INTRODUCTION

Canine ocular thelaziosis is caused by a spirurid nematode of genus *Thelazia* commonly known as eye worms (Naem, 2014). *Thelazia californiensis* and *Thelazia callipaeda* are the species of eyeworms that mostly affect dogs. Apart from infecting dogs, both the species of worms can infect cats and human as well (Chanie and Bogale, 2014). The former is reported from Western North America and the later from Far East, Russia and other parts of the world (Chanie and Bogale, 2014; Otranto and Torres, 2015). Thelaziosis in canine is characterized by blepharospasm, epiphora, conjunctivitis, keratitis and intense lachrymal secretion (Hermosilla et al., 2004). The depth of the clinical symptoms may vary depending upon the number of parasites present in the sac as well as individual. Sometimes behavioural changes like aggression, dullness are also encountered.

CLINICAL HISTORY

A 3 years old male German shepherd dog weighing 32kg was presented in the veterinary hospital at Jorhat with a history of chronic epiphora, shaking of head and chronic itching of both the eyes. Moreover, clinical history revealed that the dog has become aggressive and has not been following the masters, command for the past few months. Physical examination after sedation with diazepam and...
The ketamine combination revealed conjunctivitis, thick lacrimal discharge and a few parasites (10 to 15) moving over the cornea of both the eyes (Fig. 1). Examination of cul-de-sac revealed the presence of a lump of parasites (Fig. 2). Physiological parameters were within the normal range.

**TREATMENT**

The dog was pre-medicated with atropine sulphate @ 0.04mg/kg body weight intramuscularly and diazepam @1mg/kg body weight intravenously. Anaesthesia was induced with ketamine hydrochloride @ 5mg/kg body weight intravenously. Operational anaesthesia was maintained with incremental doses of ketamine hydrochloride intravenously. After attaining the operational anaesthesia the dog was positioned in lateral recumbency. Both the eyes were cleaned properly with normal saline solution. With the help of a mosquito forceps all the visible parasites were removed first. A jet flow of normal saline was applied to remove the few parasites which were present deep in the cul-de-sac. All the parasites collected from both the eyes were preserved in the 10% formalin solution. Approximately 250 numbers of adult parasites were removed. After manual removal of all the parasites, Inj Ivermectin was administered subcutaneously @ 200mcg/kg body weight at weekly interval on four occasions. Antibiotic eye drop containing Gentamicin was instilled @ two drops four times a day for seven days to overcome conjunctivitis. A course of Pheniramine maleate @ 1.5 ml was injected intramuscularly for three days. The animal was managed in intensive care unit in a fly free environment.

**PARASITIC DESCRIPTION**

The worms are creamy white in colour. The male is about 8-12 mm in length and the female measures about 12-18 mm in length (Naem 2014, Chanie and Bogale 2014). The cuticle bears prominent transverse striations. In the male, the spicules are unequal. The tail is curved ventrally and there are a number of pre and
post cloacal papillae. The adult female *T. callipaeda* will measure about 12-18.5 mm in length. The vulva which has a short flap, is located in the anterior region of the body near the oesophagus. The vagina opens near the oesophago-intestinal junction (Naem 2014). The females are viviparous and they lay first stage larvae (L₁) into the lachrymal secretions from where they are picked up by the vectors (Dipteran flies) (Naem 2014). A dipteran fly *Phortica variegata* (Family- Drosophilidae) acts as intermediate host (Otaševiæ et al., 2014, Otranto and Torres 2015) for *Thelazia callipaeda*, the eyeworm prevalent mostly in the Far East including India (Otranto and Torres 2015). The sites of predilection of these worms are under the eyelids, nictitating membranes and lachrymal ducts (Naem 2014).

**EPIDEMIOLOGY**

Canine thelaziosis caused by oriental eyeworm (Anderson 2000) has been reported from many parts of the world including former Soviet Union and countries from the Far East which includes China, Korea, Myanmar, Japan, Indonesia, Thailand, Taiwan and India. The disease has also been reported from cats and humans from these countries (Naem 2014). In Korea, military dogs were found to be acting as reservoir host (Seo et al., 2002), however, the main final reservoir host of *T. callipaeda* seems to be the farm dogs (Naem 2014).

*T. callipaeda* has been reported from different climatic conditions varying from tropical and subtropical regions from the Far East to the temperate in the Russian federation (Naem 2014). In Italy, a high prevalence (60%) of *T. callipaeda* was reported in dogs from a comparative study with cats and foxes conducted in Southern Italy (Otranto et al., 2003). Thelaziosis in dogs has also been reported in France and Germany (Chermette et al., 2004; Hermosilla et al., 2004). First case of *T. callipaeda* in a dog was reported in Southern Switzerland in the year 2000 (Malacrida et al., 2008). In wild animals such as beech marten, wild cats and brown hares, the infection was reported for the first time by Otranto et al. (2009).
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(Fig.1) Thelazia Parasites over Cornea (Fig.2) Lump of Thelazia Parasites in the cul-de-sac
TENORRHAPHY OF SUPERFICIAL DIGITAL FLEXOR TENDON IN A GS MULE USING THREE LOOP PULLEY SUTURING TECHNIQUE

Capt Rishi Sharma*, Maj Sameer Faruquie* and Col Deep Ahalawat**, VSM

ABSTRACT

A three year old mule GS was presented with history of lacerated wound due to entanglement in paddock boundary wire. Clinical examination revealed complete rupture of superficial digital flexor tendon. As animal was unable to bear weight, the surgery was performed under GA using Xylazine-Ketamine combination. The ruptured ends of tendons were sutured using three loop pulley suturing pattern to maintain the strength required for flexor tendon function after repair along with application of calkin shoe to provide support to the injured tendon. The animal successfully recovered after 4 months of care and treatment and was discharged from the hospital.

INTRODUCTION

Lacerations of the flexor tendons in the horses is a life threatening and potentially career ending event. The superficial digital flexor tendon (SDFT) originates on the distal humerus and caudal radius and becomes a tendinous unit at the level of the distal radius. As the tendon courses distally at the level of the metacarpus, it is covered by a very little soft tissue, making it vulnerable to traumatic injury. Tendons in the equine forelimb and hindlimb act mainly to position the limb correctly during locomotion. Some tendons have an additional function, acting as springs to store and release energy as they are stretched and recoil during the stance and swing
phase of each stride and so decrease the energetic cost of locomotion (Alexander, 1991). The superficial digital flexor tendon (SDFT) and suspensory ligament (SL) are the main energy storing structures in the equine limbs and are subjected to higher strains than the deep digital flexor tendon (DDFT) and common digital extensor tendon (CDET), which do not contribute significantly to energy storage (Wilson et al. 2001). Flexor tendon lacerations are common injuries that can occur in a variety of ways including kick injuries, lacerations from environmental obstacles and other accidents. Surgical repair of these lacerations is the current recommendation if greater than 50% of the cross sectional area of the tendon is lacerated. The SDFT plays a significant role in locomotion by experiencing a significant load (up to 844 N) and strain (2.2-4.6%), at the walk (Dowling and Dart, 2005). This information coupled with the immediate weight bearing required after tendon laceration repair makes equine flexor tendon tenorrhaphy challenging.

Tendon injury is one of the most common causes of wastage in the performance horses, the majority of tendon injuries occur to the superficial digital flexor tendon (SDFT) Injuries to the musculoskeletal system have been found to account for 82% of all injuries to race horses competing in hunt and flat races, and of these 46% involved tendons or ligaments (Williams et al. 2001, Ely et al. 2004).

Some tendons are much more prone to injury than others, the majority of tendon injuries (97-99%) occur to the forelimb tendons (Kasashima et al. 2004, Lam et al. 2007), with the superficial digital flexor tendon (SDFT) being injured in 75-93% of cases and the remaining injuries occurring to the suspensory ligament (SL) (Ely et al. 2004. Kasashima et al. 2004).

The three pulley and the compound locking loop are the two most popular suture patterns (Romero et al. 1997). Currently the three-loop pulley (3LP) pattern is recommended for repair of equine flexor tendons. The 3LP compares favorable in biomechanical studies by resisting gap formation when compared to a compound
locking loop pattern (Easley, 1990 and Jann, 1990). Polydioxanone (PDS) is a synthetic monofilament absorbable suture that has been recommended for tenorrhaphy and has been used in multiple in vivo studies in animals for tenorrhaphy (Valdes et al. 1996). The suture has been reported to retain 86% of its tensile strength at 8 weeks (Ray et al. 1981).

CASE HISTORY AND DIAGNOSIS

A three year old Mule GS was presented with a transversely oriented, 4cm x 2.5cm, lacerated wound on the plantar surface of the right hind limb at mid-level metatarsal due to entanglement in boundary wire while running in paddock. Clinical examination revealed a horizontal tear in SDFT at mid level of metatarsal. There was evident dropping of hoof with marked lameness. The case was diagnosed as Superficial Digital Flexor Tendon rupture based on clinical signs, palpation and visual examination of injury.

Ruptured Superficial Digital Flexor Tendon
TREATMENT AND CASE MANAGEMENT

The animal was sedated with Inj Xylazine @ 1.1mg/kg BW i/v followed by induction of anesthesia with Inj Ketamine @ 2.2mg/kg BW i/v and was cast into lateral recumbency. Anesthesia was maintained with Xyalzine-Ketamine combination with the ratio of 1:1 given i/v till effect. The wound was properly shaved and cleaned with antiseptic solution. The wound was exposed to reveal the torn ends of SDFT.

The tendon was appropriately sutured using (Monocol) No 2 absorbable monofilament polydixanone suture material of Stericat Gutstrings in three loop pulley suturing pattern to maintain the proper strength and gliding action of tendon.

![Three Loop Pulley Suturing Pattern](image)

The skin was sutured with simple interrupted sutures using surgical silk black braided no. 1. In the end aseptic dressing with pressure bandaging was applied over the wound and limb to reduce the internal motility of the tendon and animal was put under stall rest. Parenteral administration of Inj Tentnus Toxoid @1ml i/m, Inj Ceftriaxone and Tazobactam @ 10mg/kg bw bid, Inj Meloxicam @ 0.3mg/kg bw i/v bid along with multivitamin infusion was given for 7 days.
Pressure Bandaging of Limb and Application of Calkin Shoe

A modified calkin horse shoe was applied on the affected limb to give additional support to limb and to relive pressure till its recovery. The animal was kept with the shoe for three months. Skin sutures were removed on 12th day post-operation. The animal was kept under stall rest for 21 days and it showed marked recovery. After 4 months the animal made full recovery and was discharged being fit for duty.

CONCLUSION

Flexor tendon lacerations in horses are traumatic injuries that can be career ending and life threatening. In the horse, a strain is placed on the tenorrhaphy by immediate weight bearing and locomotion post-operatively. Despite the use of external cooptation, such strains can lead to significant gap formation, construct failure, longer healing time and poor quality of the healed tendon. Early return to exercise and decreased gap formation has been shown to
reduce adhesion formation. Based on these concerns, the ideal tenorrhaphy suture pattern for equines would provide:-

(1) High ultimate failure load.

(2) Resistance to gap formation.

(3) Minimal alteration in blood supply.

(4) Minimal adhesion formation.

Historically, various suture patterns and materials have been evaluated for equine flexor tendon repair. Results of equine studies suggest the three-loop pulley pattern (3LP) compares favorably to other patterns and is recommended for primary tenorrhaphy. However, this pattern still experiences significant gap formation and can result in failure. As a result, a technique which decreases the problems inherent in the 3LP is warranted for tenorrhaphy of equine flexor tendons.

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CANINE PERIODONTAL DISEASE: A CASE REPORT

Lt Col Rakesh Sharma* and Col Manoj Batra**

INTRODUCTION

Regular cleaning of teeth in canines post feeding prevents plaque formation. Left un-cleaned, the bacteria develop in the plaque leading to halitosis, periodontitis and gingivitis (Hilson 2005). Afterwards, minerals in the saliva harden the plaque into dental calculus (tartar), which gets firmly attached to the teeth. The tartar once formed is visible on teeth and gum line and cannot be removed by simple brushing; it requires scaling and debridement. Subsequently plaque and calculus spread under the gum line, damaging the supportive tissue around tooth leading to loosening of bone and loss of tooth (periodontitis). (Frunk 2011) Bacteria under gum line can cause extensive tissue damage by secreting toxins causing inflammation and reddening of gums (gingivitis). Combination of gingivitis and periodontitis form Periodontal Disease which is a common manifestation of improper dental care (Cecelia 2008).

HISTORY AND CLINICAL SIGNS

A 04 years old, male, German Shepherd dog was referred to the clinic with history of halitosis, swelling on right cheek and loss of body condition. The swelling had been initially observed about six months ago by the handler. On examination, it was found that the dog had large tartar deposits on all teeth and had pan gingivitis. A large lemon sized plaque mass, deep pocketed was found accumulated over upper right pre molar. There were signs of purulent exudate and the soft palate had

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been invaded by the growth. The color of gums was purplish. Epulis growth was evident around the calculi. There was a general recession of gums by 2-6 mm. The dog was in fair body condition and showed reduced food intake lately.

**TREATMENT AND SURGICAL REMOVAL OF THE TARTAR CALCULI**

The dog was put on 5 days antibiotic course (Ceftriaxone @ 4mg/kg body weight IV s.i.d) along with anti-inflammatory medication (Meloxicam @ 0.2 mg/kg body weight IM). Tartar plaque removal and periodontal debridement was carried out on other teeth under sedation and the mouth was rinsed with mouthwash solution (Morefresh Red®. Chlorhexidine).

Ultrasonic scaling was also done to remove the finer plaques. To remove the major deposit mass, the dog was fasted for 12 hours and anaesthetized using xylazine(@ 2mg/kg body weight IM) and ketamine (@ 10 mg/kg body weight IM). The tartar calculi was manipulated and it was found that complete premolar tooth was engulfed in the mass. After reducing the size by chipping with bone cutter, a walnut sized mass was extricated. A blind cavity between gums, maxilla and soft palate was exposed. The cavity was lined by granulating tissue and pyogenic membrane which was curetted and freshened to remove the pyogenic layer. It was then flushed with 2% H₂O₂ sol along with Metronidazole sol and povidone iodine (Betadine®) impregnated gauge was packed in it. A single retention suture (black braided silk no 1) was applied to keep the gauge in place. Inj Metronidazole (Metrogyl®) (@ 20 mg/kg body weight IV sid) was administered for 5 days. Anti-inflammatory medication (Meloxicam) was given for 4 days. Liver supplement (Belamyl 0.5ml IM q48h) was also given on three occasions on alternate days. Daily gauge removal and mouth rinsing was done using mouth wash for 7 days (Chlorhexidine). The dog was kept on liquid diet (milk, mix veg soup) during these 7 days. Postoperatively, the dog showed recovery from gingivitis, periodontitis and halitosis. No gingivectomy was performed and the cavity left by the tartar calculi
mass was left as such for natural contraction, as it was not interfering in feeding or drinking. The dog was discharged with instructions for soft food feeding for two weeks and daily brushing with soft brush.

**DISCUSSION**

Gingivitis and periodontitis lead to an irreversible periodontal disease which needs to be treated usually under anesthesia (Frank 2011). Left untreated, it may cause loss of bone and fistulation as was evident in the present case. In more severe sequel even bacteremia may arise due bacteria from the site entering blood stream (Cecelia 2008). Though the initial calculi is on teeth (supragingival) it may soon invade the gum line and develop below the gums too (subgingival). Heavier accumulation of calculi in maxillary teeth can even cause oro-nasal fistula (Hilson 2005). Dogs on soft and pasty diet left un-brushed after food are more susceptible to this disease. Dogs having some component of diet as abrasive/fibrous feed rarely form thick deposits. For the same reason ruminants or equines don’t suffer from tartar deposits. Dogs chewing on various toys or dental chews are also less prone to tartar deposits (Deekwish 2001). Smaller breeds have been reported to be affected more than the larger breeds because of crowding of teeth. More cases have been reported in older dogs than in young puppies or adults (Cecelia 2008).

Acidic saliva and bacteria (*Actinomyces viscosus, Porphyromonas spp, Prevotella spp, Peptostreptococcus spp and Fusobacterium spp*) present in mouth also determine the onset of mineralization of plaques (Diekwich 2001). Dogs that breathe with open mouth are also more prone due to dehydration of oral cavity.

Gum recession, acute gingivitis, loose teeth or missing teeth are other accompanying symptoms of periodontal disease. Sometime drooling and gastrointestinal involvement may also be seen (Frank 2011). Such dogs are also temperamentally irritated and upset. Anorexia and loss of condition can be seen in
majority of the cases. Diagnosis may need radiography in some cases to estimate the depth and involvement of bone.

Daily cleaning of teeth is essential as the plaques may start to mineralize as soon as 4-5 days. Routine off the shelf fluoride toothpaste may be used. Weekly mouthwash and brushing with chlorhexidine or listerine should be practiced. Susceptible dogs should undergo ultrasonic scaling every 5-6 months. Many associated health problems can be prevented by just adhering to a routine of brushing and cleaning teeth after feeding the dog.

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PLATE-1 LARGE PLAQUE MASS AND DENTAL CALCULI

PLATE-2 CAVITY LEFT AFTER SURGICAL REMOVAL OF CALCULI & PLAQUE MASS
PLATE -3: SURGICALLY REMOVED LARGE CALCIFIED PLAQUE CALCULI

PLATE-4: DOG 6 WEEKS POST SURGERY.
DIAGNOSIS OF HEPATOZOOON CANIS IN MILITARY WORKING DOGS BY POLYMERASE CHAIN REACTION

Maj Surender Kumar*, Maj Rahul Dubey*, Maj Chhabil Singh*, Maj Mayur Varshney*, Maj DS Mandhotra*, Lt Col Manav Deshwal* and Col Amit Kumar**

ABSTRACT

Background: *Hepatozoon canis* is a widespread tick-borne protozoan affecting dogs. The objective of the study was to standardize a PCR assay for detection of clinical/sub clinical cases of *Hepatozoon canis*.

Results: The study was conducted utilizing extracted DNA from 321 samples from Military working dogs suspected to be infected with vector borne diseases. A *Hepatozoon canis* PCR protocol was standardized and then the similar protocol was used in routine for all the samples suspected for protozoan infections. Out of the 321 samples tested for *Hepatozoon canis*, 05 (1.5%) samples tested positive. Further, all *Hepatozoon canis* positive samples were co-infected with *Babesia spp* except in case of one dog which was positive only for *Hepatozoon canis*.

Conclusions: The present study successfully standardized a PCR assay for diagnosis of *Hepatozoon canis* from clinical samples of working dogs. This is the first report of development of a diagnostic PCR test against *Hepatozoon canis* in India.

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INRODUCTION

Despite its wide geographical distribution and the fact that it was described in the early 20th century (James SP, 1905), there are still knowledge gaps concerning canine hepatozoonosis caused by *Hepatozoon canis* (Adeleorina: Hepatozoidae), including insufficient understanding of its pathogenesis and the best diagnostic methods to employ for diagnosing this infection. In contrast to other tick-borne protozoa, *Hepatozoon canis* infects leukocytes and parenchymal tissues and is transmitted to dogs by the ingestion of ticks containing mature oocysts (Baneth et al., 2001). The brown dog tick, *Rhipicephalus sanguineus* (Ixodida: Ixodidae), is the main vector of *Hepatozoon canis* (Baneth et al., 2001; Christophers SR, 1907), although oocysts of this protozoan have also been detected in other tick species feeding on dogs, including *Haemaphysalis longicornis* and *Haemaphysalis flavas* in Japan (Murata et al., 1991) and *Amblyomma ovale* in Brazil (Forlano et al., 2005, Rubini et al., 2009). *Hepatozoon canis* is probably one of the most widespread canine vector-borne disease (CVBD)-causing pathogens due to its close association with *R. sanguineus* and the cosmopolitan distribution of this tick species (Dantas-Torres F, 2008 & 2010). A study by Abd Rani et al., (2011) investigated the occurrence and distribution of canine tick-borne disease pathogens in four cities of India. The study concluded that the *Hepatozoon canis* was the most common pathogen infecting dogs in India (30% prevalence rate).

The diagnosis of hepatozoonosis is frequently based on the detection of intracytoplasmatic ellipsoid-shaped gamonts in stained blood smears by microscopy and on the histopathological visualization of meronts and/or monozoic cysts in tissues (Klopfer et al., 1973, Baneth et al., 2003). Molecular diagnosis based on both conventional (Baneth et al., 2000) and real time polymerase chain reaction (PCR) (Li et al., 2008), developed during the last decade, greatly contributed to understanding the spread of this protozoan in canine populations. Although PCR is
considered the most sensitive detection method for canine hepatozoonosis, microscopic examination of blood smears is a simple technique frequently used for the diagnosis of this infection. Nonetheless, few studies have compared these methods (Karagenc et al., 2006) and a diagnostic gold standard has not been clearly established.

The objectives of the study were firstly to standardize a PCR assay for detection of clinical/sub clinical cases of *Hepatozoon canis* and to assess the utility of this PCR assay in detection of *Hepatozoon canis* from blood and tissue samples. Secondly, the assay shall also be used to document the incidence of *Hepatozoonosis* in Military working dogs as information on the prevalence of *Hepatozoon canis* and the extent of incidence of this organism is presently unknown.

**MATERIALS AND METHODS**

**Samples**

The study was conducted utilizing extracted DNA from EDTA blood and necropsy tissue samples from Military working dogs suspected to be infected with vector borne diseases. The study also included the DNA samples positive for other vector borne diseases in the past which had been stored in repository of Central Military Veterinary Laboratory (CMVL). A total of 321 samples spanning a time period from April 2013 to Oct 2016 were used. Approximately 200uL of EDTA blood or 100 mg of tissue samples were used for extraction of DNA.

**Polymerase chain reaction (PCR)**

DNA was extracted individually from EDTA blood and tissue samples using a commercial kit (DNeasy Blood and Tissue kit, Qiagen®) following the manufacturers’ instructions. A fragment of the 18S rRNA gene (666 bp in size) was amplified by PCR, using the primers HepF (5’-ATACATGAGCAAAATCTCAAC-3’) and HepR
(5'- CTTATTATTCCATGCTGCAG-3') (Inokuma et al., 1992). PCR amplifications were carried out in a total volume of 25μl, including 5μl of genomic DNA, 10 pmol of each primer and 12.5 μl of DreamTaqgreen PCR mastermix®(2X)(Thermo Scientific, Foster City, CA, USA). The amplification protocol was employed in a gradient thermal cycler®(Eppendorf, Germany) as following: 95°C for 3 min (for polymerase activation), followed by 40 cycles of 95°C for 30 sec (denaturation); 57°C for 30 sec (annealing); 72°C for 1 min and 30 sec (extension), followed by 15 min at 72°C (final extension), as previously described (Inokuma et al., 1992). Negative controls (no DNA template) were also included in all PCR reactions. Amplicons were resolved in ethidium bromide-stained agarose gels (1.5%) and sized by comparison with Gene RulerTM® 100-bp DNA Ladder (MBI Fermentas, Vilnius, Lithuania) as molecular marker. Gels were photographed using Gel Doc 2000® (Bio-Rad, Hercules, CA, USA).

Sequencing

Amplified product from one representative Hepatozoon canis positive clinical sample was sequenced to confirm the specificity of the PCR assay. Sequences were determined in both directions (double pass sequencing; using the same primers individually as for the PCR). Sequence was compared with 18S rRNA gene sequences of Hepatozoon canis available in National Centre for Biotechnology Information (NCBI) GenBank.

RESULTS AND DISCUSSION

During PCR standardization, a positive amplification of size 666 bp was obtained from one of the DNA samples from repository of vector borne disease positive DNA stored at CMVL biotechnology section (fig 1). On repetition, the results showed consistency and the size of the amplicon was in accordance with the earlier reports by Otranto et al. (2011). The identity of the amplified product from the positive clinical sample was further confirmed to the species level by sequencing the 666
bp amplified PCR product. The sequencing reaction from the sample gave a good read and on BLAST analysis the sequenced amplicon was identical with that of *Hepatozoon canis* sequences available in GenBank (AY461378, AF176835). The sequence has since been submitted to National Centre for Biotechnology Information (NCBI), Bethesda and is available online with accession number KX236166. The sequence of the product is depicted in fig 2. The phylogenetic relationship of the sequence with other similar sequences was also traced (fig 3).

After successful standardization of *Hepatozoon canis* PCR, the protocol was used in routine for all the EDTA blood and tissue samples suspected for protozoan infections received in the lab. Out of 321 samples tested for *Hepatozoon canis*, 05 (1.5%) samples tested positive for *Hepatozoon canis* and all positives were from EDTA blood. Further, all *Hepatozoon canis* positive samples were co-infected with *Babesia spp* except in case of one dog which was positive only for *Hepatozoon canis*. As suggested from previous studies, in endemic areas, CVBD-causing pathogens may infect the same dog with two (*Hepatozoon canis* and *Ehrlichia canis*) (Baneth *et al*., 1997), three (*Hepatozoon canis, Babesia spp., E. canis*) (Karagenc *et al*., 2006, Sasanelli *et al*., 2009) or even four agents (*Hepatozoon canis, Babesia spp., E. canis, Leishmania infantum*) (Otranto *et al*., 2010).

As no information is available on the incidence of *Hepatozoon canis* infection in working dogs, the data thus presented here is of interest in indicating that the Military working dogs in the country are exposed to this infection. This is of more relevance in geographic areas where CVBD-causing pathogens co-infect the working dogs resulting in complex disease manifestations, impairing the achievement of a definitive diagnosis and the selection of proper therapeutic agents.

**CONCLUSIONS**

The present study standardized a PCR assay for diagnosis of *Hepatozoon*
canis from clinical samples of working dogs. The results presented here suggest that the PCR on EDTA blood and tissue samples is specific assay for the detection of Hepatozoon canis infections. This PCR technique may be also used as an epidemiological tool for studies in areas where canine hepatozoonosis is endemic or where it is suspected. Finally, the achievement of a prompt diagnosis of hepatozoonosis is pivotal in areas where other CVBD-causing agents occur in order to reduce the clinical effects of simultaneous pathogen infections and to select the best therapeutic drug. This is the first report of development of a diagnostic PCR test against Hepatozoon canis in India.

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Figure 1. Agarose gel electrophoresis of *Hepatozoon canis* 18S rRNA gene specific 666 bp PCR product. Lane 1: Clinical sample; Lane M: 100bp molecular wt marker

>CMVL_ Hcanis  
KX236166  

CAGGCCGATAAATCATTCAAGTTTCTGACCTATCAGCTTTTCGACGGTATGTTATGGCTTACCGTGACCGGTCCACGGGATTAGGGTTCGGATTCCGGAGAGGGA  
GCCCTGAGAAACGGCTACCACATCTAAAGGAAGGCAGGCAGGCGGCAAATTACCACCAATTCTAACAGTTTTGGAGAGGTAGTAACAAGAAATAACAAGGCAGTTAAATGGCTTTGTAA  
TTGGAATGATAGAAATTTAACCCTTTTAAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCTATATTTAAATGGTGCAGTTAAAAAGCTC  
GTAGTTGAAGTTCTGCTAAAAGTAACCGGTCTGCTTTTAATAAAGGTGGTATCTTTGTGATTCTTTTAGCAATGATGTCCTTTGAAGTGTTTTTTACTTTATTGTAATAATTCAGGACTTTTACTTTGAGAAATAGAGT

Figure 2. Partial sequence of 18S rRNA of *Hepatozoon canis*
Figure 3. Neighbour-joining tree depicting relationship between multiple *Hepatozoon canis* sequences.
STANDARDIZATION OF A DIFFERENTIAL MULTIPLEX PCR ASSAY FOR EQUINE HERPESVIRUS 1 AND 4 AS A DIAGNOSTIC TOOL

Maj Surender Kumar*, Maj Rahul Dubey*, Maj Chhabil Singh*, Maj Mayur Varshney*, Maj DS Mandhotra*, Lt Col Manav Deshwal* and Col Amit Kumar**

ABSTRACT

In the present study, a multiplex polymerase chain reaction (mPCR) procedure was standardized for detection and differentiation of equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4) strains. Specific oligonucleotide primers were combined to amplify the glycoprotein C (gC) gene region of EHV-1 and EHV-4, which would yield fragments of different lengths for each virus in the same amplification reaction. Once the protocol was established and standardized, it was used for all the samples received from equine abortion and rhinitis cases. The mPCR proved to be a fast and sensitive method for detecting EHV-1&4 isolates and for differentiation of both viruses in field samples. The multiplex PCR protocol has been used successfully to detect EHV-4 infections from mild rhinitis cases from equine breeding stud. The specificity of the mPCR amplicon for EHV-4 was confirmed by sequencing. Moreover, it was the first case report of detection & identification of EHV-4 in India.

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INTRODUCTION

Equine herpes viruses (EHV) are found in most horses all over the world. To date, nine EHV's have been identified worldwide. The two most common strains of these, viz, EHV-1 and EHV-4 pose health risks for domesticated horses. EHV-1 is a major cause of abortion, perinatal foal mortality, respiratory disease and neurological disorders in horses (O’Callaghan et al., 1983, Campbell et al., 1983, Sabine et al., 1983, Matsumura et al., 1992). In contrast, the closely related EHV-4 primarily causes respiratory disease and rarely produces abortion or neurological outcomes (Allen and Bryans, 1986; Matsumura et al., 1992). In view of the serious economic losses caused by these two viruses, EHV-1 in particular due to its propensity to cause abortion storms, it is important that methods employed to diagnose and discriminate between these two viruses be both rapid and sensitive so that early intervention strategies aimed at reducing the devastating effects of virus spread can be implemented.

Differential diagnosis of EHV-1 and EHV-4 is hampered by their overlapping disease spectra and by the close genetic and antigenic relationship shared by the two viruses. The genomes of EHV-1 and EHV-4 share approximately 85% nucleotide sequence homology, and encode proteins which share up to 90% amino acid sequence homology (Whalley et al., 1989, Riggio et al., 1989, Nicolson et al., 1990, Robertson et al., 1991). Conventional rapid serological methods such as ELISA, which uses polyclonal antisera, cannot reliably distinguish between EHV-1 and EHV-4 due to the high degree of antibody cross-reactivity to shared viral epitopes. The viruses can be distinguished by monoclonal antibodies (Yeargan et al., 1985), restriction enzyme digestion of viral DNA (Sabine et al., 1981, Studdert et al., 1981) and by differences in their in vitro host cell range (Studdert and Blackney, 1979), all of which involve laborious cell culture techniques.
A rapid and sensitive differential assay for these viruses is therefore necessary for better detection and screening of the epidemiology of these infections in breeding horses. With this objective in focus, development of a multiplex PCR for detection and simultaneous differentiation of EHV-1 and EHV-4 in a single reaction was undertaken. The other benefits of mPCR include cost effectiveness, saving on time and less input material. In this paper we have described an mPCR, which employs one set of EHV-1 specific forward primer, an EHV-4 specific forward primer and a virus-common backward primer. The primer set amplify region of the glycoprotein C (gC) gene (Allen and Coogle et al., 1988, Nicolson and Onions, 1990) of both viruses to yield PCR products of different sizes for each virus.

**MATERIALS AND METHODS**

**Samples**

The development of multiplex PCR protocol was conducted utilizing extracted DNA from Pneumabort-k®+1b vaccine (Zoetis) and EHV reference strains. Once the protocol was established and standardized it was used for all the samples received from equine abortion and rhinitis cases. Total of two hundred thirty samples in all were used; which included EDTA blood, nasal swabs, cervical swabs, placenta swabs and tissues; collected over a time period from Mar 2016 to Nov 2016.

**Multiplex polymerase chain reaction (mPCR)**

DNA was extracted individually from 200ìl of Pneumabort-k®+1b vaccine or 200ìl of EDTA blood or 100mg of tissue samples or nasal/cervical/placenta swabs mixed in 200ìl of 1X Phosphate buffer saline (PBS) using a commercial kit (DNeasy Blood and Tissue kit, Qiagen®) following the manufacturers’ instructions. The PCR primers used in this study were selected from the glycoprotein C (gC) gene of the EHV-1 and EHV-4, based on the study by Lawrence et al. (1994). The oligonucleotide
primers used were: EHV-1 specific forward (5’-GCGAGATGTGGTTGCTTAATCTCG-3’), EHV-4 specific forward (5’-AGCCACGAACACTCAACCGATGT-3’) and virus common reverse (5’-GAGACCGTAA CGCTGGTACTGTTAA-3’). PCR amplifications were carried out in a total volume of 25 µl, including 5 µl of genomic DNA, 10 pmol of each primer and 12.5 µl of DreamTaq green PCR mastermix® (2X) (Thermo Scientific, Foster City, CA, USA). The amplification protocol was employed in a gradient thermal cycler® (Eppendorf, Germany) as following: 95°C for 3 min (for polymerase activation), followed by 40 cycles of 95°C for 30 sec (denaturation); 58°C for 30 sec (annealing); 72°C for 1 min (extension), followed by 15 min at 72°C (final extension), as previously described (Lawrence et al., 1994). Negative controls (no DNA template) were included in all PCR reactions. Amplicons were resolved in ethidium bromide-stained agarose gels (1.5%) and sized by comparison with Gene Ruler™ 100-bp DNA Ladder (MBI Fermentas, Vilnius, Lithuania) as molecular marker. The amplification of fragment 649 bp for EHV-1 and 507 bp for EHV-4 were obtained. Gels were photographed using Gel Doc 2000® (Bio-Rad, Hercules, CA, USA).

Sequencing

The EHV-1 products obtained in mPCR were not sequenced as we used Pneumabort-k®+1b vaccine as positive control for EHV-1 and the sequence of the vaccine strain is already published by the company and it is copyrighted. For EHV-4, amplified product from one representative EHV-4 positive clinical sample was sequenced to confirm the specificity of the PCR assay for EHV-4. Sequences were determined in both directions (double pass sequencing; using the same primers individually as for the PCR). Sequence was compared with glycoprotein C (gC) gene sequences of EHV-4 available in National Centre for Biotechnology Information (NCBI) GenBank.
RESULTS AND DISCUSSION

The present study standardized a multiplex PCR-based differential diagnostic test in which EHV-1 and EHV-4 virus-specific PCR products were distinguished on the basis of size in a single gel step, thereby reducing the time and number of steps required for differential EHV-1/EHV-4 diagnosis. The specificity of the primers for the EHV-1 & 4 gC genes was confirmed from the positive isolates and vaccine strains assayed by multiplex PCR (Lane 1 & 2; Fig 1). In addition, when both EHV-1 & 4 DNA were present in a reaction which contained the complete gC gene primer set, target sequences of both DNA species were amplified (Lane 3; Fig 1). Together these results show that, if present, either or both viruses could be detected in a single PCR reaction containing gC primer set and that the viruses could be distinguished by size differences of their respective PCR products on an agarose gel.

After successful standardization of multiplex PCR for EHV-1 & 4, the protocol was used in routine for all equine abortion and rhinitis cases samples received at CMVL. The types of samples tested for the presence of either or both viruses by mPCR included; EDTA bloods (n=73), nasal swabs (n=96), cervical swabs (n=15), placenta swabs (n=03) and tissues (n=43). Out of 230 samples tested for EHV-1 & 4, the multiplex PCR protocol was successful in detecting EHV-4 from 03 nasal swabs of stallions. On repetition, the results showed consistency and the size of the EHV-4 (507bp) was in accordance with the earlier reports by (Lawrence et al., 1994). The 507 bp amplified mPCR product targeting glycoprotein C (gC) gene of EHV-4 from one representative positive sample was further confirmed to the species level by sequencing. The sequencing reaction from the sample gave a good read and on BLAST analysis the sequenced amplicon was identical with that of EHV-4 sequences available in GenBank. The sequence has since been submitted to National Centre for Biotechnology Information (NCBI), Bethesda and is available
The facility for diagnosis of EHV-1 infection in horses by traditional PCR was available at CMVL but not for EHV-4 infections. As both viruses are responsible for equid acute respiratory disease, abortive outbreaks in late pregnancy and encephalitis; it was important that methods be employed to diagnose and discriminate between these two viruses. In view of the above, multiplex PCR assay was developed to detect EHV-1 & 4 viruses and the protocol proved to be sensitive and specific for improving the diagnosis of field isolates of both the viruses. It may be suitable for use as a rapid method for screening large numbers of horses in epidemiological surveys.

CONCLUSIONS

The multiplex PCR test reported here allows the rapid detection and identification of EHV-1 and EHV-4 in clinical samples. The new multiplex PCR protocol has been used successfully to detect EHV-4 infections from the samples of mild rhinitis cases in a equine breeding stud. Moreover, it was the first report of detection & identification of EHV-4 at CMVL. The speed with which a positive diagnosis could be made would allow the rapid implementation of stable management procedures aimed at curbing virus spread.

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Allen, GP. Coogle LD. (1988) Characterization of an equine herpesvirus type 1 gene encoding a glycoprotein (gp13) with homology to herpes simplex virus
glycoprotein C. J. Viral. 62, 2850-2858.


Figure 1. Agarose gel electrophoresis of mPCR products amplified from the gC gene of EHV-1 and EHV-4. Lane M: 100bp molecular wt marker; Lane 1: EHV-1 positive control DNA; Lane 2: EHV-4 positive control DNA; Lane 3: both EHV-1 and EHV-4 positive control DNA.

>CMVL_EHV-4 492 bps Accession No.
KY204084

CGAACAACCTCAACCGATGAAACCGCACCAGCTACACCAACGCCGAGTCA
CCCACATTCAATGAAAATACAAATTACATGCAACAAATAGTCTCATATCAGGTTCCTA
CTACACATCTGTTACCATTAACTGTCTTACTACAACAGTAAGTGTAATCAGATGA
ATACAGACTAGAAATTACCTAACCAGCGACCCCCATTTTCAGA CACGC CT
CCTGGTGACCCAGAAAACTATGTGTTACCACCAACCGCTACCACAAAGACCAACCC
TGCTGTTATTTCACCTAACCAGACATTCTAGCGCACAAATCTCGAAGGGTTGGCCA
GCTGGGCCTTAGTCTCCAGACAGGTCTACCTAAGCGTCAGACTGTTCAACCTCCCG
Figure 2. Partial sequence of glycoprotein C (gC) of Equine Herpes Virus-4

Figure 3. Neighbour-joining tree depicting relationship between multiple EHV-4 sequences.
SUCCESSFUL CORRECTION OF PARTIAL BI-LATERAL NASOLACRIMAL DUCT OBSTRUCTION (NLDO) IN A MALE LABRADOR: A CASE REPORT

Dr RUP JYOTI LASKAR* and LT COL SJ PREMKUMAR**

INTRODUCTION

The nasolacrimal system is important for health and function of the eye. In healthy animals tears are produced by lacrimal glands and are drained from the eye by nasolacrimal duct system (Gionfrido, J.R. 2003). Diseases of the tear drainage system may not have severe consequences, but they usually lead to epiphora, conjunctivitis and periocular alopecia (Gionfrido, J.R. 2003). Diseases of the nasolacrimal drainage system may be caused by either congenital or acquired factors. Regardless of the cause, clinical signs of the disease result from either compromised drainage of the tear film from the ocular surface or inflammation in the system. Clinical signs may consist of epiphora, mucopurulent discharge from the puncta, conjunctival inflammation, swelling of the medial canthal region and a drainage fistula ventral to the medial canthal area. Epiphora is the most common clinical sign and is due either to complete or partial obstruction at any level of the drainage system (Grahn, BH., 1999). Diseases of the nasolacrimal and tear systems occur frequently in small animals and, to a lesser extent in horses (Gelatt, K.N., 2011). Diseases of the nasolacrimal apparatus that require surgical intervention are associated with partial-to-complete obstruction.

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The nasolacrimal drainage system consists of upper and lower puncta, a lacrimal sac, a long nasolacrimal duct, and a nasolacrimal punctum. The upper and lower puncta in dogs and cats are in the palpebral conjunctiva, just inside the mucocutaneous junction about 3 to 5 mm from the medial canthus. In the dog, the puncta are slitlike. The puncta are 0.5 to 1.00 mm in diameter in dogs (Gionfrido, J.R. 2003). Therapy of the tear drainage system diseases is usually a combination of medical and surgical modalities, and re-establishment of patency. The nasolacrimal drainage apparatus conveys tears and other debris from the external eye to the nasal cavity. This process appears to be only a passive capillary-like activity in animals, and the valve-like structures within the human nasolacrimal apparatus that prevent reverse flow have not been identified in small animals (Gionfrido, J.R. 2003).

HISTORY AND CLINICAL OBSERVATION

A male Labrador Retriever dog was reported to the Assam Rifles Dog Training Centre, Jorhat for continuous lacrimation from last six months. During routine physical examination marked bilateral lacrimation and partially blocked lacrimal duct in both the eyes were observed (Fig.1). It was reported that the dog was being administered Ciprofloxacin (Ciplox) eye drops @ two drops twice a day for last six months but there was no clinical improvement.
TREATMENT

The partial bilateral nasolacrimal duct obstruction was cleared by normograde nasolacrimal flush with 30 ml of Normal saline solution mixed in 3 ml of Povidone iodine using 22 G intravenous cannula (Fig. 2 and 3). Nasolacrimal flush tests for the anatomic patency of the nasolacrimal system was carried out under general anesthesia. The head of the dog was firmly restrained and either the upper or lower lacrimal punctum is located, cannulated with a 20–22 G blunt stainless steel needle, and flushed with 1–3 mL of sterile saline (Gelatt, K.N., 2011). General anesthesia was achieved with a combination of Inj. xylazine @ 1 mg/kg body weight and Inj. Ketamine @ 5.5 mg/kg body weight. Prior to giving the general anesthetic, the dog was pre-anaesthetized with Inj. Atropine sulphate @ 0.04 mg / kg body weight. After flushing, the patency was checked by inserting nylon thread (0 size Prolus suture material of lotus) into the upper lacrimal puncta to nasal puncta (Fig. 4). Both the nasolacrimal ducts were flushed by above described procedure for 10 days and the dog was administered Chloramphenicaol with Dexamethasone (Chlorocol plus) eye drops @ three drops thrice a day along with Serrapeptase proteolytic enzyme tablet (Serrase 10 mg tablet) one tablet OD for 10 days. The above said procedure showed marked improvement by 3\textsuperscript{rd} & 7\textsuperscript{th} (Fig. 5 & Fig. 6) day of nasolacrimal flushing and after 10\textsuperscript{th} day of flushing it showed complete cure (Fig. 7.)
Fig. 2

Fig. 3
Fig. 4
SUMMARY

The procedure described and the medicine used resulted in uneventful management, treatment and correction of Partial Bilateral Nasolacrimal Duct Obstruction in the Labrador retriever dog of Assam Rifles Dog Training Centre, Jorhat, Assam.

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HAEMATO-BIOCHEMICAL PROFILE OF DOGS WITH PROSTATIC AFFECTIONS

Maj Chandan Singh¹, S.K. Mahajan², S.S. Singh² and J. Mohindroo³

ABSTRACT

The present study was conducted on 12 dogs suffering from prostate affections to evaluate various haemato-biochemical changes. The blood and serum samples were collected upon presentation of case for estimation of Hb, TLC, DLC, platelet count, liver function tests, kidney function tests, total protein and albumin. The diagnosis was confirmed by clinical, radiographic, ultrasonographic and ultrasound guided fine needle aspiration biopsy (USG-FNAB) findings. Hematology, total protein, albumin, renal function tests were not useful for diagnosis of prostatic affections. In case of benign prostate hyperplasia (BPH) prostatitis, and prostatic carcinoma the blood SGPT and SGOT were moderately elevated. The alkaline phosphatase values were markedly elevated in animals suffering from prostatic carcinoma. The haemato-biochemical parameters are not diagnostic for dogs suffering from BPH and prostatitis. However, elevation of AKP values may be indicative of prostatic carcinoma when supported with clinical, radiographic and ultrasonographic findings.

INTRODUCTION

Prostatic disorders are common in middle-aged and older sexually intact male dogs (Olsen et al., 1987) and have been categorized as hyperplasia, cyst,
inflammation, primary and metastatic neoplasia. The diagnosis of prostatic disease in the past has been problematic and had relied primarily on prostatic fluid analysis, commonly collected through prostatic massage, blind percutaneous fine needle aspirate and radiographic imaging (Olsen et al., 1987). Radiography and ultrasonography are the major tools for confirming the prostatic disease. However, haemato-biochemistry is also considered as an important preliminary tool for proceeding towards the correct diagnosis and treatment protocol of prostatic affection. There is paucity of literature about the haemato-biochemical profile of dogs suffering from prostatic affections.

MATERIALS AND METHODS:

The present study was conducted on 12 clinical cases of male dogs aged 1.5 - 10 years and body weight ranging from 9 to 36 kg presented at the small animal teaching hospital, GADVASU, Ludhiana with varying symptoms.

Irrespective of the organ involved, all the animals were subjected to systematic evaluation for diagnosis of disease conditions. Hematological and biochemical parameters including Hb (g/dl), TLC (x10^3 per ml), DLC (%), serum AKP, SGPT, SGOT (μ/L), BUN, Creatinine (mg/dL), total protein and albumin (g/dL) were determined. Radiography and ultrasonography was performed in all the animals to evaluate the status of the prostate. Confirmatory diagnosis was made by ultrasound guided fine needle aspiration biopsy (USG-FNAB). The haemato-biochemical parameters were correlated with the cytological findings.

RESULTS AND DISCUSSION

The USG-FNAB was done in 12 animals. Fine needle aspiration of the prostate gland under ultrasound guidance is an effective means of localizing and diagnosing prostatic lesions (Zinkl, 1999). The animals were divided into 4 groups
according to clinical, haematological, radiological, ultrasonographic and cytological findings. The groups were classified as:-

1) Benign Prostate Hyperplasia (N=3)

2) Prostatitis (N=3)

3) Prostatic Carcinoma (N=4)

4) Non diagnostic sample (N=2)

The survey radiography of the caudal abdomen showed that there was an increase in the soft tissue density caudal to the neck of the urinary bladder with cranially displaced and distended urinary bladder. Ultrasonographic findings showed that the prostate was enlarged with normal or generalized increase in echogenicity in case of BPH, mixed echotexture in prostatitis and hyperechoic/ mixed, non uniform echotexture mass were evident at the neck of urinary bladder in the region of prostate in case of prostatic carcinoma. Rectal temperature was within normal range and heart rate and respiration rate were moderately elevated in most of the cases (Table 1). Anorexia, hematuria, history of urolithiasis, constipation, and pasty faeces etc. are the common clinical findings in prostatic affections. Similar clinical signs were recorded by Singh (2006), Paclikova et al., (2006), Mahajan (2007) and Kraft et al., (2008) in prostatic affections in dog. The prostate was found enlarged on per-rectal examination in all cases of prostatic affections.

In case of BPH, the blood SGPT, SGOT and AKP values were moderately elevated in two and markedly elevated in one animal (Table 2). The total platelet count was adequate in two cases and was moderately high in one case. BUN and creatinine levels were normal in all except in one case in which BUN and creatinine level were markedly elevated (124 mg/dL and 3.1 mg/dL respectively). Total protein and albumin level were within the normal range in all animals. In majority of cases
there was mild neutrophilic leucocytosis which might be due to concurrent inflammatory conditions (Paclikova et al., 2006). The blood Hb was normal in one case, slightly low in one case and was very low in one case.

In case of prostatitis the blood SGPT, SGOT and AKP values were moderately elevated in all animals (Table 2). The increase in AKP value might be due to inflammation and degeneration of prostate cells (Paclikova et al., 2006). Total platelet count was adequate in all the animals. BUN, creatinine, total protein and albumin level were within the normal range in all animals. In majority of cases there was neutrophilic leucocytosis as also reported by Hanson et al., (2001) and Paclikova et al., (2006). The blood Hb was normal in two cases and was low in one case.

In case of prostatic carcinoma, the digital rectal examination of prostate tumors commonly reveals an enlarged, irregularly asymmetrical, usually non-painful, gland (Zinkl, 1999). Dogs generally do not show clinical signs until late in the course of the malignancy (Hanson et al., 2001). The blood SGPT, SGOT values were moderately elevated in all animals but AKP value was markedly elevated in all animals (Table 2). Total platelet count was adequate in two animals and was moderately high in rest of the two animals. Renal function tests, total protein and albumin level were within the normal range in all except one animal in which BUN and creatinine level was very high. In majority of cases there was marked neutrophilic leucocytosis. The blood Hb was within normal range in two cases and was low in rest of the two cases.

**CONCLUSION**

The Haemato-biochemical profile was found to be useful adjunct in diagnosis of prostatic affections in dogs. BPH and prostatitis were characterized by moderate elevation of SGPT and AKP values along with mild to moderate anemia and neutrophilic leucocytosis. Mild to moderate increase in SGPT, SGOT along with
marked elevated AKP values were recorded in neoplastic conditions of prostate (prostatic carcinoma).

**Table 1: The mean ± SE values of rectal temperature, pulse rate and respiratory rate in cases of prostatic affections.**

<table>
<thead>
<tr>
<th>Affections of prostate</th>
<th>Rectal Temperature (°F) Mean ± SE</th>
<th>Respiratory Rate (Breaths/min) Mean ±SE</th>
<th>Heart Rate (Beats/ min) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG-FNACBPH (N=3)</td>
<td>102.87±0.406</td>
<td>42.00±3.055</td>
<td>98.00±5.774</td>
</tr>
<tr>
<td>USG-FNAC Prostatitis (N=3)</td>
<td>103.13±2.659</td>
<td>41.33±8.110</td>
<td>95.33±7.333</td>
</tr>
<tr>
<td>USG-FNAC Prostate carcinoma (N=4)</td>
<td>101.92 ±0.522</td>
<td>40.00±3.162</td>
<td>96.00±5.228</td>
</tr>
<tr>
<td>USG-FNAC Non Diagnostic sample (N=2)</td>
<td>102.80±0.200</td>
<td>41.00±5.000</td>
<td>91.00±1.000</td>
</tr>
</tbody>
</table>
Table 2: Hematobiochemical parameters in prostatic affections.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Benign Prostate Hyperplasia (BPH)</th>
<th>Prostatitis</th>
<th>Prostatic Carcinoma</th>
<th>Normal reference ranges*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (X g/dL) Mean ± SE</td>
<td>9.43± 2.88</td>
<td>10.33± 1.29</td>
<td>9.00± 2.70</td>
<td>12-18</td>
</tr>
<tr>
<td>TLC (X10^9/μL) Mean ± SE</td>
<td>22.03± 3.96</td>
<td>19.72± 4.48</td>
<td>15.52± 4.02</td>
<td>6-17</td>
</tr>
<tr>
<td>DLC (%) Mean±SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>78.67± 10.35</td>
<td>92.33± 2.03</td>
<td>87.50 ± 1.71</td>
<td>60-70</td>
</tr>
<tr>
<td>L (%)</td>
<td>14.00± 5.03</td>
<td>6.67± 1.33</td>
<td>12.50 ± 1.71</td>
<td>12-30</td>
</tr>
<tr>
<td>M (%)</td>
<td>1.67±0.33</td>
<td>0.33±0.33</td>
<td>-</td>
<td>03-10</td>
</tr>
<tr>
<td>E (%)</td>
<td>5.67± 5.67</td>
<td>0.67± 0.67</td>
<td>-</td>
<td>02-10</td>
</tr>
<tr>
<td>SGPT (μ/L) Mean ± SE</td>
<td>103.67± 8.95</td>
<td>131.00± 5.57</td>
<td>147.50± 20.56</td>
<td>8.2-57</td>
</tr>
<tr>
<td>SGOT (μ/L) Mean ± SE</td>
<td>147.00± 51.21</td>
<td>123.67± 12.73</td>
<td>118.25± 17.85</td>
<td>8.9-49</td>
</tr>
<tr>
<td>AKP (μ/L) Mean ± SE</td>
<td>229.67± 75.70</td>
<td>256.33± 23.68</td>
<td>296.25± 64.53</td>
<td>10.6-101</td>
</tr>
<tr>
<td>BUN (mg/dL) Mean ± SE</td>
<td>57.33± 33.41</td>
<td>100.93± 76.56</td>
<td>88.00± 44.87</td>
<td>8.8-26</td>
</tr>
<tr>
<td>Creatinine (mg/dL) Mean ± SE</td>
<td>1.83± 0.66</td>
<td>8.47± 7.62</td>
<td>4.65± 3.52</td>
<td>0.5-1.6</td>
</tr>
<tr>
<td>Total Protein (g/dL) Mean ± SE</td>
<td>6.70± 0.10</td>
<td>6.90± 0.06</td>
<td>6.30± 0.33</td>
<td>5.5-7.5</td>
</tr>
<tr>
<td>Albumin (g/dL) Mean ± SE</td>
<td>3.53±0.66</td>
<td>3.87± 0.03</td>
<td>3.40± 0.23</td>
<td>2.6-4.0</td>
</tr>
</tbody>
</table>
REFERENCES


INTRODUCTION

Equine fractures usually result in serious conditions manifested by lameness. A comprehensive fracture classification is important for the veterinary orthopaedist who would comprehend the method of reduction and subsequent treatment and fixation in such cases (Bramlage, 1983). Fractures of the scapula and femur are seldom to occur because of the protection afforded to these bones due to position and the extensive surrounding soft tissues (Stashak, 1987). Incomplete humeral fractures are most commonly recognized in race horses and are particularly difficult to diagnose (Nixon et al., 1996). Radial fractures are significant orthopaedic emergencies in adult horses (Watkins, 2006).

Fractures of the ulna are relatively common in foals and adult horses (Levine and Meagher, 1980). Carpal bone fractures frequently occurs as a result of heavy falls or run over by street cars (Auer et al., 1990). Distal limb fractures are the most common cause for equine euthanasia in all types of race horses.

The basics of fracture management in the horse have the same guidelines
established for human and small animals. Many techniques can be derived from them, but some principles are unique in the treatment of equine long bone fractures (Auer 1999; Fackelman et al., 2000; and Johnson, Houlton et al., 2005). Principles of equine fracture treatment depend mainly on prevention of further soft tissue damage, debridement of necrotized and contaminated tissues, and stabilization of fracture fragments. Nonsurgical management techniques include stall rest and external Co-aptation (Fessler and Turner, 1983) while surgical management includes; trans-skeletal fixation (Nunamaker and Richardson, 1992, McClure et al., 2000), internal fixation including pins (Nixon et al., 1996 and Fitch et al., 2001), lag screw (Perren and Buchanan, 1981), and bone plates (Auer and Stick, 2006).

In this case study, management of fractures in equines with the conservative methods of external loaptation using fiberglass reinforced with metal splint for multiple fracture of radins in a mare and multiple fracture of metacarpal in a horse young stock are reported.

HISTORY AND CLINICAL FINDINGS

A 19 year old Mare and a 1 yr old young stock were admitted to veterinary wing with history of kick injury on forelimb followed by swelling at site along with non weight bearing on affected limb. Signs of swelling and mild diffuse pain were elicited in response to palpation of the radius-ulna right fore region in the first case (mare) and at metacarpal region in the second case (young stock). Crepitating sound was present at the site of fracture in both cases. Radiographic evaluation of right radius and ulna region revealed multiple fractures of radius along with few small bone chips at the site of fracture in mare and multiple fracture of proximal third metacarpal bone along with few small bone chips at the site of fracture in the young stock. (Fig. 1 & 2)
TREATMENT

Fracture in both cases was managed with conservative treatment in-situ in the paddocks. The affected limb was stabilized with modified Robert Jones bandage reinforced with aluminum splint and fiber glass. Immediately after admission Inj Meloxicam was administered for pain management. Animals were casted in lateral recumbency by administering Inj Xylazine @ 1.1 mg/kg BW as sedative followed by Inj Ketamine @ 2.2 mg/kg BW after 5 min. Counter traction was applied on the affected limb from both ends. After cleaning the entire limb, the limb was dusted with powder calamine. Modified Robert John bandage was applied on the affected limb followed by a layer of fiber glass. A 3mm aluminum splint was moulded in limb shape ('U' shape) and was enforced over the layer of fibreglass. The splint was tightened with cotton rope and again a layer of fibreglass was applied on the affected limb. Both the animals recovered very well after anesthesia and got up on its own after 45-50 min (Fig.3). Animals were kept in a loose box with sandy surface. Radiographic evaluation was carried out after 21 days of application of fiber glass done to evaluate the formation of callus (Fig. 4 and 5). The cast was removed after 45 days and uneventful recovery was noticed in the both the cases.

DISCUSSION

Long bone fractures in horses are considered as a major problem for owners, trainers, and veterinarians for long. The practice of humanitarian euthanasia of horses with long bone fracture particularly open comminuted fractures was the only acceptable management of choice till many years ago (Nunamaker, 2002). Management of equine fractures and prognosis are determined by different factors such as the affected region, the fractured bone, age, sex, weight and temperament (LeJeune et al., 2003 and Fürst et al, 2008).

Successful outcome of an injury requires prompt recognition of the injury
and appropriate treatment at the earliest. Equine fracture in particular, each step of treatment, beginning immediately after injury (first aid and stabilization), is critical for improving the chances of a successful outcome. Without appropriate first aid and emergency splinting, the overall prognosis for survival can be poor. However, if emergency first aid is performed properly, the chances for a successful outcome can be increased, some time significantly so. In the present case immediately after injury modified Robert Johns bandage along with metal splint reinforced with fibre glass was applied before transferring the animal to veterinary hospital which could have helped in comfortable transportation of the animal and successful outcome in this case.

Management of equine fractures can be divided into conservative (non-surgical), surgical methods and euthanasia. Conservative treatment includes stall rest, medical treatment and external coaptation like splints or casts. The surgical treatment consists of trans-skeletal fixation and internal fixation using lag screw, bone plate, pins, cerclage wire either alone or in combinations. The prognoses of all cases managed at right time were found to restore the normal function of the fractured region. Euthanasia may be required only in cases of bad prognosis, old open fractures and some complicated cases. This finding is in agreement with that of Auer (2006). External fixation of limb fractures through application of splints or casts are still the most common method of treatment employed in veterinary practice either as first aid or less frequently as complete treatment (Nemeth and Black, 1991).

In the present cases, conservative treatment via application of casting materials gave encouraging results in both distal radial and one metacarpal fractures. The prognosis was favorable in both cases. External fixation by using a fiberglass cast showed advantages as a better fixation than plaster of Paris, in terms of being harder, allowing the skin to breath, lighter in weight and requires shorter time for
curing in management of distal limb fractures. This is in agreement with the characteristics listed by Auer et al., (1990). Full limb cast along with metal splint was applied in both the cases. The use of metal splint coaptation gave excellent results in terms of optimal healing with good consolidation of the callus across the fracture line particularly in multiple fractures. These results are in agreement with those reported by Janicek et al., (2013). The recorded complications with conservative management of fractures were in terms of instability soft, tissue adhesions, tendons laxity and cast soreness. The same observations were obtained by Bramlage, (1983).

CONCLUSION

The management of equine fractures begin with careful assessment of the fracture as well as the temperament of the patient. Proper stabilization of the fracture is essential for a successful outcome, and an improperly placed splint can cause significant soft tissue damage or worsening of the fracture. Although fracture stabilization and, ultimately, repair of the fracture are central components of fracture management, the medical treatment of the patient also plays a key role in the morbidity and mortality of these cases. In the present study, conservative treatment via application of fiberglass enforced with metal splint gave encouraging results in two cases of fracture in horses involving radius & metacarpal bone respectively.

REFERENCES


Fig. 4 (Case 1-after 21 days)

Fig. 5 (Case 2 after 21 days)
SUCCESSFUL CLOSED REDUCTION OF UNILATERAL LUXATION COXOFEMORAL JOINT IN A LABRADOR RETRIEVER DOG OF ASSAM RIFLES

DR RUP JYOTI LASKAR*, LT COL S J PREM KUMAR**

INTRODUCTION

Coxofemoral (CF) or hip luxation is a traumatic displacement of the femoral head from the acetabulum. Coxofemoral luxation is the most frequently encountered luxation in veterinary practice. Craniodorsal is the most common type of CF luxation, seen in 78% of affected dogs. In Craniodorsal hip luxation the head of the femur rests dorsal and cranial to the acetabulum making the affected limb shorter than the opposite limb when positioned ventrally and extended caudally. The thigh is adducted, and the stifle is rotated outward and the hock inward. On palpation, the trochanter major is elevated compared with the normal side and the space between it and the tuber ischii is increased. Traumatic coxofemoral luxations cause disruption of the joint’s supportive structures, resulting in damage to soft tissues (such as the round ligament, joint capsule and surrounding musculature) and articular surfaces (Bone et. al. 1984). Most affected animals have the history of trauma, such as motor vehicle accidents. Hip luxation should be treated as quickly as possible to prevent continued damage of the soft tissue surrounding the hip joint and degeneration of articular cartilage (Evers et. al. 1997). Early reduction allows rapid return of the nutrient source of the articular cartilage. Animals with hip luxation usually show a non-weight-bearing lameness associated with trauma.

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Diagnosis of hip luxation should be confirmed with ventrodorsal and lateral radiographs. The goals of treatment for luxation of the hip are to reduce the dislocation with as little damage to the articular surfaces as possible and to stabilize the joint sufficiently to allow soft tissue healing, with the expectation of normal clinical function. Most patients can be treated by closed reduction. In certain cases, hip luxation is irreparable because of conditions like pre-existing dysplasia, severe abrasion to the articular cartilage of the femoral head, and irreparable concomitant fractures of the acetabulum or femoral head.

HISTORY AND CLINICAL OBSERVATION

A female Labrador retriever dog reported to the Veterinary Hospital of Assam Rifles Dog Training Centre, Jorhat from Manipur. Handler reported that she had a fall in the hilly area during duty and after that limping was observed during walking. The thigh area of left hind limb was swollen and evinced pain on palpation. She was showing typical posture of cranio-dorsal hip dislocation along with shortening of left hind limb in comparison to the right hind limb (Fig.1). Physical examination revealed cranio-dorsal hip dislocation of left hind limb which was confirmed by radiographic examination (Fig.2)

TREATMENT

After physical and radiographic continuation the dog was administered pre anaesthetic medication with atropine sulphate @ 0.02 mg per kg BW and Xylazine hydrochloride injection @ 0.5 mg per kg BW. After sedation the dog was anaesthetised with Ketamine Hydrochloride injection @ 5 mg per kg BW. The dog was then placed on left lateral recumbancy on a table and closed reduction of left hip joint was done. A rope was placed in the inguinal area and gently it was pulled dorsally. To provide countertraction, the distal portion of the limb was grasped and pulled gently in the opposite direction (Fig.3). With one hand positioned on the greater trochanter and the other hand positioned distally on the stifle or hock,
reduction of the coxofemoral joint was achieved by externally rotating the limb, during which firm and consistent traction was applied (Basher et al. 1986). When the femoral head was in the area of the acetabulum, the limb was internally rotated, with the hand on the greater trochanter, pushing caudally with slightly abducted. Firm pressure was applied to the greater trochanter, as this motion will allowed the femoral head to gently “toggle” over the cranial lip of the acetabulum and resume its normal position. It was confirmed as both the hind limbs became equal in length.

After closed reduction, Ehmer sling was applied as per standard procedure (Fig. 4). The dog was treated with Cap. Crtigen @ one cap BID for three months along with Intacal pet syrup @ 10 ml BID for one month. The dog was on complete rest for the next three months and it had an uneventful recovery (Fig: 5).

SUMMARY AND CONCLUSION

Successful management and treatment of coxofemoral hip luxation in one Labrador retriever of Assam Rifles. It is proved that closed reduction management along with Ehmer sling gives good results in coxofemoral hip luxation.

REFERENCES


INSTRUCTIONS TO AUTHORS

1. The Journal of Remount Veterinary Corps is an official publication of Remount Veterinary Corps of Indian Army and is published half yearly in the month of Jan & Jul every year. It publishes papers on original works, general and clinical articles and reviews on all aspect of veterinary science with special reference to equines, canines and dairy animals.

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EDITORIAL

The Journal of RVC has been churning out precious scientific articles & reviews in the field of equines and mil working dogs. It is a matter of great pride that possibly this is the only Mil Vet Journal still under publication in the entire world. True to its tradition, the current issue of the Journal also has an interesting mix of rare case reports, articles on latest Vet diagnostic techniques and scientific research reports dealing exclusively with equines and mil working dogs.

Accurate diagnostic tools form a very important pillar for sound Veterinary Treatment. Articles in this issue titled “Diagnosis of Hepatozoon canis in working dogs by polymerase chain reaction” and “Standardization of a differential multiplex PCR assay for equine herpesvirus 1 and 4 as a diagnostic tool” are sure to throw light on ways to improve diagnostic capability of canine & equine diseases.

Case reports on “Canine periodontal disease” “Successful correction of partial bilateral Nasolacrimal duct obstruction (NLDO) in a male labrador” and “Canine Ocular Thelaziosis” are noble efforts in the direction of accumulating information for better and improved canine practice.

Articles on Veterinary orthopedics & surgery titled “Conservative management of radius and metacarpal fracture in equines with external coaptation using fiber glass cast enforced with metal splint” and “Tenorrhaphy of superficial digital flexor tendon in a GS mule using three loop pulley suturing techniques” reflect the sincere efforts on the part of authors to contribute to the advancement in equine orthopedics.

We are happy to inform that Jan 2016 issue of The Journal of RVC has been made available online at https://indianarmy.nic.in (knowledge.online). Soon we will be uploading the old issues of the Journal from the archives.

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