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EVALUATION OF DIFFERENT DIAGNOSTIC TESTS FOR *TRYPANOSOMA EVANSI* INFECTION AMONG HORSES AND CAMELS IN THE PUNJAB REGION, PAKISTAN

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ABSTRACT

Parasitological, serological and biochemical tests were used to determine *Trypanosoma evansi* infection in 170 horses and 150 camels sampled from Punjab region, Pakistan. The micro-Haematocrit Centrifugation Technique was used as gold standard method. Wet blood films, thin stained smears and thick stained smears showed a sensitivity of 0.8 and a specificity of 1. Positive and negative predictive values were 1 and 0.99, respectively. Serology using Suratex® showed a sensitivity of 1 and a specificity of 0.99. Positive and negative predictive values were 0.83 and 1, respectively. Biochemical tests showed a very low positive predictive value (around 0.38). None of the equines resulted positive at any method. In contrast, 5 (3.3%) and 6 (4%) camels were positive at parasitological and serological examination, respectively. These results seem to indicate that *T. evansi* infection has a relatively low prevalence in the Punjab region.

Keywords: Camels, horses, Pakistan, prevalence, surra, *Trypanosoma evansi*

Animal trypanosomosis caused by *Trypanosoma evansi*, commonly known as "surra", is a frequent health problem of animal species in a wide range of climate and vegetation zones in Asia, the Middle East, the Far East, Central and South America and usually outside the tsetse belt in Africa (Wernery and Kaaden, 2002). Trypanosomosis causes huge economic losses due to production losses and a high mortality rate, specially in dromedary camels (Rutter, 1967; Wilson, 1984) and horses. The diagnosis of *Trypanosoma evansi* infection is based on clinical signs and on the demonstration of the parasites by direct or indirect methods. The clinical signs of surra are indicative but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods (OIE, 2004). However, sensitivity/specificity of the diagnostic methods used to detect *T. evansi* infection can also vary due, among other reasons, to strains present.

The purpose of this study was to assess different serological and parasitological methods in horses and camels sampled from different areas of Punjab, Pakistan.

Materials and Methods

A total of 320 animals (170 equines and 150 dromedary camels) of both genders and different ages were randomly selected for diagnosis of *T. evansi* infection. The sample size for this cross-sectional study was calculated in base to the following formulae for an estimation of a proportion:

$$N = \frac{k^2\, p\, (1-p)}{e^2}$$

where k represents the confidence level (0.95), p is the estimated prevalence (0.08) and e represents the maximum error (0.03).

The animals belonged to several herds located at various areas of the Punjab region and they had not previously been introduced from other regions. Selected animals underwent a physical examination, and the minimum number of sampled animals per herd was 10.

Blood samples were collected from jugular vein using tubes containing EDTA (2 mg/mL) and without anticoagulant for serum extraction. Blood samples were maintained at 4°C until their arrival

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at laboratory. Samples containing EDTA were ultra-centrifuged (10000 rpm) to obtain packed volume cells (PCV). All samples without anticoagulants were centrifuged (3000 rpm) for serum collection, were distributed in aliquots and frozen at -80ºC until processing.

In all cases, trypanosomes were searched using micro-Haematocrit Centrifugation Technique (m-HCT) at low magnification (x10),[10] (considered as gold standard method) as well as examination of wet blood films, thin stained smears and thick stained smears (Giemsa), following the protocol described by OIE (2004).

Suratex® detects trypanosome-circulating antigens. The Suratex® reagent is a suspension of latex particles that have been sensitised with a monoclonal antibody against a *T. evansi* internal antigen (Nantulya, 1994) on a plastic card. The test was performed as described by the manufacturers (AccuPharma, Inc, USA).

Biochemical tests included formol-gel test, mercuric chloride precipitation, and thymol turbidity test, and were performed following procedures described by the OIE (2004).

Results

Results obtained by the different diagnostic methods are summarised in Table 1. The presence of *Trypanosoma evansi* was only demonstrated in dromedary camels (5/150 animals, 3.3%). Serum antigens detection was also evident in dromedaries, with a prevalence of 4%. No horses were found to be positive at any diagnostic method.

All seropositive camels were found to be also parasitemic except one, in which *T. evansi* was not discovered. On the other hand, all parasitemic and seropositive camels were also positive to biochemical tests. In contrast, 7 positive cases at formol gel test, 10 at mercuric chloride test and 6 at thymol turbidity test resulted negative to *T. evansi* diagnostic specific methods.

At physical examination, all 6 seropositive camels showed one or various clinical signs related to *T. evansi* infection. The clinical signs most commonly found were atrophy of the hump and petechial hemorrhages in mucosae.

Discussion

From the diagnostic point of view, only visualisation of the trypanosomes by microscopy could confirm the patency of infection. Parasitological tests revealed the presence of parasites in 5 camels. Sensitivity and specificity of parasitological tests were 0.8 and 1, respectively. Predictive values resulted very high (1 and 0.99 for positive and negative, respectively).

Serological tests detected 6 positive camels, 5 of which were parasitemic too. Its sensitivity resulted higher that the parasitological tests (1) but specificity resulted lower (0.99). Negative predictive value resulted 1 but positive predictive value was 0.83. Olaho-Mukani *et al* (1996) found that Suratex and Antigen-ELISA could diagnose the camels with sub-patent infections. In a later study (Olaho-Mukani *et al*, 1997), a comparison of card latex agglutination test (Suratex) with the haematocrit centrifugation technique (HCT) and mouse inoculation (MI) found that Suratex was much more sensitive than HCT.

Biochemical tests detected 16 camels using mercuric chloride precipitation, 13 by formol-gel and

Table 1. Results of the different diagnostic methods to detect *T. evansi* infection in the examined camels (n = 150) in the Punjab (Pakistan).

Test	+	-	%	Sens	Spec	PV +	PV -
Parasitological tests							
HCT*	5	145	3.3				
Wet blood film	4	146	2.7	0.8	1	1	0.99
Thin stained smears	4	146	2.7	0.8	1	1	0.99
Thick stained smears	4	146	2.7	0.8	1	1	0.99
Serological test							
SURATEX (Latex Agglut.)	6	144	4	1	0.99	0.83	1
Biochemical tets							
Formol gel test	13	137	8.7	1	0.94	0.38	1
Mercuric chloride precipitation	16	134	10.7	1	0.92	0.31	1
Thymol turbidity test	12	138	8	1	0.95	0.42	1

* Considered as the gold standard method. Sens: Sensitivity; Spec: Specificity; PV+: Predictive value +; PV-: Predictive value

12 by thymol turbidity test. All parasitologically and serologically positive camels were also positive at biochemical tests. The sensitivities were very high (1), which means that the tests are able to detect all diseased animals, but their specificities falls at 0.94, 0.92 and 0.95. Thus, positive cases at biochemical tests that resulted negative at parasitological tests should be considered as false positive. The most important disadvantage observed using biochemical tests was the positive predictive value, which resulted very low.

Biochemical tests detect increase in serum globulins as the result of infection, but this increase can not be specific for *T. evansi* infection. There are no data on the levels of globulins that are likely to produce a positive reaction (OIE, 2004). Biochemical tests are commonly used in field conditions because they are relatively quick, simple and cost-effective methods. However, in view of poor coorelation, more specific diagnostic methods should be preferred.

The prevalence of surra in camels observed in the Punjab region ranged between 3.3% and 4% and can be considered as low compared with other regions considered as endemics (56% in Somalia -Baumann and Zessin, 1992; 48% in Kenya-Olaho-Mukani and Wilson, 1983; or 16.2-25.2% in Mauritania- Dia *et al*, 1997). Previous surveys employing different serological tests carried out on several areas of Pakistan reported surra prevalence in Faisalabad of 5.18% and 9.09% in horses and donkeys respectively (Khan *et al*, 1987) and 3% in camels (Butt *et al*, 1996), 7.41% in horses in North West Frontier Province (Bano and Jan, 1986), and 3.35% in camels in Gujranwala (Waheed *et al*, 2003). The results seem to indicate that parasitological and serological tests are adequate for *T. evansi* diagnosis in the region and that the disease in the Punjab zone has a relatively low prevalence compared with the more endemic regions. The lower prevalence recorded herein compared to earlier studies may be due to epidemiological factors and possible effects of control measures.

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