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BABESIOSIS IN DONKEYS: AN UPDATE

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Introduction

Despite the increase in mechanization throughout the world, donkeys are still well deserving of the name "beasts of burden". They act as life line in arid and semi-arid parts of the world by a way of economical mode of transport for the people and goods. This is evident by the widespread use of donkeys in rural and urban areas of Asian and African continent and parts of Central America. The overall population of equids has decreased over the last two decades; however the population of donkeys has remained unchanged, emphasizing the value of the donkey as draught animal despite the mechanization of agriculture and allied field. The donkey, being the work animal, is mainly employed in transport of goods over a short distances either as a pack animal or in pulling cart. Besides this, the donkey is preferred over other animals due to its docile nature, disease resistance, easy to put on work, less health care and source of livelihood to the poorest of the poor.

The genus *Babesia* secured a place in the protozoological history due to the classical work of Smith and Kilborne (1893), which first proved that the cattle tick *Boophilus annulatus* act as a vector for the transmission of Texas fever, caused by a blood protozoa *Babesia bigemina*. In equines two different protozoa, *Babesia caballi* and *Babesia equi* are known to cause infection and the disease was described by Wiltshire as Anthrax fever (Castellani and Chalmass, 1910). In African continent, prior to 1910, equine babesiosis used to be clinically confused with African horse sickness (cited by Roberts et al., 1962). It was the Laveran in 1901, who first discovered an intraerythrocytic parasite in the peripheral blood smears prepared from South African horses. He accordingly named it *Piroplasma equi*. Later on in 1902, Theiler, confirmed the finding by reporting that the equine piroplasm and African horse sickness were two separate distinct entities (cited by Robert et al, 1962). Nutall and Strickland (1910) reported that two distinct species of the protozoa were involved in equine piroplasmosis. They demonstrated a large intra-erythrocytic parasite, which was distinct from *Piroplasma equi*, and was responsible for causing piroplasmosis in horses. They named the smaller species *Nuttallia equi* (Laveran) and large species *Piroplasma caballi* (Nuttall). Further, Nuttall and Strickland (1912) reported that *N. equi* is more prevalent and virulent than *P. caballi*. Later on, these organisms were classified under the genus *Babesia* as *Babesia equi* and *B. caballi*.

Equine babesiosis has posed a threat to the international movement of equids (Friedhoff et al., 1990), because when horses, donkeys from a *Babesia*-free are introduced into an endemic area they suffer clinically from babesiosis resulting into their death. Hence, restriction was imposed on the entry of piroplasm seropositive horses into American territory during Atlanta Olympic Games, 1996 (Losson, 1994)

Epidemiology and etiology:

Babesiosis is one of the most important tick-transmitted haemoprotozoan disease in equids (horses, donkeys, mules and zebras). *Babesia equi* is a small piroplasm, measuring 2.0 x 1.0 µm, whereas *Babesia caballi* is a large form of the parasite and measures approximately 3.0 x 2.0 µm. The shape of the *B. equi* parasite in the infected erythrocyte varies from spherical to ovoid to the Maltese-cross. The latter form is characteristic to *B. equi* (Fig. 1) in contrast to *B. caballi* parasite. Occurrence of both *Babesia equi* and *Babesia caballi* have been reported from many European countries including Portugal, Spain, France, Belgium, Italy (Chevrier et al, 1979; Friedhoff, 1982), large parts of Russia (Friedhoff and Soule 1996), throughout the African and Asian continents, South and Central America. *Babesia equi* is known to be more virulent and tends to cause a fulminating parasitaemia (Gerstenberg et al., 1998). *Babesia equi* infection in specially donkeys has been reported from tropical and subtropical courtiers like (India : Malhotra et al, 1978; Kumar et al 1997; Brazil : Kerber et al, 1999; Arabian countries :Turnbull et al 2002). The distribution of this disease in Southern Africa has not been studied extensively, but probably coincides with the distribution of the respective tick vector (de Waal and van Heerden, 1994). *B. caballi* is supposed to be more widespread in Sothern African countries than *B. equi*. Equine babesiosis observed to precipitate in the event of strenuous exercise (Hailar et al 1997), hence carrier donkeys when used as pack/draught animals become more prone to this alarming infection.

Ixodid ticks of the genera *Hyalomma*, *Dermacentor* and *Rhipicephalus* have been identified as vectors for the transmission of either *B. equi* or *B. caballi* protozoa to natural host. In Tropical countries including India, ticks of *Hyalomma* species seems to be a potential vector for the transmission of disease to donkeys and horses. Ticks of the species *Hyalomma anatolicum anatolicum* (both sex in same ratio) were released on the experimental donkeys showing gradual rise in per cent *B. equi* parasitaemia, it was observed that acini of the male ticks were more infected than the female ticks (Phogat 2001). The average infected acini per tick in male were 23.95% as compared to the females (13.19%). These ticks were able to transmit infection to the natural host-donkeys.

Pathogenesis:

The events in pathogenesis responsible for haemoglobinuria and subsequent anaemia are obscure in equine babesiosis (de Waal and Heerden, 1994). Development of progressive anaemia and haemoglobinuria in the last clinical phase of the disease is pathognomonic sign in *B. equi* infection in horses and also in donkeys (Holbrook et al 1973). The pathogenesis of anaemia has not been fully elucidated. The adverse effects which *B. equi* and its metabolites inflict on donkey's erythrocytes have been studied (Ambawat et al 1999). At high parasitaemia *B. equi* organisms were observed in neutrophils indicating phagocytosis of the infected erythrocytes by the neutrophils. They reported progressive increase in donkey's erythrocyte membrane proteins, total phospholipids and plasma malondialdehyde thereby suggesting lipid peroxidation during acute phase of the disease. Gradual decrease in haemoglobin and PCV values with the clinical progression of *B. equi* parasitaemia in experimental donkeys was also observed. Further scanning electron microscopy of *B. equi*-infected donkeys revealed spherocytes, sphero-echinocytes, sphero-stomatocyes, kinizocytes, and acanthocytes with pitting and fine granulation on their cell surface (Ambawat et al 1994). This limited study concludes the occurrence of

morphological changes and lipid peroxidation on the cell surface leading to erythrocyte-phagocytosis and subsequent anaemia during *B. equi* infection in donkeys.

Clinical manifestations:

Infection caused by *B. equi* is more pathogenic and widespread than by *B. caballi* protozoa. Mixed infections are not uncommon, makes diagnosis difficult. The incubation period following an infective tick bite varies from five to 21 days (Theiler 1905, Gautam and Dwivedi 1976). The disease condition start with intermittent fever up to 40°C followed by listlessness, depression, marked thirst, in appetence, watering from the eyes, and swelling of the eyelids. Affected donkeys are constipated, passing small, hard balls of faeces covered with yellow mucus, may loose the health condition. Sometimes donkeys can show colicy symptoms viz. looking at flank, pawing, kicking at belly region, lying down and rolling, due to sluggish intestinal peristaltic movements and constipation. The most characteristic sign is the development of icterus, mucous membrane vary from pale pink to pale yellow to bright yellow in colour. Petechiae or ecchymosed hemorrhages are seen on the mucous membranes of nasal passage, vagina and third eyelids. Extremely large spleen in the affected animals is very common symptom. Lately urine color becomes dark yellow to orange/brown indicating presence of haemoglobin and bile pigments as a result of severe haemolysis of the infected erythrocytes. Untreated or neglected cases becomes severely anemic, malaise, reluctant to move, down neck, in appetence, disinterest in surroundings and shows sign of general weakness. Equines usually have higher threshold for escape of haemoglobin through urine from the circulation, hence haemoglobinuria is observed as last irreversible clinical sign signifying nephrosis and subsequent renal failure.

Chronic cases of equine babesiosis are more common in donkeys and clinical signs are usually non-specific including mild appetence, poor work performance or poor body weight gain. Splenomegaly has been observed as unusual common finding in affected donkeys. Neonatal babesiosis has not been observed by the author, its presence has been reported with scanty literature. Severe hepatomegaly, splenomegaly, icterus and internal hemorrhages have been reported (de Waal and Heerden, 1994).

Clinical pathology: Haemoglobin concentration, PCV level, red blood cell counts reduces significantly in donkeys infected acutely with *B. equi* parasite (Ambawat et al 1999, Kumar et al 2002). Acute infection is also characterized by severe leucocytosis, lymphopenia, high absolute neutrophils count (Kumar et al 2002, Singh et al 1980). Donkeys that died of *B. equi* infection show varying degree of emaciation, gross enlargement of liver and spleen; flabby kidneys (Sengupta et al 1999). Small pin-point petechial hemorrhages are also present on liver, spleen and cortical surface of the kidneys. Lungs are oedamatous, congested and enlarged lymph nodes. Microscopically, Kupffer cells revealed deposition of haemosiderin pigments (Sengupta et al 1999, Gautam and Dwivedi 1976).

Diagnosis:

I. **Microscopy:** It is difficult to diagnose the clinical disease condition in donkeys on clinical symptoms. Although high fever, anaemia, jaundice, with or without haemoglobinuria are important clinical signs and animal showing symptoms should be preceded with blood smear examination. The clinical form of the disease can be confirmed by detecting *Babesia* protozoa inside the erythrocytes in blood smear stained with

Romanowsky's stain (viz. Giemsa stain). The organism may be found either singly, in pairs, or in tetrads. Formation of four daughter parasites generally called as Maltese-cross' is very characteristic feature of *B. equi*. Irrespectively *B. caballi* organisms are commonly seen singly or in pairs and are pyriform, round or oval in shape. Maltese-cross form is absent in *B. caballi*. In *B. equi* infection clinical parasitaemia may exceed 20 per cent but 1-5 per cent parasitaemia is more commonly observed in field conditions. In *B. caballi* parasitaemia is even lower than that observed in *B. equi* infection. In latent carrier donkeys it is very difficult to demonstrate the parasite in stained blood smears as the parasitaemia is extremely low. Hence, serological and other tests are used to detect the carrier status in the donkeys.

II. **Serological tests:** A variety of serological techniques have been standardized and used for specific detection of antibodies in carrier donkeys and horses. At this time, complement fixation test (CFT) is the only official test approved by USDA, Canada, Australia and Japan for screening of horses imported from countries where equine babesiosis is endemic (Holbrook et al 1971). Kumar et al (1997) reported that CFT should be standardized separately for donkeys as the condition optimized on horse samples failed on donkey's samples. They reported that donkey's serum activates non-specifically the complement (from Guinea pig serum) and hence requires higher concentration of complement in CF test than that of horse. They also advocated that the donkey serum should be in-activated at 59°C for 30 min instead of conventional 56°C for 30 min. This practice reduces the non-specific fixing of complement by the donkey's serum. ELISA's have also been standardized for detecting *B. equi* antibodies in carrier donkeys. Among this, Dot-ELISA (Kumar et al 1997), serial dilution ELISA and single dilution ELISA (Kumar et al 2003) are most important. Dot-ELISA could detect *B. equi* antibodies in experimentally infected donkeys as early as 3-6 days post-infection and remained positive showing high antibody titre until 90 days experimental period. Serial dilution ELISA was found to be more time consuming, require large quantities of antigen/conjugate/reagents as compare to single dilution ELISA, when the end titre of a sample is to be determined (Kumar et al 2003, Manuja et al 2001, Singh et al 2001). The advantage of single dilution ELISA is that exact antibody titre can be determined by testing the serum sample at only one single particular dilution. The single dilution ELISA was found to be non-cross-reactive with *B. caballi* and *Trypanosoma evansi* positive serum and more sensitive than conventional serial dilution ELISA (Kumar et al 2003). Among these Dot-ELISA promises to be a field oriented test as it is simple to perform, require less expertise/reagents and obliterate the need of expensive ELISA reader. Other serological tests like capillary tube agglutination test (CAT, Malhotra et al 1978; Kumar et al 1997), counter current immunoelectrophoresis test (CIEP, Dhar et al 1997) were also used and standardized for diagnosis of *B. equi* infection in donkeys, but these are less sensitive and specific.

III. **PCR and DNA probe:** Routine microscopic and serological examinations are not sensitive enough in determining the status of the animals if the parasitaemia/antibody titre is very low. Hence direct detection of the specific parasitic DNA by a way of DNA probes and polymerase chain reaction are desirable. PCR based detection tests and DNA probes have been designed for detection of *B. equi* and *B. caballi* parasite in horses and we do not imagine any impediment in their application on donkey's samples. Posnett et al (1991) and Posnett and Ambrosio (1989; 1991) defined DNA probe as a diagnostic test capable to detecting the parasitaemia as low as 0.0028% (*B. equi*) and 0.0016% (*B. caballi*) in the blood samples. Recently PCR based diagnostic tests have also been standardized for detection of *B. equi* and *B. caballi* parasitic DNA in the blood (Bashiruddin et al 1999; Rampersad et al 2003). PCR methods have also been used to detect the *B.*

equi infection in the host tick-vector (*Boophilus microplus* and *Dermacentor nuttalli*) capable to transmitting *B. equi* infected sporozoites to the equid population in the field (Battsetseg et al 2001, 2002).

Treatment and Control:

I. **Chemotherapy:** The *B. equi* infection is resistant to chemotherapy as compare to *B. caballi* infection and usually frequent repeated doses are required. A variety of drugs have been used for the treatment of *B. equi* infection in donkeys. Most of the drugs improve the clinical signs but unable to completely eliminate the infection from the body. At the same time it is seldom mandatory in endemic areas, but entails to serious relapse of the disease condition in the event of physiological/physical stressful conditions. Tetracycline like chlortetracyclins hydrochloride (Aureomycin®, Ledera Laboratories) and oxytetracycline hydrochloride (Terramycin®, Pfizer) are effective only against *B. equi* when given intravenously daily for two or more days at a dosage rate of at least 5.5 mg/kg body weight (Jansen 1953). The time interval between doses seems to be important. Four intramuscular doses of imidocarb dipropionate (Imizol® Burroughs Wellcome and Co., Gr. Britain), 72 h apart at 4 mg/kg body weight cleared *B. equi* from horses but not from the infected donkeys, which died even after treatment (Frerichs et al 1973; Baturina and Lutsuk 1975). Two dose therapies with imidocarb, 48 h interval at 5 mg/ml was found quite effective in bringing about the clinical recovery in infected donkeys (Singh et al 1980).

Dennig (1965) reported that diminazene diaceturate (Berenil® Hoechst Pharmaceuticals Ltd.) was the only drug that was successful against mild-to-moderate *B. equi* infection in horses and donkeys, but that it was not effective against acute infection. Further diminazene diaceturate was effective in eliminating *B. caballi* infection but not *B. equi* parasite (Taylor 1972, Kuttler 1981). Singh et al (1980) applied diminazene diaceturate to donkeys infected with *B. equi* parasite at a dose rate of 12 mg/kg body weight, two intramuscular injection 24 h apart, they observed that parasitaemia could only decline two to three days after treatment. It was opined that some time is required for initiation of drug action against multiplying parasite which should preclude its field use. Copper glycinate has been proved successful in treating clinical cases due to *Babesia bigemina* infection in cattle (Randawa et al 1992). But similar trial in splenectomised and non-splenectomised donkeys infected with *B. equi* parasite with intravenous injection of copper glycinate at 1.5 mg/kg, two injection 24 h apart, proved unsuccessful in reducing fulminating parasitaemias (Kumar et al 1999).

Encouraged with the high therapeutic efficacy of artemisinin (qinghaosu) derivatives viz. artesunate, arteether and artemeter against multiple drug resistant cases of falciparum malaria (Bunnag et al 1992; Pittler and Ernst 1999; Taylor et al 1997) and buparvaquone against tropical bovine theileriosis by *Theileria annulata* (Wilkie et al 1998), we tested these drugs for their therapeutic efficacy (alone or in drug combination) against *B. equi* infected splenectomised donkeys (Kumar et al 2003). Individually, arteether (5 mg/kg daily for 3 days, intramuscularly) and buparvaquone (5 mg/kg daily for 4 days, intravenously) were found to have no parasite clearing efficacy and the treated donkeys died within 5-6 days after showing high parasitaemia and clinical symptoms of the disease. However, artesunate (2.5 mg/kg daily for 4 days, intramuscularly) treated animals were able to restrict the parasite multiplication but only during the treatment period, after that all the treated donkeys died. Animals treated with imidicarb and arteether+buparvaquone combination were able to clear the parasite from the blood circulation after 2-5 days post-treatment (PT), but recrudescence (in both these groups) of *B. equi* parasite was observed after 55-58

days PT and mean survival period post-treatment was 66-69 days. Haemato-biochemical parameters on these animals had shown that imidocarb had deleterious effect on the liver function while on the other hand arteether+buparvaquone combination was found to be safe. This limited study indicates that arteether+buparvaquone combination could be a better choice than imidocarb for treating *B. equi* infection in donkeys (Kumar et al 2003).

II. **Immunological:**

Very scanty efforts were made with regards to development of a suitable and potent vaccine for the control of equine babesiosis in horses and donkeys. After having diagnosed the infection in equids, the second strategy come into affect is its curtailment and control. Some efforts towards vaccinological control have been made using crude *B. equi* immunogen in donkeys. Singh et al (1980) immunized donkeys with *B. equi* infected erythrocytes lysate followed by boosted inoculation. Immunized donkeys survived after challenge infection, but become carrier to *B. equi* parasite. Salem et al (1990) also tried crude vaccine on donkeys and reported protection upon challenge. Recently Kumar et al (2002) immunized the donkeys with immunogen so that each dose of immunogen should contain lysate of 2×10^{10} parasitized erythrocytes. The immunogen was mixed with adjuvant Quil A and injected and one boosted inoculation was repeated. The immunized donkeys survived after challenge infection (1×10^{11} parasitized erythrocytes), simultaneously a very high humoral and cell mediated immune response was mounted by the immunogen in the immunized donkeys by the immunonogen as monitored by ELISA and lymphocyte stimulation assay, respectively during the experimental period. The most astonishing part of the experiment was absence of any parasitaemia after splenectomy of the immunized donkeys which survived after challenge infection. This experiment had proved that *B. equi* crude immunogen can elicit a strong immune response against *B. equi* infection and also possibility of a vaccinological control of the infection. Further 112, 45, 33 and 18 kDa polypeptides were identified as immunodominant in *B. equi* merozoite antigen and reacted strongly with the serum collected from immunized challenge survived donkeys (Kumar et al 2002). More future vaacinilogical experiments are required to come out with a potent subunit vaccine.

References:

Ambawat, H. K., Malhotra, D. V., Bhardwaj, R. M., Dhar, S., Rana, J. P. and Bhattacharyulu, Y. 1994. Scanning electron microscopy of *Babesia equi* infected erythrocytes. *Indian J. Vet. Med. 14:* 13-15.

Ambawat, H. K., Malhotra, D. V., Kumar, S. and Dhar, S. 1999. Erythrocyte associated haemato-biochemical changes in Babesia equi infection experimentally produced in donkeys. *Vet. Parasitol. 85:* 319-24.

Bashiruddin J. B, Camma C, Rebelo E. 1999. Molecular detection of Babesia equi and Babesia caballi in horse blood by PCR amplification of part of the 16S rRNA gene. *Vet. Parasitol. 84*:75-83.

Battsetseg B, Xuan X, Ikadai H, Bautista J. L, Byambaa B, Boldbaatar D, Battur B, Battsetseg G, Batsukh Z, Igarashi I, Nagasawa H, Mikami T, Fujisaki K. 2001. Detection of *Babesia caballi* and *Babesia equi* in *Dermacentor nuttalli* adult ticks. *Int. J. Parasitol. 31*:384-6.

Battsetseg B, Lucero S, Xuan X, Claveria FG, Inoue N, Alhassan A, Kanno T, Igarashi I, Nagasawa H, Mikami T, Fujisaki K. 2002. Detection of natural infection of *Boophilus microplus* with *Babesia equi* and *Babesia caballi* in Brazilian horses using nested polymerase chain reaction. *Vet Parasitol. 107*:351-7.

Baturina, F. M., Lutsuk, S. N. 1975. Action of dimidin on the causative agents of piroplasmosis and nutalliosis in horses. *Veterinariia. 9:*70.

Bunnag, D., Karbwang, J., Harinasuta, T. 1992. Artemether in the treatment of multiple drug resistant falciparum malaria. *Southeast Asian J. Trop. Med. Pub. Health. 23:* 762-7.

Castellani, A. and Chelmess, A. J. 1910. *Babesia equi* Laveran 1899. In: *Manual of Tropical Medicine*, Tindall and Cox,University Series, London, p 287.

Chevrier, L., Soule, C. and Dorchiers, P. H. 1979. Les piroplasmoses equines inapparentes. *Bull. Acad. Vet. Fr. 52:* 37-43.

Dennig, H. K. and Brocklesby, D. W. 1965. Splenectomy of horses and donkeys. *Veterinary record. 77:* 4045.

Dhar, S., Kumar, S., Malhotra, D. V., Chaudhary, S. S. and Bhattacharyulu, Y. 1997. Comparative study of counter-current immunelectrophoresis test for serodiagnosis of *Babesia equi* infection with capillary tube agglutination test. *Indian J. Vet. Med. 17:* 1-5.

Frerichs, W. M., Allen, P. C. and Holbrook, A. A. 1973. Equine piroplasmosis (*Babesia equi*): Therapeutic trials of imidocarb dihydrocholride in horses and donkeys. *Vet.Rec.. 93:* 73-75.

Friedhoff, K. T. 1982. Die piorplasmen der Equiden-Bedeutung fur den Internationalen Pferdeverkehr. *Berl. Munch. Tierartz. Wochenschr.95:* 368-374.

Friedhoff, K. T., Tenter, A. M. and Muller, I. 1990. Haemoparasites of equines: impact on international trade of horses. *Revne Scientifique et Technique – Office International des Epizooties. 9:* 1187-1194.

Friedhoff, K. T. and Soule, C. 1996. An account on equine babesiosis. *Revne Scientifique et Technique – Office International des Epizooties. 15:* 1191-1201.

Gautam. O. P. and Dwivedi, S. K. 1976. Equine babesiosis: A severe outbreak in stud farm at Hisar. *Indian Vet. J. 53:* 546-551.

Gerstenberg, C., Allen, W. R. and Phipps, L. 1998. Mechanical transmission of *Babesia equi* infection in a British herd of horses. In : *Equine Infectious Diseases VIII, Proceedings of the Eighth International Conference, Dubai.* 217-222.

Hailat, N. Q., Lafi, S.Q., al-Darraji, A.M., al-Ani, F.K. 1997. Equine babesiosis associated with strenuous exercise: clinical and pathological studies in Jordan. *Vet. Parasitol. 69:*1-8.

Holbrook, A. A., Cooperider, D. E., Roby, T. O., Kirkham, M. W. and Martin, W. H. 1971. Protocol for the complement fixation test for equine piroplasmosis. *Proc. 75th Ann. Meeting US Anim. Health Ass.* 617-627.

Holbrook, A. A., Frerichs, W. M. and Allen, P. C. 1973. Laboratory disgnosis of equine piroplasmosis. *Proceedings of the Third International Conference on Equine Infectious Diseases.,* Paris: S. Karger, Basel.

Jansen, B. C. 1953. The parasiticidal effect of aureomysin (Lederle) on *Babesia equi* (Laveran 1899) in splenectomised donkeys. *Ond. J. Vet. Res.. 13:* 103-106.

Kerber, C. E., Ferreira, F. and Pereira, M. C. 1998. Efficacy of imidocarb dipropionate against natural *Babesia equi* infections in horses. In: *Equine Infectious Diseases VIII, Proceedings of the Eighth International Conference, Dubai.* 552-554.

Kumar, S., Malhotra, D. V. and Dhar, S. 1997. Serodiagnosis of *Babesia equi* infection – a comparison of Dot-ELISA, complement fixation test and capillary tube agglutination test. *Vet. Parasitol. 69:* 171-176.

Kumar S, Malhotra DV, Dhar S, Nichani AK. 2002. Vaccination of donkeys against Babesia equi using killed merozoite immunogen. *Ve.t Parasitol. 106:*19-33.

Kumar S, Kumar Y, Malhotra DV, Dhar S, Nichani AK. 2003. Standardisation and comparison of serial dilution and single dilution enzyme linked immunosorbent assay (ELISA) using different antigenic preparations of the *Babesia* (*Theileria*) *equi* parasite. *Vet. Res. 34:*71-83.

Kuttler, K. L. 1981. Chemotherapy in babesiosis : a review. In: Ristic, M., Kreir, J. P., eds. *Babesiosis*. New York: Academic Press Inc. 65-85.

Losson, B. 1994. Equine piroplasmosis, a check on International movement of horses: relevance to the Atlanta Olympic Games. *Annalesp-de-Medicine-Veterinaire. 138:* 357-359.

Malhotra, D. V., Gautam, O. P. and Banerjee, D. P. 1978. Prevalence of latent cases of *Babesia equi* infection in some parts of North-West India as measured by capillary tube agglutination test. *Equine Vet. J. 10:* 24-26.

Manuja, A., Nichani, A. K., Kumar, R., Sharma, R. D., Kumar, B. 2001. Single dilution ELISAs using piroplasm, cellular schizont and soluble schizont antigens for the detection of antibodies against *Theileria annulata.*. *Vet. Res. 32:* 165-173.

Nuttall, G. H. F. and Strcikland, C. 1910. Die Paeasiten des Pferdepiroplasmose resp. der 'Biliary fever'. *Centralblatt fur Bakteriologie Parasitenkunde und Infektionskrankheiten Abteilung I. 56:* 524-525.

Phogat, S. K., Malhotra, D. V., Nichani, A. K., Sangwan, A. K. and Kumar, S. 2001. Assessment of infectivity of *Hyalomma anatolicum* ticks fed on *Babesia equi* infected donkeys at different levels of parasitaemia. Proceedings: *XIX Annual Convention of Indian Society for Veterinary Medicine.* p95.

Pittler M.H., Ernst E. 1999. Artemether for severe malaria: a meta-analysis of randomized clinical trials. *Clin. Infect. Dis.. 28:*597-601.

Posnett E. S, Fehrsen J, De Waal D. T, Ambrosio R. E. 1991. Detection of *Babesia equi* in infected horses and carrier animals using a DNA probe. *Vet. Parasitol. 39*:19-32.

Posnett E. S, Ambrosio R. E. 1991. DNA probes for the detection of *Babesia caballi. Parasitology. 102 Pt 3*:357-65.

Posnett E. S, Ambrosio R. E. 1989. Repetitive DNA probes for the detection of *Babesia equi. Mol. Biochem. Parasitol. 34*:75-8.

Rampersad J, Cesar E, Campbell M. D, Samlal M, Ammons D. 2003. A field evaluation of PCR for the routine detection of Babesia equi in horses. *Vet. Parasitol. 114*:81-7.

Randhawa, S. S., Randhawa, C. S. and Joshi, B. P. 1992. Dual efficacy of coper-glycinate – treatment of clinical babesiosis and nutritional haemoglobinuria in crossbred cattle. *Indian J. Vet. Med. 12:* 74-75.

Roberts, E. D., Morehouse, L. G., Gainer, J. H., McDaniel, H. A. 1962. Equine piroplasmosis. *J. Amer. Vet. Med. Assoc. 141:* 1323-1329.

Salem, A. A., Samia, A. H., El-Seify, M. A. and El-Battawy. 1990. Further studies on the efficacy of the locally prepared *Babesia equi* vaccine on equine piroplasmosis compared with imported one. *Assiut-Vet. Med.l J. 23:* 144-158.

Sengupta, P. P., Virmani, N. and Yadav, M. P. 1999. Pathological changes in the donkeys experimentally infected with *Babesia equi. Indian J. Anim. Sci.. 69:* 169-170.

Singh, B. P., Gautam, O. P. and Banerjee, D. P. 1980. Activity of imidocarb dipropionate against experimental *Babesia equi* infection in donkeys. *Indian J. Parasitol. 4:* 51-53.

Singh S, Khatri N, Manuja A, Sharma RD, Malhotra DV, Nichani AK. 2001. Impact of field vaccination with a *Theileria annulata* schizont cell culture vaccine on the epidemiology of tropical theileriosis. *Vet. Parasitol. 101:*91-100.

Smith, T. and Killborne, F. L. 1893. Investigation into the nature, causation and prevention of Texas or Southern cattle fever. *US Department of Agriculture Bur. Anim. Ind. Bull. 1:* 1-301.

Taylor, W. M. 1972. Chemotherapy of equine piroplasmosis. *Proceedings of the Third International Conference on Equine Infectious Diseases,* Paris: S. Karger, Basel.

Taylor T. E., Wills B. A., Courval J. M., Molyneux M. E. 1997. Intramuscular artemether vs intravenous quinine: an open, randomized trial in Malawian children with cerebral malaria. *Trop. Med. Int. Health. 3:* 3-8.

Theilor, A. 1905. Further notes on piroplasmosis of horse, mule and donkey. *J. Comp. Pathol. . Ther.. 18:* 229-239.

Turnbull, A., Werney, U., Werney, R., Anandh, J. P. and Kinne, J. 2002. Survey of six infectious diseases of feral donkeys in the United Arab Emirates. *Equine Vet. Edu. 14:*33-38.

de Waal D. T, and van Heerden J. 1994. Equine babesiosis. In: *Infectious diseases of livestock with special reference to Southern Africa.* Ed. Coetzer, J.A; Thomson, G. R.; Tustin, R. C. Oxford University Press. 295-304.

Wilkie G. M., Brown C. G., Kirvar B. E., Thomas M., Williamson S. M., Bell-Sakyi L. J., Sparagano O. 1998. Chemoprophylaxis of *Theileria annulata* and *Theileria parva* infections of calves with buparvaquone. *Vet. Parasitol. 78:*1-12.