COMPARATIVE EFFICACY OF VARIOUS DRUGS USED AGAINST NATURALLY INFECTED HORSES WITH BABESIOSIS

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ABSTRACT: Equine Piroplasmosis or Babesiosis is a serious hemoparasitic disease of horses. It is caused by two protozoal piroplasms i.e. Theileria equi and Babesia caballi. The disease can cause excessive destruction of red blood cells, anemia, jaundice, in-appetence and some times colic, which can be fatal. The present research was conducted to study the effect of three anti-protozoan (Babesicidal) drugs on the reversal of clinical signs of the disease. A total of 200 horses were included in this experimental study. Out of these, 80 horses exhibiting classic clinical symptoms of the disease and confirmed positive through blood smear examination, were randomly selected and divided into four groups i.e. A, B, C and D. Horses of group A, were treated with pentamidine methunsulphate at a dose rate of 3 mg/kg body weight intravenously, horses of group B were treated with imidocarb dipropionate at a dose rate of 2.4 mg/kg body weight intramuscularly, while horses of group C were treated with diminazene diaceturate at a dose rate of 3.5mg/kg bodyweight intramuscularly. Horses of Group D served as control. Efficacy of drugs was detected by the reversal of clinical signs and negative blood smear test. Pentamidine methunsulphate gave 100% results while efficacy of Imidocarb dipropionate proved to be 90%, whereas Diminazene diaceturate possessed 80% efficacy against babesiosis.

Key words: Equine Piroplasmosis, Babesiosis, *Theileria equi, Babesia caballi*, Pentamidine, Imidocarb, Diminazene.

INTRODUCTION

Horse is a valuable animal and can be easily infected by various bacterial, viral and protozoan parasites. These diseases adversely affect production and performance in addition to death of the animal. Babesiosis or Equine Piroplasmosis is one of the protozoan diseases which causes serious problems for horse husbandry and impairs the equine industry considerably [1].

Equine babesiosis is caused by *Babesia equi* (*Theileria equi*) and *Babesia caballi*, which are intracellular obligate parasites of the phylum Apicomplexa and cause hemoparasitic disease in horses [2,3]. Both species of the parasite are transmitted by fourteen species of ixodid ticks belonging to three genera *Dermacentor*, *Hyalomma* and *Rhipicephalus*, and are endemic in most tropical and subtropical areas of the world [4,5]. In nature, babesiosis is transmitted biologically by ixodid ticks, but other means such as biting flies and fomites that transfer blood from the infected carrier to susceptible animals may also be involved in the mechanical transmission of these intra-erythocytic parasite. Contaminated needles and surgical instrument can also transmit the infection physically. The ease with which infection can be transmitted in this way depends largely on the degree of parasitaemia [6]. After recovery, horses may become carriers for long periods. Animals infected with *B. caballi* can remain carriers for up to 4 years, but might be able to clear the organism eventually. Equids infected with *T. equi* appear to remain permanently infected [7]. Equine babesiosis is widely distributed throughout the tropics and subtropics and to a lesser extent in temperate regions [8]. This disease was not known to occur in Australia until 1976 and since then the disease has been common in South America, U.S.A., France, Spain, Portugal, Hungary, Yugoslavia, Greece and Italy [9].

The initial signs associated with babesiosis are rise in temperature, anemia, haemoglobinuria, malaise, depression, in appetence and poor performance of game horses. Edema of the head and legs is not unusual. Colic and passing of mucous covered hard faeces is also observed occasionally. The incubation period is 8 –10 days. Acute cases in adults show a sudden onset of immobility and reluctance to move, while some animals exhibit lateral recumbancy and do not respond to stimuli. Affected horses may die within 24 – 48 hours of the first appearance of clinical signs. Chronic cases may survive for months and become carriers [6]. The clinical symptoms are almost pathognomonic in areas with an enzootic occurrence of piroplasmosis. Diagnosis is based on the clinical signs, the presence of tick vectors, prevalence of the disease in the surrounding area and demonstration of the organism in peripheral blood. The most preferred site to obtain blood sample is the ear vein [10]. The severity of infection is related to number of parasites in blood of the infected animals. The acute and sub-acute forms of piroplasmosis can be diagnosed by microscopic examination of stained blood smears. For staining purposes 10% Giemsa's stain was used [11].

Many drugs including amicarbalide isothionate, diminazene, Pirevan, Berenil, euflavine, Diampron and oxytetracycline have been used with variable success to treat babesiosis in horses. Imidocarb more than 3 mg/kg body weight has been used for treatment of babesiosis. It can effectively depress parasitaemia and usually eradicates *B. caballi* infection. *T. equi* is much more resistant and Imidocarb therapy is only effective in eliminating the infection in 50-60% of the cases.

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Imidocarb is an aromatic dimidine. It has a widespread distribution in body fluids and tissues. The precise mode of action is not clearly understood [12,13,14]. Imidocarb also inhibits the entry of inositol into the parasitized erythrocytes and therefore, leads to 'Starvation' of the parasite [15]. Side effects include restlessness, colic, abdominal pain, sweating, diarrhea and anorexia sometime leading to death [16]. These are common at higher doses of imidocarb treatment and may also prove toxic. Pentamidine is a drug used in the treatment of protozoal infections. Treatment with Pentamidine may cause sudden cardiac death [17]. The mechanism of action of pentamidine is unclear and may differ for different organisms. Trypanosomes actively transport pentamidine intracellularly and the drug may then interfere with DNA biosynthesis. The drug appears to be concentrated in kidneys and excreted in urine. Immediate adverse effects have included hypotension, nausea and vomiting. Local pain or formation of abscess at injection site, mild azotemia, leucopenia, abnormal finding from liver function test, and hypoglycemia may also occur [18]. Diminazene diaceturate has been used to eliminate *B.caballi* infections [13]. Diminazene is an aromatic dimidine. The exact mechanism of action and *in vivo* behavior of Diminazene is poorly understood. Its effect on the babesia parasite appears to relate to interference with aerobic glycolysis, as well as, with synthesis of DNA in the parasite [19]. The side effect of Diminazene includes swelling and necrosis at the site of injection, respiratory distress and depression [13].

The present study was designed to determine: the best available drug against Babesiosis, which will save the life of horses, and will provide help to the veterinarians working in field in future as well.

MATERIALS AND METHODS

In this experimental study, 80 horses suffering from babesiosis were randomly selected from Game animals (Lahore Race Club and Atchison College) and Tonga ponies (SPCA, University of Veterinary and Animal Sciences, Lahore formerly College of Veterinary Sciences, Lahore) out of a total 200 horses that were examined. The horses were of either sex, and aged between 6-10 years. Outbreak or presence of blood protozoan disease in the surrounding area, usual feed, managemental practices and any previous medication done were questioned. A detailed clinical examination was performed and clinical signs like onset and duration of high body temperature, color and duration of passing dark colored urine, texture of the faeces, and signs of colic, malaise, depression, inappetence, debility, and loss in endurance and stamina of game horses was noted. Examination of conjunctiva, mucous membranes of buccal cavity and vagina, presence of any swelling on the extremities and presence of ticks was also observed. Examination of the blood smear was performed for identification of the parasite by slide method, in the laboratory of Dept. of Clinical Medicine & Surgery and confirmed in University Diagnostic Laboratory, University of Veterinary & Animal Sciences, Lahore (formerly Central Diagnostic Laboratory, College of Veterinary Sciences, Lahore).

After being certain that all the selected horses were naturally infected with babesiosis and no other disease or health problem was evident, the 80 horses were randomly divided into four groups named A, B, C, and D. Groups A, B and C were treated with three different antiprotozoan (Babesicidal) drugs i.e. pentamidine methunsulphate, imidocarb dipropionate, and diminazene diaceturate, while group D was kept as control and no medicine was given to horses of this group. The horses of group A were treated with pentamidine methunsulphate at a dose rate of 3mg/kg body weight intravenously. The horses of group B were treated with imidocarb dipropionate at a dose rate of 2.4 mg/kg bodyweight intramuscularly, while the horses of group C were treated with diminazene diaceturate at a dose rate of 3.5 mg/kg body weight intramuscularly.

Statistical Analysis

The data obtained in this experimental study was analyzed by percentage efficacy method [20].

RESULTS

The horses of group A were treated with pentamidine methunsulphate. Eighteen out of twenty horses recovered after first dose, while two horses recovered when another dose was given on the 3rd day. All the horses showed complete recovery, appeared normal, healthy and active. The colour of the mucous membrane of mouth, conjunctiva and urine returned to normal on the 5th day.

The horses of group B were treated with imidocarb dipropionate. Fourteen out of twenty horses recovered after first dose, while four recovered after the second dose which was given on 3rd day. Four out of twenty horses showed severe anaphylactic shock upon intramuscular administration of the drug. Out of these animals, two were saved by administering atropine sulphate and dexamethasone, while the other two horses could not survive. Two horses out of twenty also developed abscess at the site of the injection. This was treated surgically and the wound healed after about 14 days. Except the dead horses all the treated ones showed complete recovery at the end of treatment regime of two doses.

The horses of group C were treated with diminazene diaceturate. Eight out of twenty horses recovered after the first dose, four horses recovered after the second dose, four horses recovered after the third dose, while four horses did not show any improvement and died due to the chronicity of the disease. All alive horses recovered after full treatment regime of three doses.

The horses of group D were kept as control and were not given any treatment. Nine out of twenty horses developed chronic babesiosis and became carriers, while eleven expired due to chronicity of the disease. Overall percentage efficacy of all the three drugs is shown in Table-I:

Table-1. Overall percentage efficacies of drugs used.

Groups	Drug used	No. of horses treated	No. of horses cured after the dose:			No. of expired animals	Total %age efficacy of drug
			1st	2nd	3rd		
A	Pentamidine methunsulphate	20	18	2	-	0	100%
B	Imidocarb dipropionate	20	14	4	-	2	90%
C	Diminazene diaceturate	20	8	4	4	4	80%
D	Control	20	Untreated group			11	-

DISCUSSION

Equine babesiosis or equine piroplasmosis is caused by *Theileria equi* (*Babesia equi*) and *Babesia caballi*. It is considered to be the most important tick borne disease of horses in tropical and subtropical regions of the world [21]. Babesia is a protozoan parasite present within the red blood cells of the host. The disease can prove highly fatal if not treated properly in time [22,23,24] which has also been confirmed in the present study.

The present study was conducted in Lahore, Pakistan. In this experiment, horses exhibiting the relevant clinical symptoms were included. They were examined both physically and clinically. Confirmation of infection was done by examining their blood smears. The blood used for making smear was taken from the tip of ear, and stained by Giemsa's staining method, which is commonly used for detection of babesia parasite in the red blood cells. Similarly, various scientists have observed clinical symptoms and confirmed babesiosis by Giemsa's stained blood smears [25,26].

Horses of group A (20) were treated with Pentamidine methunsulphate, and gave excellent response to the drug. Eighteen horses out of 20 were cured after the first dose and two animals were cured after the second dose. Efficacy of the drug was found to be 100 %. Pentamidine has also been used by French workers in North Africa, for the treatment of *Babesia equi* [27]. Pentamidine isethionate and Diminazene aceturate has also been used for the treatment of Babesia gibsoni and Babesia canis [28]. These drugs were effective in halting and reversing the clinical progression of the disease. Pentamidine has also been used for the chemoimmunisation of *Babesia bigemina* infection in cattle and observed that a single dose of Pentamindine led to clinical recovery of splenectomised calves infected with *Babesia bigemina* [29]. Contrary to this study, found pentamidine combined with DFMO (DL alpha difluromethyl Qionithine) was found to be less effective using mouse model for CNS-trypanosomiasis [30]. The reason could be that the drug was used for the treatment of CNS-trypanosomiasis in mouse-model while in the present study work was conducted on horses naturally infected with babesiosis.

Horses of group B (20) were treated with Imidocarb dipropionate. The drug was found good in its effect against the disease and proved to possess 90% efficacy, but two animals expired due to adverse reaction of the drug. Similarly, this drug was also used in an experiment on donkeys and horses, and the drug was found to be highly toxic as all donkeys died and also found it ineffective in curing the disease in some horses [31]. Reason for the difference in results of two studies could be the dose rate i.e. 4mg/kg body weight which proved fatal in donkeys having lower plasma volume. The present study is also supported by a study in which five groups of equines wee treated with Imidocarb dipropionate at different dose rates (0.5, 1.0, 2.0, 4.0 and 8.0mg/kg), each animal received two injections at 24 hours intervals. Satisfactory results and minimal reaction of drugs at dosage up to and including 2 mg/kg rate were found [32]. The therapeutic efficacies of imidocarb was also tested and found ineffective in eliminating *B. equi* carrier infection in 9 mature geldings [33]. The reason of failure of imidocarb in this study could be the environment (European origin carrier horses) or splenectomy could also have affected the efficacy of drug, because tin that study splenectomized equines while in the present study naturally infected horses were included. Efficacy of the drug was reported to be excellent [34], which is in accordance with the present study. In contrast to the present study, it was reported that high dose treatment with imidocarb may not be capable of eliminating B. caballi and B. equi infections from healthy carriers [35].

Horses of group C (20) were treated with Diminazene diaceturate at a dose rate of 3.5 mg/kg body weight. Efficacy of the drug was found to be 80% in curing the horses. Four horses out of 20 died due to chronicity of the disease. Previously, the efficacy of drug was found to be 98% in an experiment [36]. It has also been reported that diminazene diaceturate is effective in chemo-sterilization of *B. caballi* and in elimination of clinical signs of *B. equi* infection [13]. Diminazene aceturate has been used at dose rate of 3.5 to 5.0mg/kg body weight and satisfactory results were found but supportive therapy was also given [37]. It has also been reported that the efficacy of Diminazene diaceturate was 80% in a trial on naturally infected horses [10].

From the present study it is concluded that the three treatment regimes discussed above can be adopted to treat horses infected with babesiosis. Pentamidine methunsulphate is very effective with 100% efficacy against equine babesiosis and can be recommended for routine use. Imidocarb dipropionate also proved to possess 90% efficacy, is cheaper than pentamidine methunsulphate and is available in the local market.

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