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Use of physiological biomarkers in diagnosis along with field trials of different trypanisidal drugs in camels of Cholistan desert

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ABSTRACT

The point prevalence of trypanosomiasis with different physiological biomarkers along with evaluation of the most responsive trypanosidal drug against trypanosomiasis under field conditions was studied. For this purpose a total of 300 free range camels were selected at different grazing and watering point in Cholistan desert. The study population of camels included 150 clinically suspected camels for trypanosomiasis and 150 healthy camels with normal temperature, pulse and respiration. For therapeutic trials 36 positively diagnosed animals were randomly divided into three experimental groups for therapeutic trials. Group A was treated with Imidocarb dipropionate (ID) @ 1.2 mg kg^{-1} body weight; Group B was treated with Diaminazine aceturate (DA) @ 3.5 mg kg^{-1} body weight and Group C was treated with Isometamidium chloride hypochloride (IC) @ 0.75 mg kg^{-1} body weight of camels. Data on risk factors of age,sex, ectoparasites, housing was also collected. Results revealed an overall 15% point prevalence of trypanosomiasis. There was significant ($P < 0.05$) decline in the values of physiological biomarkers of total erythrocyte counts, hemoglobin concentration, packed cell volume, serum total proteins and albumin while erythrocyte sedimentation rate was increased in infected camels as compared to healthy ones. Different hepatic enzymes including aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase and alkaline phosphatase were also significantly increased in the infected animals. Therapeutic trials indicated that Isometamidium chloride hypochloride (IC) was more effective than Imidocarb dipropionate (ID) and Diaminazine aceturate (DA). It is concluded that haemato-biochemical parameters were important physiological biomarkers and IC was the most responsive therapeutic agent against trypanosomiasis in camels in field conditions. The risk factors analysis showed older camels (>5 years) showed highest infection while infection was found to be lowest in less than 1 year age group.
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1. Introduction

Camels are highly valued by the desert dwellers. Camels are not only2] useful for transportation and loading purposes, but its skin and wool are also quite worthwhile. Camel wool is spun and woven into beautiful woolen blankets known as falsies and into stylish and durable rugs. The camel's leather is also utilized in making kuppies, goblets, and expensive lampshades. Camel milk and meat are important food items [1]. Cholistan development authority data shows that the desert area is inhabited by 0.08 million camels. Infectious diseases and particularly vector-borne protozoan diseases are serious threat for camel health and productivity [2] Among vector-borne protozoan diseases, Trypanosomiasis or Surra is principally important, caused by *Trypanosoma evansi* (*T. evansi*) and transmitted principally by Tabanus flies in camels [3]. .The pathological consequences of this disease include anemia, emaciation, lymphadenopathy, edema and sudden death [4]. After an outbreak of trypanosomiasis during 1985–1986 in camels of Baluchistan, various studies have been directed on camel trypanosomiasis in numerous areas of Pakistan [5]. Under

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microscope *T. evansi* are seen as slender parasites with an undulating membrane [6]. The physiological biomarkers of hemato pathological effects in its hosts include significant variations in the values of blood parameters along with the alterations in serum chemistry [7].

So far the disease is being treated by the use of trypanocidal drugs. Vaccine against this disease is not available so far. *T. evansi* can be treated by introducing several trypanocidal drugs, depending upon the lethal concentration of the drug in the serum for parasite. Failure in responsive therapy for trypanosomiasis could be due to development of drug resistance in parasite along with failure to control the vectors. The most extensively administered trypanocidal in surra is diminazene aceturate. The aim of the study was to record point prevalence of trypanosomiasis along with hematological and biochemical biomarkers for *T. evansi* and to evaluate responsive therapy under field condition in camels of Cholistan desert.

2. Materials and methods

2.1. Study area

The Cholistan Desert locally known as Rohi covers an area of 26,300 km2 (10,200 sq mi) and extends from Bahawalpur, Punjab province to Sindh province and into India. In Cholistan desert, mean annual temperatures ranges from 20 °C to 40 °C. Geo-location includes 27° 42′ to 29°45′N latitudes and 69° 52′ to 75° 24′E longitudes. The annual rainfall in Cholistan, ranges from under 100 mm in western areas to 200 mm in eastern areas [8].

2.2. Study animals

The study included the camel population of dromedary breed of all ages in Cholistan that are kept open with no specified housing system. In the all three districts of Cholistan, specific sites near “tobas” were preferentially selected where the camels share common drinking water and browsing from wild shrubs and trees. A total of n = 300 camels from the different grazing and watering point were selected including n = 150 clinically suspected camels for trypanosomiasis and n = 150 healthy animals to compare normal blood and serum biochemical parameters. The age of individual animals was documented on the basis of information provided from the owners. The animals were distributed in four age groups. (Table 1). The data regarding animals' clinical history and treatment protocols, vaccination, deworming and vaccination were also entered in data capture form.

2.3. Inclusion and exclusion criteria of animals

Camels showing signs of fever, anorexia, dullness, depression, pale mucous membranes, anemia, weight loss, facial paralysis, thin hump and dropped to one side and females with history of abortions were suspected for being infected with trypanosomiasis [9] while all other animals except these signs and with normal temperature, pulse and respiration were considered in exclusion criteria i.e., healthy.

2.4. Collection of blood samples and smears examination

Approximately 5 ml blood sample was collected aseptically by Jugular vein puncture from each camel and transferred into anticoagulants vacationers containing EDTA as an anticoagulant for hematological examination. Preparation of blood smear slides was done from the drops of blood collected from ear tip puncture for parasitological examination. A drop (3–5 μl) of blood was positioned at a margin of a microscope slide and a thin smear was prepared. Slides were air-dried, fixed in methanol for one minute and air dried. The smears were stained with Giemsa (Giemsa stain 1drop + PBS 1 ml, pH 7.2) for duration of 25 min, subsequently washed and air dried. The thin smears were observed at magnifications of 400× to1000× [10]. Trypanosomes were identified by their morphological characteristics [11].

2.5. Hematological examination and serum biochemistry

Blood sample for hematological studies were processed at Clinical Medicine Laboratory, UVAS Lahore. Hemoglobin (Hb) estimation; Erythrocytes Sedimentation Rate (ESR) Total Erythrocytes Count (TEC), Total Leukocytes count (TLC) were done by hematology analyzer. Differential Leukocytes Count (DLC) was obtained by stained-blood smear with Wright-Giemsa stain and Hematocrit (Hct) estimation by capillary tube centrifugation for 5 min at 14,000 rpm [10] Serum biochemical tests including total serum proteins, serum albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) were conducted as per documented methods [13].

2.6. Therapeutic trials

A total of thirty six (n = 36) camels positive for trypanosomiasis with no history of prior treatment/prophylaxis were divided randomly into three treatment groups. Group A was treated with Imizole® (Imidocarb dipropionate or ID) at a dose rate of 1.2 mg kg^{-1} of body weight of animal; Group B was treated with Diaminazine aceturate (DA) at a dose rate of 3.5 mg kg^{-1} of body

Table 1
Prevalence of trypanosomiasis in camels in Cholistan desert.

Sex/age	No. of camels	Positive		Negative	95% CI	Odd Ratio/P value
		n	%			
Sex						
Female	140	20	14.29	120	9.20–20.83	OR = 0.90 [reciprocal = 1.11]
Male	160	25	15.63	135	10.61–21.88	
Overall	300	45	15.00	525	11.29–19.38	–
Age groups						
Less than 1 year	64	7	10.94	57	4.91–20.44	Mantel-Haenszel chi-sq P < 0.053
2 years	68	8	11.76	60	5.62–21.12	
3–5 years	81	11	13.58	70	7.35–22.37	
Above 5 years	87	19	21.84	68	14.10–31.43	
Overall	300	45	15.00	255	11.29–19.38	–

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