter (Units)	11.29.21	12.15.21	Reference interval
RBC (M/µL)	6.88 N	2.74 L	6.54-12.2
Hematocrit (%)	31.2 N	10.9 L	30.3-52.3
Hemoglobin (g/dL)	10.0 N	3.5 L	9.8-16.2
MCV (fL)	45.3 N	39.8 N	35.9-53.1
MCHC (%)	32.1 N	32.1	28.1-35.8
RDW (%)	21.3 N	24.9	15- 27
Reticulocyte (%)		0.6	3.0-50.0
Reticulocyte-Hb (pg)		13.6	13.2-20.8
WBC (K/µL)	17.5 H	7.6	2.87-17.0
Neutrophil (%)	23.7	67.6	
Lymphocyte (%)	9.8	18	
Monocyte (%)	2.5	10.9	
Eosinophil (%)	57.1	3.2	
Basophil (%)	-	0.3	
Neutrophil (K/µL)	41.7 H	5.14	2.87-17.02
Lymphocyte(K/µL)	17.1 H	1.37	0.92-6.88
Monocyte (K/µL)	4.37	0.83	0.05-0.57
Eosinophil (K/µL)	10.0. H	0.24	0.17-1.57
Basophil (K/µL)		0.02	0.01-0.26
Thrombocyte (K/µL)	287 N	376	151-600

Table 2. Sequential blood chemistry profile

Parameter (Units)	11.29.21	12.15.21	Reference interval
Glucose (mg/dL)	108 N	160 H	74-159
SDMA (µg/dL)	23 N	27 H	0-14
Creatinine (mg/dL)	0.9 N	1.0 N	0.8-2.4

BUN (mg/dL)	29 N	19 N	16-36
Phosphate (mg/dL)	8.5 H	4.7 N	3.1-7.5
Calcium (mg/dL)	8.0 N	8.9 N	7.8-11.3
Total protein (g/dL)	7.3 N	6.2 N	5.7-8.9
Albumin (g/dL)	2.7 N	2.3 N	2.3-3.4
Globulin (g/dL)	4.6 N	3.9 N	2.8-5.1
A/G ratio	0.6	0.6	
ALT (U/L)	46 N	15 N	12-130
ALKP (U/L)	<10 L	66 N	14-111
GGT (U/L)	44 H	0 N	0-4
Amylase (U/L)	932 N	872 N	500-1500
Lipase (U/L)	826 N	709 N	100-1400
Total Bilirubin (mg/dL)	0.4z	<0.1N	0.0-0.9
Cholesterol (mg/dL)	109	93 N	65-225
Na+ (mmol/L)	147	159N	150-165
K+ (mmol/L)	7.8	5.6 N	3.5-5.8
Cl- (mmol/L)	109	119N	112-129
TT4 (µg/dL)	0.9	2.1	0.8-4.7

Pathologist's Report

Necropsy Sample (Fig. 3a, 3b, 3c, 3d,) Feline patient's stomach tissue with ulcer.

Microscopic Description The gastric wall is extensively ulcerated intramurally, extending deep into the submucosa, muscularis externa, and serosa **(Fig. 4a).** Inflammatory infiltrates, transgressing transmurally, form an extensive thick serosal band with the maximum cell density **(Fig. 4b).** The submucosa is visibly expanded by the lymphoid follicular aggregates, dilated lymphatic channels, hemorrhagic foci, and occasional edematous open spaces **(Fig. 4c,4d).** Within the lamina propria, proliferation of spindle cells is conspicuous. Numerous blood vessels lined with hypertrophied endothelial cells indicate

the presence of granulation tissue. Infiltrating neutrophils (ranging from perivascular to interstitial), histiocytes, plasma cells, and lymphocytes are clearly discernible (Fig. 4f). The mild to moderately hyperplastic mucosa exhibits occasional tortuous crypts. There is no benchmark evidence of malignancy like neoplastic cells, and no pathogenic microorganisms are seen. Interpretation: advanced chronic transmural, locally extensive, ulcerative gastritis with the presence of granulation tissue, hemorrhagic foci, and edema (Fig. 4e). Deranged histoarchitecture, in the absence of neoplasm, may be related to foreign body trauma, gastrin-secreting pancreatic tumors,

Survey radiographs

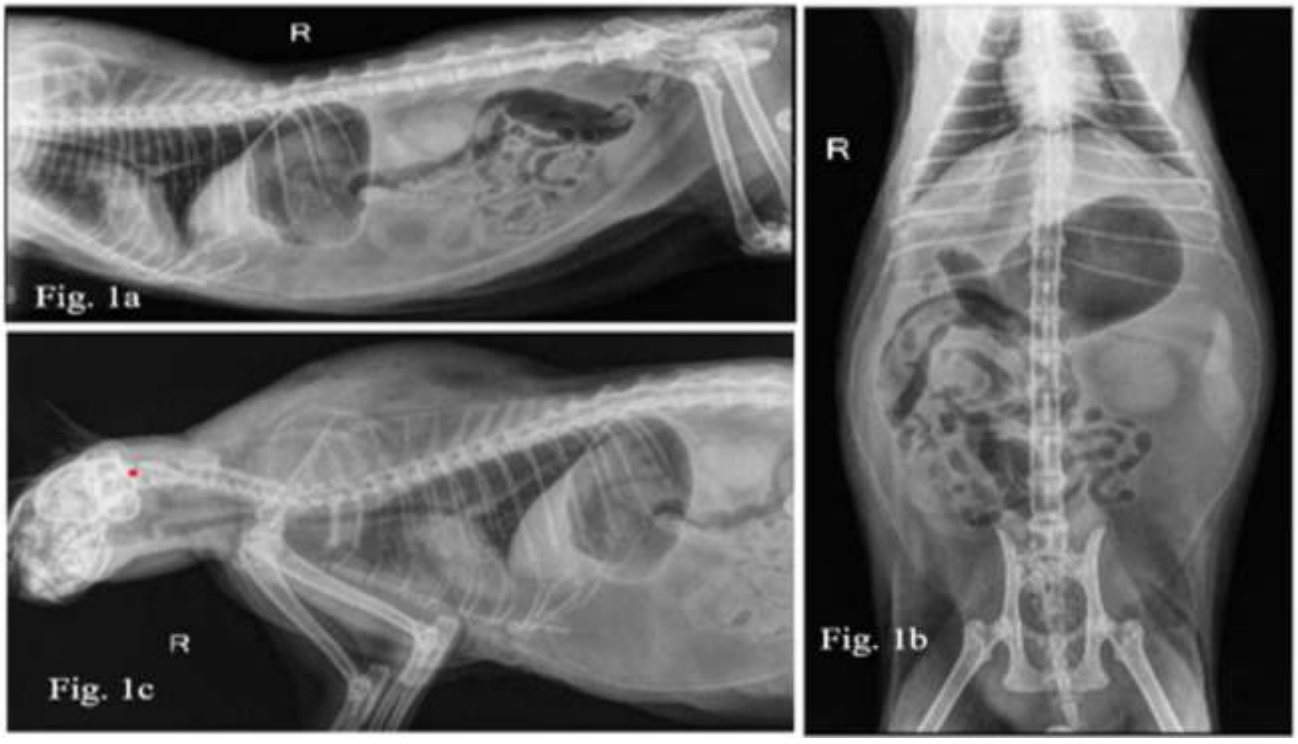

Fig. 1a. RL view no evidence of tumor seen Nov. 29, 2021.
Fig. 1b. VD view no obvious tumor seen Nov. 29, 2021.
Fig. 1c. RL head view no evidence of tumor

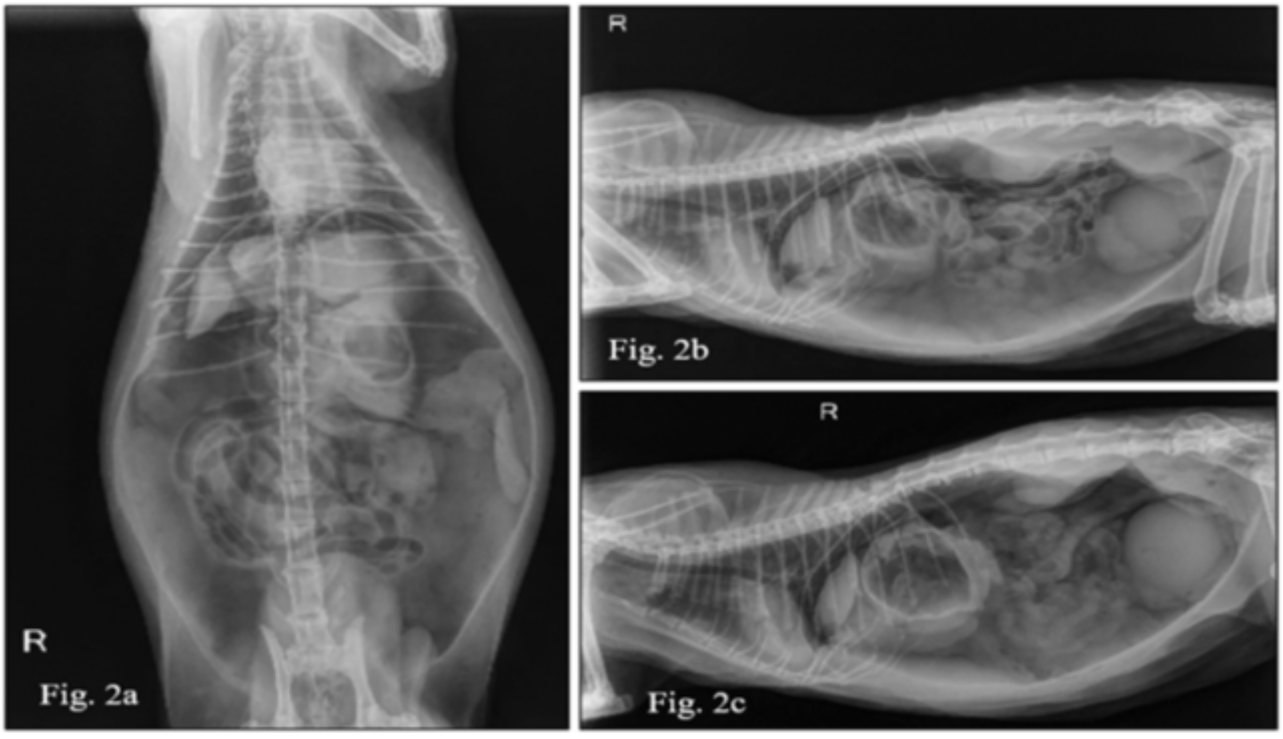

Fig. 2a. VD view- Pneumoperitoneum Dec. 14, 2021
Fig. 2b. RL view- Pneumoperitoneum after tapping air Dec. 14, 2021
Fig. 2c. RL view- Pneumoperitoneum after tapping air Dec. 16, 2021

Necropsy findings

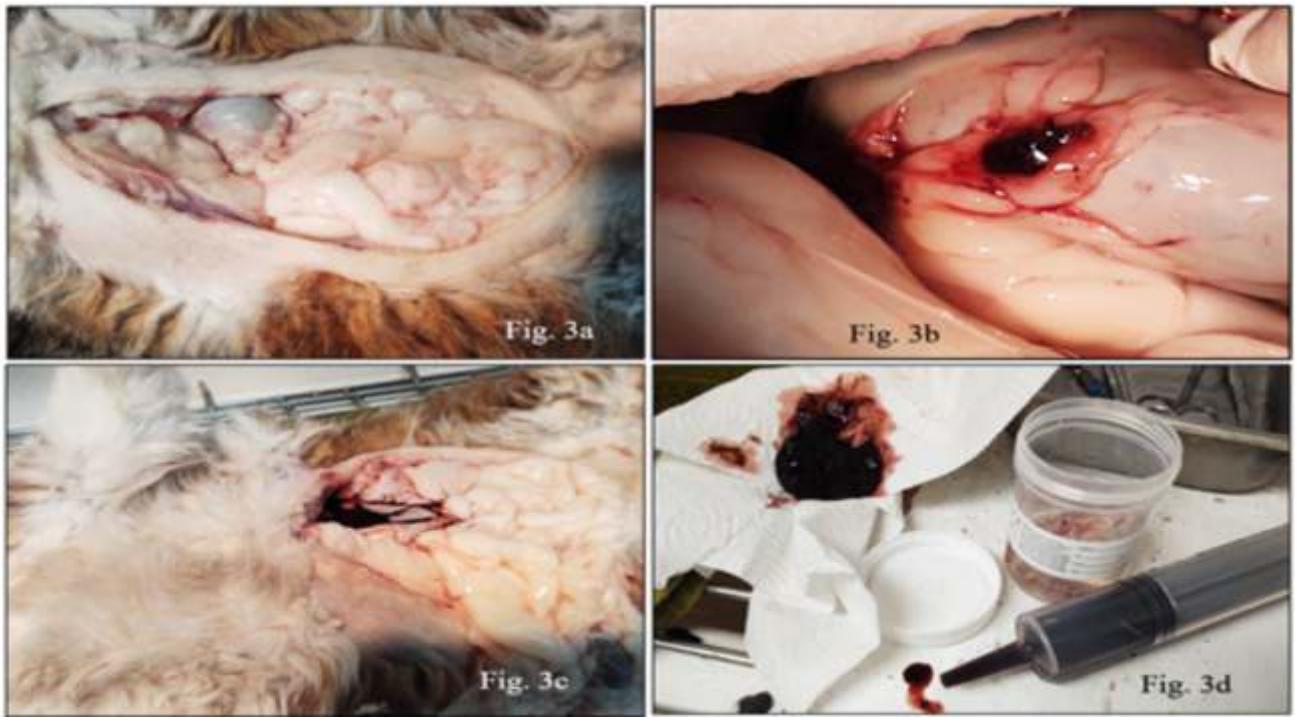

Fig. 3a. Abdominal cavity opened.
Fig. 3b. Stomach tissue with a solitary prominent ulcer.
Fig. 3c. Blood oozing out from the ulcer crater.
Fig. 3d. Collection and Preservation of sample for histopathology

mastocytomas, hepatic, or renal dysfunction.

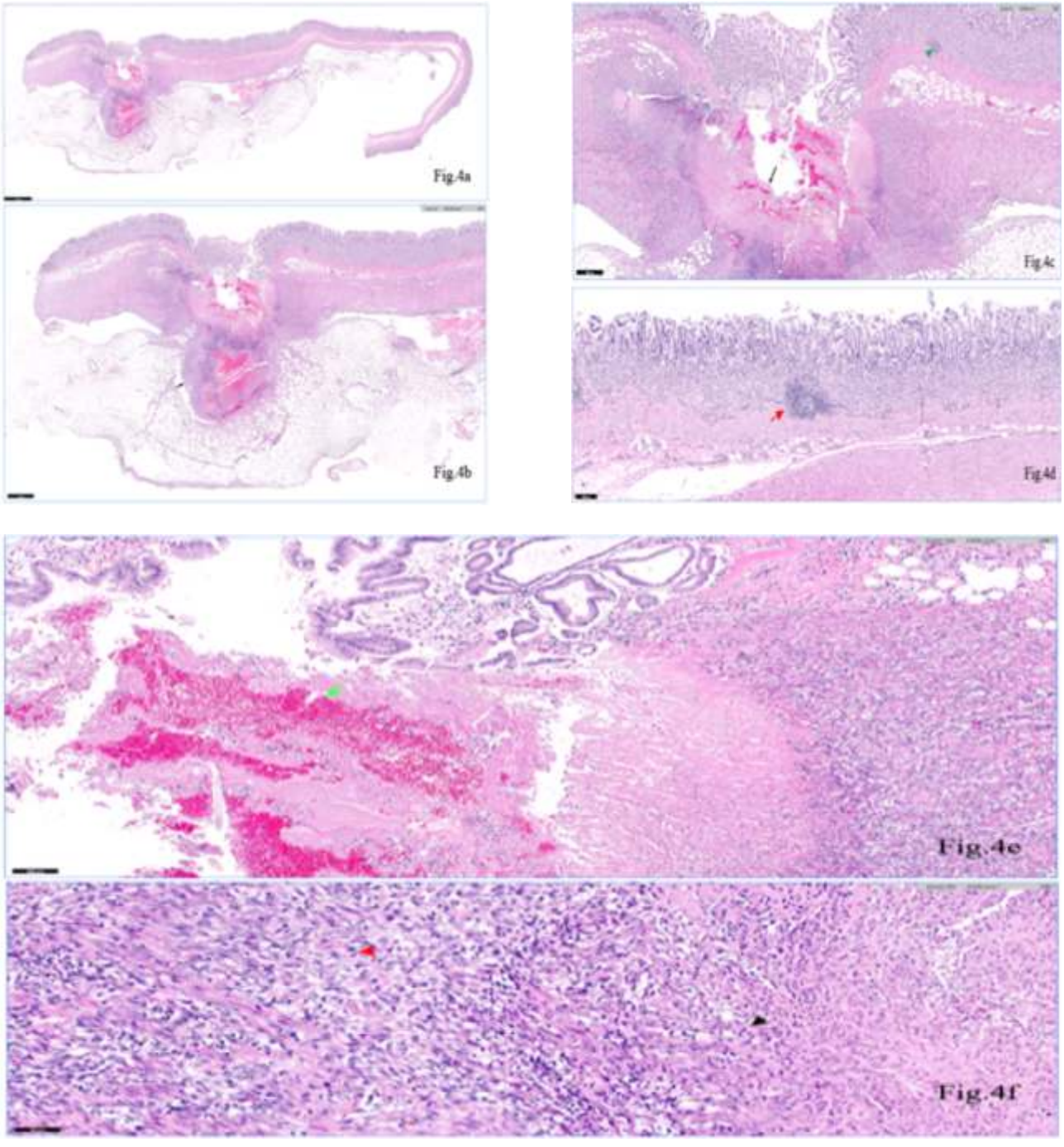

Fig.4a. The gastric wall, intramurally ulcerated, extending deep into the underlying submucosa, muscularis externa, serosa (H&E, 0.5x).

Fig.4b.The inflammatory infiltrates, extending transmurally, form an extensive thick serosal band (Black arrow) (H&E, 1x).
Fig.4c. Ulceration and necrotic debris (black arrow), lymphoid aggregate (green arrowhead) (H&E, 2.0x).
Fig. 4d. Lymhofollicular aggregates (red arrow) (H&E -5x).
Fig. 4e. Hemorrhage (Green arrow) (H&E -10x).
Fig. 4f. Spindle cells (Reactive fibroplasia red arrowhead, neutrophils black arrowhead (H&E -20x

Discussion

Survey radiographs revealed no radiopaque patches, suggestive of malignancy in any internal organ in the instant case. Increased circulatory SDMA concentration indicated early renal dysfunction. The patient was hospitalized and kept on antibiotic-nutraceuticals combination regimen after surgical management of deep abscess in the left cheek. Fecal smear from watery diarrhea revealed TNTC Clostridium spp. The patient recovered and was discharged with advisory on home care medicaments. On scheduled recheck visits the patient exhibited near normalcy in body condition. However, the sudden deterioration in the overall health status was totally unexpected. Acute respiratory distress on December 15, 2021 was of much clinical concern. Repeat radiographs **(Fig. 2)** revealed pneumoperitoneum and hemogram **(Table 2)** accentuated anemia (PCV 10%); Differentials: hemolytic anemia, GU ulceration with blood loss. The patient, hospitalized again, remained under constant medical surveillance. The abdominal gas was tapped out carefully. On December 16, 2021 the patient was apparently comfortable. However, on December 17, 2021 the cat suddenly collapsed and died.

Resume´ of the published reports highlights the indispensability of judicious surgical intervention. GU ulcers, often leading to wall perforation are named 'spontaneous' in the evidence-based absence of any foreign body, gastric dilatation and volvulus, trauma, leakage from previous gastrotomy/ anastomosis site (14). Cats with GU clinical syndrome often present in a critical condition with no indication of GI tract perforation, and peritonitis. From the biomedical perspective, clinical cases of GU ulceration may be categorized into neoplastic and non-neoplastic. Endoscopic probe/ultrasonography is suggested to locate imperforated GU (15) with adjunctive medical therapy: Clavamox, enrofloxacin generic and metronidazole (11). Latent GU ulceration with subsequent wall perforation in the feline patients may result from neoplasia, mostly Adenocarcinoma (16, 17), inflammatory bowel disease (IBD)18, stress from surgery (19), gastric hyperacidity from systemic mastocytosis (15, 20, 21) or Gastrinoma (22, 23), and bacterial granuloma (24). However, in most of the reports the specific cause for ulceration/ perforation was not determined conclusively (25, 26, 27, 28). Cats with hemorrhagic GU need urgent transfusion of whole blood from a healthy donor cat, invariably after cross-matching, or blood typing: B donor→A recipient; A donor→B recipient (28). Severe gastric hemorrhage necessitates prompt surgical intervention: exploratory or curative, with biopsies referred expeditiously for histopathology (29). In our well-considered view, with no evidence of GU malignancy, or acute peritonitis, exponential accumulation of gas trapped within the peritoneal cavity, 'tension pneumoperitoneum' culminating in respiratory failure: hypoxemia/ apnea proved fatal in the feline patient in the instant case; the deep septic facial wound was totally unrelated to pneumoperitonitis.

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Gene Therapy in Humans and Livestock Species: Current Status and Future Perspectives

I C Datta, Kabita Roy, Rakesh Saindla, Srashty Singh, Sabita Rakshit and Dale Clark
College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh and
Milford Veterinary Clinic, Milford, MI, USA

Introduction

Gene, the torch bearer of heredity characters with specific base DNA sequence in humans and animals, carries information for well-defined intracellular protein biosynthesis **(Fig. 1).** Variations in this molecular code or 'mutations' often harmless, occasionally induce a serious disease cascade. Gene therapy is the upcoming biotechnology tool, designed to replace the deleterious degraded gene with the corresponding functional gene, enabling uninterrupted synthesis of functional protein (1). Homologous recombination, the conventional approach, is insertion of a normal gene into a nonspecific locus, designed to replace the defective gene within the genome, or selective reverse mutation **(Fig. 2),** aimed to repair the deformed gene, or altered regulation of the defective gene (2).

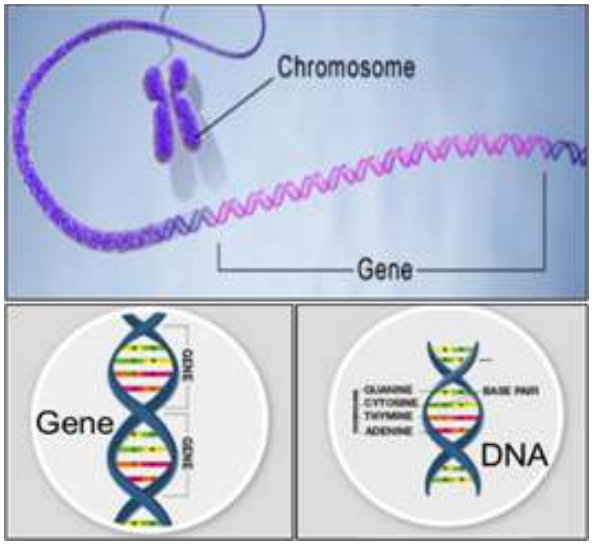

Fig. 1 Gene and DNA

Currently, the main focus is on overcoming some common human ailments at the point of origin itself.

(i) Inherited autosomal or cross-linked recessive single gene mutation resulting in cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia.

(ii) Acquired genetic diseases, e.g., acquired immunodeficiency syndrome (AIDS) and cancer.

(iii) Cardiovascular disorders, arthritis, diabetes mellitus, Parkinson's disease, Alzheimer's disease under gene therapy trials (3,4).

Evidence-based impact of genome-editing technology in development of livestock breeding and production in cattle, sheep, goats and pigs is highlighted in a comprehensive review. Pandemic disease-resistant genome modified farm animals, generated with. DNA nucleases, are being increasingly used as human disease models, production of β-lactoglobulin allergen-free cow milk, improved quality pork/goat meat, and bioreactor piglets for human serum albumin (HAS) (5).

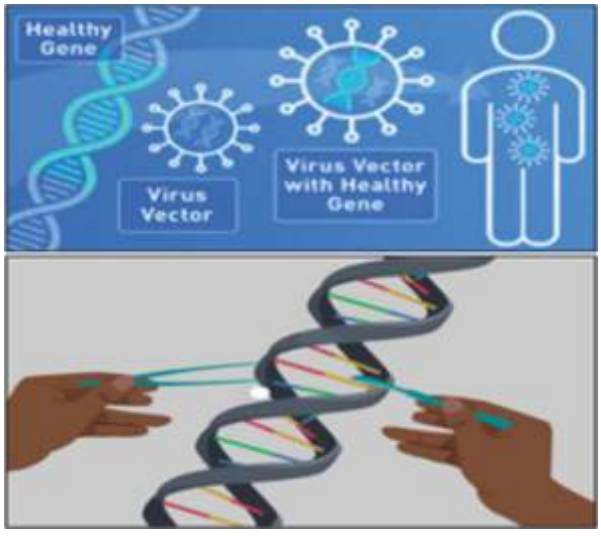

Fig. 2 Insertion of a normal gene to replace the defective gene.

Gene Therapy Protocols

(i) Somatic Gene Therapy Insertion of a functional, expressible gene into a targeted somatic cell to overcome a genetic disease permanently. This is a more conservative and safer approach, preempting the risk of inheritance by the next generation. Despite the limitations associated with apoptosis (programmed cell death) and improper gene insertion, this methodology is considered most appropriate in rectifying many human genetic disorders.

(ii) Germline Gene Therapy Functional genes are incorporated into germ cells (sperm/ovum). However, this technique is not preferred in view of the obvious risk: changes resulting from this

therapy, though long-lasting in the individual patient, are heritable (6).

Gene Therapy Procedures

I. Ex Vivo

(i) Isolate the individual patient's identified homologous cells with genetic defect from the well-prepared patient.

(ii) Multiply these cells in the selected culture medium in the optimized micro-environment and time interval.

(iii) Transfer the therapeutic gene to rectify the genetic defect in each harvested cell.

(iv) Select the genetically modified cells and multiply these in culture.

(v)Transplant the genetically modified cells in the patient as per the protocol II.

II. In Vivo

(i) Transfer the therapeutic gene directly into the target cells of the affected tissue, e.g., liver, spleen, lung, brain, muscle, blood cells, skin.

(ii) Bio efficacy evaluation parameters: magnitude of incorporation of the therapeutic gene in the target defective cells, intracellular degradation of the introduced gene, followed by uneventful integration

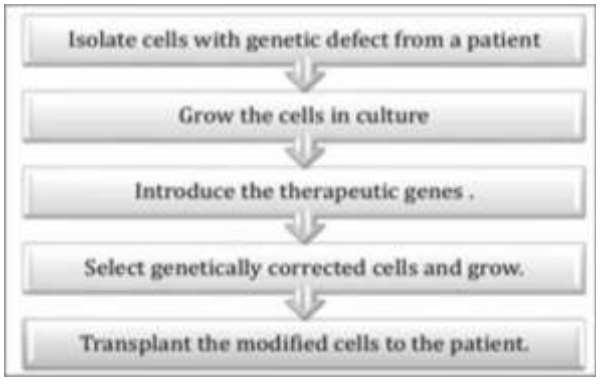

Fig. 3a *Ex Vivo* Procedure.

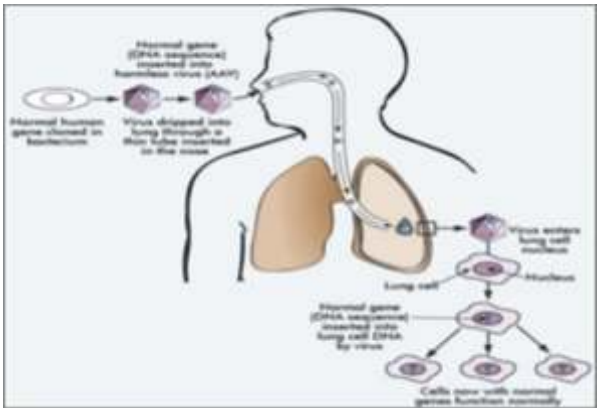

Fig. 3b In Vivo Procedure

in the functional host cell nucleus, and finally functional status of the modified gene (6) **(Fig.3a, b).**

Vectors Used in Gene Replacement

In biotechnology terminology, the carrier particles, or molecules (viral/non-viral) used in transferring the healthy genes into the target cells are named 'vectors'. The ideal vector needs to be specific, able to deliver one or more genes of the appropriate size, and capable of being recognized by the innate immune system for safe passage, and finally permit gene expression for a sufficient duration.

(i). Conventional Virus Vectors Viruses are actually genes, wrapped up with a special protein coat, capable of binding to the surface of cells before entry into the cytosol. This capability is utilized after manipulating the viral genome and replacing with the functional human gene. Once the transplanted gene is sucked and lodged in the right chromosomal locus in the diseased person, it can transmit instructions for synthesizing the specific functional proteins. Virus vectors: Retroviruses are the classic gene transfer agents (7, 8). Viral reverse transcriptase promotes synthesis of double stranded DNA copies of the RNA genomes. These bio entities are integrated in the chromosomes of the human cells. Presently, the drawbacks of this technique include (a) low in vivo efficacy (b) immunogenic limitations (c) inability to transduce the non-dividing cells (d) risk of erroneous gene insertion leading to tumour- suppressor gene inactivation and oncogenesis. However, if the genes encoding nucleases are incorporated into the chromosomal matrix; the expressed nucleases then 'edit' the chromosome disrupting genes, responsible for disease induction. Successful treatment of human x-linked severe combined immune deficiency syndrome with retroviral vector is a landmark biomedical event (6). Alternative viral vectors: Adenoviruses type 2 and type 3, Adeno-associated Viruses (AAVS), Herpes Simplex Virus (HSV), Lentivirus.

(ii) Non-viral vectors These include all physical and chemical gene transfer agents, replacing traditional viral bio entities. Though the efficacy is relatively less, the procedures with manipulative convenience (no particle size limitation) are also cost-effective. More importantly, there is less possibility of adverse immune system activation. In perspective, these are receiving increasing attention in the contemporary bio-engineering protocol. The focus is on chemical methods utilizing nanomeric complexes, e.g., negatively charged nucleic acid compacted with polycationic nanomer particles: liposome/micelle polymers. Alternatively, synthetic oligonucleotides may be used in inactivating the deleterious genes responsible for

disease onset by disrupting the transcription or blocking translation of the degenerated mRNA (9). This contention is validated by the successful treatment of Huntington's disease in humans (10).

Genome-edited Livestock Species

Significant progress has been made in genome-editing biotechnology utilizing novel programmed DNA nucleases: zinc-finger nuclease (ZFN), transcriptor activator-like endonuclease (TALEN), and the clustered regularly interspersed short palindromic repeats/ Cas9 system (CRISPR/Cas9) (11). These tools are capable of splitting DNA double helix precisely by targeting specific sequences and enable the addition, removal or replacement of nucleotides at the appropriate genomic loci. CRISPR/Cas9 tool, relying on RNA-DNA binding, able to generate multiple genomic nicks concurrently, is simpler and more cost-effective, compared to the other genome-editing tools, relying on protein-DNA interactions. Carefully scanned published reports revealed that judicious use of the appropriate genome-editing tools has significantly contributed to increased disease resistance, improved productivity, easy availability of allergen-free dairy/goat milk promoting human nutrition, commensurate with animal welfare.

Increased Disease Resistance

(I) Bovines Bovine tuberculosis continues to be hazardous to agricultural economy and public health globally. In perspective, evolution of tuberculosis-resistant cattle is a landmark in gene therapy (12). The natural resistance-associated macrophage protein-1 (NRAMP-1) gene significantly boosts the innate resistance to Mycobacterium bovis and other intracellular pathogens. Transgenic cows with the site-specific NRAMP-1 gene exhibit enhanced resistance to tuberculosis (13).

(ii) Pigs Porcine reproductive and respiratory syndrome (PRRS), a highly detrimental disease globally is refractory to the currently available vaccines. The virus receptor site in the host cell (CD163 protein) was identified as the appropriate target to block the viral infection, CD163-nullified pigs were generated with CRISPR-Cas9 genome targeting technology (14).

Improved Human Healthcare

Genome-editing: ZFN, TALEN, or CRISPR/Cas9-mediated is expected to impact the future course of breeding and production in dairy cattle, sheep, goats and pigs. This contention is strongly supported by the facilitated availability of β-lactoglobulin allergen-free dairy/goat milk (15,16,17). ZFN-mediated disruption of muscle protein (myostatin) resulted in 100% increase in muscle mass with low fat content, suitable for human nutrition in Meishan pigs (18), native to Southern China. known for the large litter size of 15-22. A similar genome-editing response in goats is on record (19). Further, specific germline-edited cell lines successfully generated hornless cattle, contributing to the safety of dairy attendants and herd mates (20). CRISPR-Cas9 gene-editing tool has been successfully used to insert human albumin cDNA gene into the pig endogenous albumin locus, and transgenic piglets with high demand human serum albumin (HAS) in their blood circulation were produced (5).

Pros and Cons of Gene Therapy

- Gene therapy is the futuristic medicine, though at present, it cannot obliterate genetic disease, before the onset of clinical symptoms.
- Multigene disorder. Low intensity disorders associated with single gene mutation are best suited for gene therapy. However, the most common diseases in humans are multigenic, for example heart disease, hypertension, arthritis, diabetes mellitus, and Alzheimer's disease.
- Insertional mutagenesis: If the therapeutic gene (DNA template) is accidentally inserted in a wrong site in the genome, e.g. in a tumour-suppressor gene it may promote tumour formation,
- Chance recovery of the capacity to cause disease with the use of viral vectors.
- Short duration of efficacy.
- Ethical, legal issues and social acceptance of genome-edited livestock products (21, 22).

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Emerging Viral Zoonotic Diseases in India: An Overview

Ayashi Sachan , Ranvijay Singh, Bhavana Gupta and Rashmi Kulesh
Dept. of Veterinary Public Health and Epidemiology, NDVSU, Jabalpur, Madhya Pradesh

Introduction

Zoonotic infectious diseases have been important concern to humans since the beginning of the domestication of animals 10,000 years ago. Infectious diseases remain a significant cause of mortality and morbidity globally. Approximately 75% of emerging infectious diseases (EIDs) are zoonoses. According to recent WHO data, more than 75% of the different zoonotic diseases that may cause illnesses in humans are transmitted through animals and/or animal products (1). Nevertheless, several zoonotic pathogens may be transmitted from various animals to man via several direct and/or indirect pathways such as close contact with the infected animals that might be shedding the infectious pathogen, when humans use contaminated sources of food or water, and/or by outdoor or indoor animal scratches or bites. The prevention and control strategies against zoonotic pathogens are considered important issues and a global challenge requiring efforts of all veterinarians and medical staff.

Emerging Zoonoses on the Rise

It is clear that there is an increased recognition of the emergence of zoonotic infections. New technologies have expanded the sensitivity and scope of our detection and diagnostic capability. However, a pathogen may still go undetected if it does not cause a significant disease outbreak. Over time, changes in human population density, mobility, lifestyle, behaviours and food choices have all influenced the dynamics of zoonotic disease emergence and have served as drivers of pathogen transmission. As the human population has grown, the influence of increased urbanization, coupled with higher incomes, has also led to greater demands for domesticated and wild animal meat products. This development has greatly expanded both our consumption of animal-source protein and our need for land to grow these animals and their feedstuffs, leading to tremendous modification of previously remote and seldom-visited regions into ever more intense livestock and agricultural production areas. This expansion of animal production into areas with greater abundances of wildlife has resulted in numerous examples of zoonotic disease emergence directly related to agricultural practices, including Henipaviruses (pigs and horses), Middle East respiratory syndrome-coronavirus (CoV) (camels), Crimean-Congo hemorrhagic fever virus (ostriches) and other tick-borne bunyaviruses (2).

Emerging Infections in India: Trends and Epidemiological Features

Developing countries such as India suffer disproportionately from the burden of infectious diseases. India, the most populous country in the world, is in the midst of a triple burden of diseases; the unfinished agenda of communicable diseases, non-communicable diseases linked with lifestyle changes and emergence of new pathogens and overstretched health infrastructure. Communicable diseases account for nearly half of India's disease burden. Many infections are associated with poor sanitation, contaminated food, inadequate personal hygiene, or access to safe water and lack of basic health services. These conditions are common to large parts of India. Favourable environmental, demographic and socio-economic factors further put India at a risk of epidemics of emerging infections. Over the years increase in cases of drug resistant malaria, CCHF, Nipah outbreak in Kerala and epidemics of avian influenza have demonstrated the vulnerability of India to the threat of evolving microbes (3).

Some of the Emerging Zoonotic Viral Infections in India are as follows

Chikungunya Fever

Chikungunya fever is a viral disease transmitted through the bite of infected Aedes mosquitoes. The disease typically consists of an acute illness with fever, skin rash, and incapacitating arthralgia. The word chikungunya means, "to walk bent over" in the African dialect Makonde and refers to the effect of the incapacitating arthralgia seen in the affected. Chikungunya virus (CHIKV) was first isolated from the serum of a febrile human in Tanganyika (Tanzania) in 1953 (4). The sudden onset of the disease including crippling arthralgia and frequent arthritis that accompany fever, chills, headache, nausea, vomiting, low back pain and rash are clinically distinctive. The disease is almost self-limiting and rarely fatal.

Etiology

CHIKV is a Group IV (+) (RNA) belonging to family Togaviridae, genus Alphavirus with icosahedral symmetry. The virion is 70 nm in diameter, and it is

composed of repeating units of the E1 and E2 transmembrane glycoproteins (240 heterodimers of E2/E1 arranged as trimeric spikes on its surface), the capsid (C), a host-derived lipid bilayer, and a single molecule of genome RNA. The genome is approximately 12 kb in length and encodes the non-structural proteins (nsPs) at the 5 end and the structural proteins at the 3 ends. The nsPs are translated from genomic RNA and the structural proteins from a sub genomic RNA (5). Genetic analyses suggest that the virus originated in Tropical Africa and subsequently evolved into 3 distinct genotypes- the east African, the west African and the Asian genotypes.

Host Range

While humans serve as reservoirs during urban epidemics, several animal species are capable to attain CHIK infection. Monkeys, rodents and birds are thought to act as reservoirs for the virus, and it is likely that other vertebrate's hosts have not yet been identified.

Transmission

CHIKV is most commonly transmitted to humans through the bite of an infected mosquito, specifically mosquitoes of the Aedes genus, which usually bite during daylight hours. Aedes aegypti is considered to be the principal vector and A. albopictus (Asian Tiger mosquito) has also recently emerged as an important vector. It predominantly breeds in stored fresh water, such as desert coolers, flower vases, water-tanks, etc., and in peri-domestic areas (discarded household junk items like vehicular tyres, coconut shells, pots, cans, bins, etc.,) in urban and semiurban environments. Adult mosquitoes rest in cool and shady areas and bite humans during the daytime. Insecticide treated bed nets are, therefore, of limited use against Aedes mosquitoes. The bite of only the female mosquito is considered to be infective because a blood meal is required for the formation of the egg. Vertical maternal foetal transmission of CHIKV has also been observed (6).

Clinical Signs

The symptoms develop after an incubation period of 4 to 7 days (incubation period lies between 1 and 12 days). In most of the cases, the disease is self-limiting, and the symptoms disappear within 5 to 7 days even without treatment. A clinical triad of 'fever, rashes and arthralgia' is suggestive of chikungunya fever. The clinical features vary from high fever (more than 400C, rapid in rise and sometimes associated with rigor), severe headache, chills and rigors, nausea and vomiting. The fever may disappear to return in one or two days giving it the name of 'Saddle back fever'. The occurrence of poly-arthralgia along with myalgia is a typical feature of the illness. The joint pain is frightening in severity, completely immobilising many patients and preventing sleep in first few days of the illness. The joint becomes very painful to touch. Movement at the joints causes excruciating pain to the person forcing to make bend up position giving it the name 'Chikungunya'. The maculo-papular rashes and gingival haemorrhages are uncommon signs although more frequent in children. Rashes occur mainly on trunks or extensor surfaces of the limbs and are itching in nature. The rare complications include myelo meningo-encephalitis, Guillain barre syndrome, fulminant hepatitis, myocarditis and pericarditis.

Diagnosis

The probable diagnosis of chikungunya fever can be made on the basis of clinical signs. The virus produces neutralizing and haemagglutination inhibiting (HI) antibodies and that helps in making serological diagnosis. HI test is a simplest diagnostic test, but it identifies the group rather than specific virus. Confirmation of the illness is done by detection of the antigen or antibody to the agent in the blood sample of patient. Reverse transcriptase polymerase chain reaction (RT-PCR) is confirmatory for the identification of chikungunya virus. IgM capture ELISA is the most sensitive serologic assay and is necessary to distinguish the disease from dengue. The virus isolation procedures need to be done under bio safety level 3 (BSL-3), although, such precautions may not be necessary in the countries where chikungunya virus is endemic.

Treatment

There is no specific antiviral drug treatment for chikungunya. Treatment is directed primarily at relieving the symptoms, including the joint pain using anti-pyretics, optimal analgesics and fluids. There is no commercial chikungunya vaccine (7).

Crimean Congo Haemorrhagic Fever

Crimean-Congo Haemorrhagic Fever (CCHF) is a zoonotic viral disease that is asymptomatic in infected animals, but a serious threat to the health of humans. Human infections begin with non-specific febrile symptoms but progresses to a serious haemorrhagic syndrome with a high case fatality rate. CCHF was first recognized in Crimean Peninsula in 1944 and was first isolated at Congo in 1956. There by the current name was adapted for virus and of disease caused by it (8).

Etiology

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is a member of genus Nairovirus in the family Bunyaviridae. CCHF virus is a spherical enveloped virus with approximately 100nm diameter and has glycoprotein spikes 8-10nm in length. Under electron microscopy, the virion of CCHF can be distinguished from other members within the Bunyaviridae family, as they possess small morphologic surface units with no central holes

arranged in no obvious order (9).

Host Range

The CCHF virus may infect a wide range of wild animals and domestic ruminant animals such as hares, rats, camels, cattle, sheep and goats. Many birds are resistant to infection, but ostriches are susceptible and may show a high prevalence of infection in endemic areas. Animals become infected with CCHF from the bite of infected ticks. Human beings are the only host of CCHF in whom the disease manifestations are visible.

Numerous genera of ixodid ticks serve both as vector and reservoir for CCHFV; however, occurrence of CCHF closely approximates the known world distribution of ticks in the genus Hyalomma spp. ticks. The most important source for acquisition of the virus by ticks is considered to be infected small vertebrates on which immature Hyalomma ticks feed.

Mode of Transmission

Animal to Human Transmission

Human beings may acquire the CCHF virus by direct contact with blood or other tissues of infected livestock or they may become infected through a tick bite or crushing of infected tick. Meat itself is not a risk because the virus is inactivated by post-slaughter acidification of the tissues and would not survive cooking.

Human to Human Transmission

Humans can become infected if blood, body fluids and wastes from patients with the disease comes into contact with broken skin or mucous membranes, as occurs when medical care personnel sustain accidental needle stick injury. In advanced stages of the disease, aerosol contact of blood of the patient can also lead to transmission of the virus.

Clinical Signs

The first sign of CCHF is a sudden onset of fever and other non-specific symptoms including chills, severe headache, dizziness, photophobia, neck pain, myalgia and arthralgia and the accompanying fever may be very high. Gastrointestinal symptoms including nausea, vomiting, non-bloody diarrhoea and abdominal pain are also common. It is followed, after several days, by the haemorrhagic phase.

The hemorrhagic phase develops suddenly. It is usually short, lasting on average two to three days. A petechial rash may be the first symptom. The rash is followed by petechiae, ecchymoses and large bruises on the skin and mucous membranes. Hematemesis, melena, epistaxis, haematuria, haemoptysis and bleeding from venepuncture sites are also common. Some patients die from haemorrhages, haemorrhagic pneumonia or cardiovascular disturbances. In patients who survive, recovery begins 10 to 20 days after the onset of illness.

Diagnosis

CCHF virus infection can be diagnosed by several different laboratory test which include enzyme linked immunosorbent assay (ELISA), antigen detection, serum neutralisation, reverse transcriptase polymerase chain reaction (RT-PCR) and virus isolation. Reverse-transcriptase PCR (RT PCR) is the method of choice for rapid laboratory diagnosis of CCHF virus infection. The ELISA test is considered the most sensitive and specific. IgG and IgM antibodies may be detected in serum by ELISA from about six days of illness.

Treatment

Hospitalisation in special care unit with constant laboratory monitoring is cornerstone for treatment of CCHF. Till date, there is no FDA approved drug or definitive treatment for CCHF. According to World Health Organization (WHO), ribavirin is the anti-viral medication used to treat CCHF and the recommended dose is an initial dose of 30mg/kg followed by 15mg/kg for four days and then 7.5mg/kg for six days for a total of 10 days.

Supportive care includes fluid management by IV crystalloids, oxygen, cardiac monitoring and administration of blood and blood products as clinically indicated. Care should include careful attention to fluid balance and correction of electrolyte abnormalities, oxygenation and hemodynamic support, and appropriate treatment of secondary infections.

Hanta Virus

Hantaviruses are described as emerging pathogens as newer serotypes are being discovered in many areas non-endemic to Hantaviruses (10). Hantaviruses are the most widely distributed zoonotic rodent-borne viruses and can cause two important clinical syndromes: haemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS) in Asia and the Americas respectively. Currently up to 21 species and more than 30 genotypes of hantaviruses have been described the important species include Hantaan virus (HTNV), Seoul virus (SEOV), Puumala virus (PUUV), Sin Nombre virus (SNV) and Dobrava-Belgrade virus (DOBV) (11).

Etiology

Hantaviruses are enveloped RNA viruses (family Bunyaviridae, genus Hantavirus) that have a negative-sense, tri-segmented genome. The large (L) segment codes for the viral RNA-dependent RNA polymerase (RdRp) and the medium (M) segment for the glycoprotein precursor (GPC) which is processed into the two envelope glycoproteins (G1 and G2). The small (S) segment codes for the nucleocapsid

(N) protein. The hantavirus particle is spherical with a diameter ranging from 80-120 nm and consists of three circular nucleocapsids, each contains one RNA segment complexing with N and RdRp proteins (12).

Old World and New World Hantaviruses

Hantaviruses are classified into two main groups: Old World and New World hantaviruses. The Old-World Hantaviruses include species which cause HFRS in Asia and Europe while the New World hantaviruses cause HPS in the Americas. The New World Hantaviruses are associated with rodents belonging to the Sigmodontinae subfamily while the Old-World Hantaviruses are associated with rodents of the subfamilies Murinae and Arvicolinae.

Transmission

Transmission of infection is via the aerosols generated from virus-contaminated rodent faeces, urine or saliva. Hantavirus transmission among rodents occurs through bites and may also result in human infection. Transmission of infection may also probably occur via food or hands contaminated by rodent excreta or via rodent bites or scratches. Human to human transmission of Hantaviruses can also occur. There is an occupational risk of transmission of hantavirus infections with animal trappers, forest workers, farmers and military personnel at greater risk. Laboratory and animal facility-acquired hantavirus infections due to contact with infected animals and infected cell lines has been reported in laboratory personnel.

Clinical Signs

Hanta Virus Cardiopulmonary Syndrome (HCPS)

It can be described to occur in two phases, prodromal and cardiopulmonary phase. The prodromal stage is characterized by fever, headache, chills and myalgia. The onset of the cardiopulmonary phase is with pulmonary edema, dyspnea and hypoxemia. Patients with severe illness may progress to cardiac depression, respiratory failure and acidosis leading to fatal arrhythmias.

Hanta Virus Haemorrhagic Fever with Renal Syndrome (HFRS)

The incubation period is 1-5 weeks, and the onset of the disease is with fever and influenza-like symptoms. Hemorrhagic manifestations if present are seen as flushing of the face, injection of the conjunctiva and mucous membranes. The disease is conveniently described as having five phases: a febrile phase lasting 3-5 days followed by a hypotensive (shock) phase lasting from a few hours to a few days, a subsequent oliguric phase lasting 3-7 days and finally a diuretic phase leading to the convalescent phase. Initial symptoms begin suddenly and include intense headaches, back and abdominal pain, fever, chills, nausea, and blurred vision. Individuals may have flushing of the face, inflammation or redness of the eyes, or a rash. Later symptoms can include low blood pressure, acute shock, vascular leakage, and acute kidney failure, which can cause severe fluid overload (13).

Diagnosis

It is difficult to diagnose Hantavirus infections clinically as the early signs and symptoms of the disease are non-specific. Serology is the mainstay of diagnosis of Hantavirus infections as the viraemia in HFRS patients is short-lived. The virus is detected in cell culture by Immunofluorescence assay (IFA). Isolation of Hantaviruses from clinical specimens is difficult and hazardous as it should be performed in biosafety level-3 laboratories. Detection of virus-specific IgM is advocated for diagnosis of acute Hantavirus infections. The ELISA is used as it has a higher specificity than IFA. Reverse transcriptase-polymerase chain reaction (RTPCR) using genus and species-specific primers are also employed for diagnosis of Hantavirus infections.

Treatment

There are no effective anti-viral drugs for the treatment of all hantavirus infections. Ribavirin (1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) has been used in clinical trials for treatment of HFRS patients in the People's Republic of China and has shown reduction in fatality. However, it remains ineffective for treatment of HPS. Supportive therapy is the best to control progression towards life threatening.

Prevention and Control of Viral Zoonoses

Effective prevention and control measures can be achieved through proper diagnostics and prophylactic aids to curtail further spread in most of zoonotic viral diseases. Improved sanitary conditions such as proper treatment and disposal of human waste, higher standards for public water supplies, improved personal hygiene procedures and sanitary food preparation are vital to strengthen the control measures. A clear understanding of epidemiology of the diseases with wildlife as reservoir namely the virulence and transmissibility of many diseases could help in understanding the severity and thereby to take appropriate measures in eradication of such dreadful diseases. Research should focus on molecular biology of these viruses so as to develop diagnostics and prophylactics in a modern way to combat these infections in short time. To safeguard the public health from pathogens of zoonotic infections, application of skills, knowledge and resources of veterinary public health is essential. It is time to combat viral zoonoses with a combined effort of veterinary and public health specialists. A better understanding of avian migration patterns and their infectious diseases would be useful to forecast disease outbreaks due to emerging zoonotic infections like avian influenza (14).

Further, the control measures for emerging and re-emerging viral pathogens are demanding, as there is population explosion. Novel, highly sensitive and specific techniques comprising genomics and proteomics along with conventional methods would be useful in the identification of emerging and re-emerging viruses, thereby; therapeutic/ prophylactic/ preventive measures would be applied on time. The first line of measure to control any disease is the surveillance. Control and prevention strategies should be designed based on transmission pattern and characteristics of virus, involvement of vectors, environment and epidemiology of the disease. The European Union (EU) has established a network termed as Med-Vet-Net to develop a network of excellence for the integration of medical, veterinary and food scientists in order to develop food safety measures and to improve research on the prevention and control of zoonoses, including food-borne diseases. The network will also consider the concerns of consumers and other stakeholders throughout the food chain. Another system the Hazard Analysis and Critical Control Point (HACCP), which is regulated under FDA, and it aims at analyzing hazards associated with food and identify preventive and control measures to check spread of food-borne diseases including viral pathogens. Similarly, sanitary and phyto-sanitary measures (SPS) measures, which are to be strictly followed to have safe food in order to conserve the health of animal, human and plants due to zoonotic agents (15).

Conclusion

The emergence of viral zoonoses poses a threat to animal and public health, as well as to commerce and trade. Measures necessary to prevent further cases in human and domestic animal populations will need holistic “One Health approach”, educating people and healthcare improvements. To address the ultimate causes of spill over, a better understanding of reservoirs and the ecological and sociological drivers for disease emergence is necessary including improved medical facilities and education are important throughout the regions where it exists.

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CRM Department
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Road No. 44, Jubilee Hills,
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