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OBSERVATIONS ON THE VALIDITY OF MOUSE INOCULATION TEST IN THE SURVEILLANCE OF *Trypanosoma evansi* IN CAMEL

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ABSTRACT

Parasitological diagnostic methods for surra, one of the most important disease of camels, are slow and may miss up to 50-80% of the true infections during the chronic phase of the disease. Inoculation of laboratory rodents with blood from suspected camels is a very sensitive method for detecting low parasitaemia. The present study was conducted to assess the suitability of the test for surveillance of *Trypanosoma evansi* infection in field cases. The observations made during the study are reported. The incubation period and course of infection in the rodents is also discussed.

Keywords : Camel, mouse inoculation test, surra, validity

It is difficult to diagnose surra in camel because clinical signs are varied and non-specific. The inoculation of blood from suspected camels into rodents (rats and mice) has proved valuable in revealing incidence of subpatent infections of *T. evansi* (Pegram and Scott, 1976; Olaho-Mukani *et al*, 1993; Pathak *et al*, 1997). However, the need to maintain rodents makes this an impractical field tool. This study was conducted to determine the validity of mouse inoculation for diagnosing *T. evansi* in blood samples from clinically suspected camels.

Materials and Methods

The study was carried out in the villages of Bikaner district known to be endemic with probabilities of low level of parasitaemia in the camels, not detectable by wet blood film examinations. At the outset, 25 camels from Amarpura and Nada villages were studied between July 2002 and May 2003. The camels were examined clinically by the symptoms and blood samples were collected from those camels showing emaciation, oedema and diminution of hump. A total of 0.2 ml blood from each camel was placed on clean slides and examined microscopically (x 400) as wet blood films. Thin blood smears were also made and stained by Giemsa's stain as described by Godfrey and Killick-Kendrick (1961). A volume of 1.0 ml of blood was inoculated intra-peritoneally into healthy albino mice in duplicate immediately after collection of blood as per the method described by Godfrey and Killick-Kendrick (1962). Parasitaemia in the blood of mice was monitored daily up to 60 days post-inoculation by wet smear examination of tail-tip blood preparation starting from the second day of inoculation for the presence of *T. evansi*.

Results

The observations of wet blood film (WBF) examination, thin blood smear (TBS) examination and mice inoculation of 25 camels revealed positivity in 4 (16%), 4 (16%) and 10 (40%) animals, respectively.

The results showed that over half (60 per cent) of the detected infections were subpatent and were revealed only by the mouse inoculation. Course of infection in mice is presented in fig 1. As the test was performed in duplicate for each camel, 19 mice out of 10 pairs (one mouse died on day 4 before appearance of the parasite) showed

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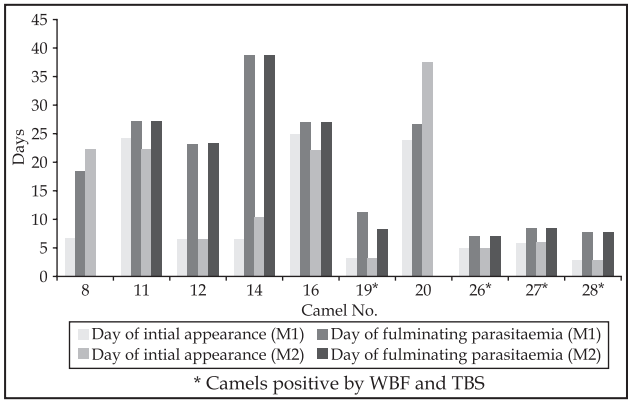

Fig 1. Course of *Trypanosoma evansi* infection in mice inoculated by camel blood.

the organisms in their blood. Out of 19 infected mice, 11 (57.8%) showed the trypanosomes in first 7 days with an average of 4.36 days and remaining 8 mice on days 10, 22, 24, 22, 25, 22, 24 and 38 post-inoculation. In 6 out of 19 mice, the first fulminating parasitaemia appeared between 7-11 days. Six of these mice were inoculated with blood from camels positive by WBF and TBS. In 10 mice the first fulminating parasitaemia appeared between 17 - 40 days. All these mice were inoculated with the blood of those camels in which trypanosomes could not be detected by both WBF and TBS.

It was observed that one mouse inoculated with blood from a WBF as well as TBS positive camel (Camel no. 27) became positive for trypanosomes on 6th day. Further, the mouse became aparasitaemic between 11-18 days but trypanosomes reappeared between 19-23 days. Thereafter, trypanosomes could not be seen up to 60 days or the end of observation period. The parasitaemia was always of very low level. The second mouse died on day 4 after inoculation without showing any trypanosomes.

One mouse each, among the inoculated mice from camel nos. 8 and 20 (both WBF and TBS negative) was killed on day 18 and 27, respectively, on appearance of fulminating parasitaemia. Trypanosomes in the second mouse inoculated with blood from these animals could only be seen once on day 22 and 38, respectively, in the entire period of study.

Discussion

The present study has revalidated the value of mouse inoculation as a diagnostic procedure for cameline trypanosomiasis since it resulted in 150% increase in the animals found positive. Further, all the mice inoculated with the blood from patent cases showed parasitaemia. This observation is in accord with the previous studies (Killick-Kendrick, 1968; Pegram and Scott, 1976; Raisinghani and Lodha, 1989; Pathak *et al*, 1997). Whereas Olaho-Mukani *et al* (1993) observed that blood from one camel positive by buffy coat examination (BCE) did not cause infection in the mice.

Our study found that blood from camels with a high level of parasitaemia caused the most acute infections in rodents, which agrees with the findings of Godfrey and Killick-Kendrick (1962). However, in the present study, trypanosomes in the second mouse of two camels (WBF and TBS negative) turned positive on 22 and 38 days, respectively. The trypanosomes could never be seen thereafter, in these two mice during the entire period of study, i.e., 60 days. Furthermore, in another mouse inoculated with blood from WBF and TBS positive camel, trypanosomes initially appeared on 6th day, could not be seen between 11-18 days, reappeared from 19-23 days and thereafter, could not be seen during the entire period of study. The infectivity of *T. evansi* for rat and mice may vary, and it has been postulated that loss of infectivity and virulence of an isolate for mice and rats may be associated with chemotherapy of the natural host.

Holland *et al* (2001) observed in their study that one mouse became positive only after 29 days of its inoculation with blood from experimentally infected animals. They also found that extended time between experimental infection and blood sample inoculation in the mice resulted in increase of sensitivity as well as shorter pre-patent period with consistent detection after 5 weeks post infection (PI) with a sensitivity of 74% which reached 100% after week 6 PI. However, in the present study no comments can be made regarding the duration of infection in the camels examined as samples from field animals. The findings indicate that difference in duration of infection in camels might have resulted in the broad range of the first appearance (6 to 38 days) of detectable parasitaemia in the mice in addition to other factors such as the level of parasitaemia in the host. The other possible explanation might be the immunological responses in the hosts, which may affect the adaptability of the parasite in the new

biological system (mice/rats), leading to the earlier establishment of the infections of longer duration.

The proportion of trypanosome infection detected by mouse inoculation test (40%) was better or identical to that found by Godfrey and Killick-Kendrick (1962), i.e., 27.6% and Pegram and Scott (1976), i.e., 36.5%. In case of 6 camels the trypanosomes though invisible in WBF and thin blood smears, induced the disease in mouse when inoculated. This may be due to the undetected developing stages, which probably assumed full trypanosomal forms in the mice.

The incubation periods and course of infection in mice in the present study were comparable to the findings of Pegram and Scott (1976) who found that acute form of the disease seems to occur only with the strains adapted to laboratory rodents. In present study 57.8% of the mice became positive within one week, which is in contrast with the earlier observations of Monzon *et al* (1990), Verloo *et al* (2000) and Holland *et al* (2001) who had reported 98, 100 and 85%, respectively.

The findings of present study support the view of Reid *et al* (2001) who had suggested inoculation of at least two mice for each test sample to be examined, to achieve a high level of sensitivity in endemic regions as there is probability that one mouse may not become infected. In the present study though the infections established in all duplicate groups but the course of disease in the mice of different groups was quite variable necessitating the need of inoculation of at least two mice for each test sample.

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References

Godfrey DG and Killick-Kendrick R (1961). Bovine trypanosomiasis in Nigeria. 1: The inoculation of blood into rats as a method of survey in the Donga Valley, Benue Province. Annals of Tropical Medicine and Parasitology 55:287.

Godfrey DG and Killick-Kendrick R (1962). *Trypanosoma evansi* of camels in Nigeria: A high incidence demonstrated by the inoculation of blood into rats. Annals of Tropical Medicine and Parasitology 56:14-19.

Holland WG, Claes F, My LN, Thanh NG, Tam PT, Verloo D, Büscher P, Goddeeris B and Vercruysse J (2001). A comparative evaluation of parasitological tests and a PCR for *Trypanosoma evansi* diagnosis in experi-mentally infected water buffaloes. Veterinary Parasitology 97:23-33.

Killick-Kendrick R (1968). The diagnosis of trypanosomosis of livestock: a review of current techniques. Veterinary Bulletin 38:191-197.

Monzon CM, Mancebo OA and Roux JP (1990). Comparison between six parasitological methods for diagnosis of *Trypanosoma evansi* in the subtropical area of Argentina. Veterinary Parasitology 36:141-146.

Olaho-Mukani W, Munyua WK, Mutugi MW and Njogu AR (1993). Comparison of antibody and antigen-detection enzyme immunoassays for the diagnosis of *Trypanosoma evansi* infection in camels. Veterinary Parasitology 45: 231-240.

Pathak KML, Singh Y, Meirvenne NV and Kapoor M (1997). Evaluation of various diagnostic techniques for *Trypanosoma evansi* infection in naturally infected camels. Veterinary Parasitology 69:49-54.

Pegram RG and Scott JM (1976). The prevalence and diagnosis of *Trypanosoma evansi* infection in camels in Southern Ethiopia. Tropical Animal Health and Production 8: 20-27.

Raisinghani PM and Lodha KR (1989). Incidence of *Trypanosoma evansi* infection in camels of Rajasthan. Indian Journal of Animal Science 59:1390-1392.

Reid SA, Husein A and Copeman D B (2001). Evaluation and improvement of parasitological tests for *Trypanosoma evansi* infection. Veterinary Parasitology 102:291-297.

Verloo D, Holland WD, My LN, Thanh NG, Tam PT, Goddeeris B, Vercruysse J and Büscher P (2000). Comparison of serological tests for *Trypanosoma evansi* natural infections in water buffaloes from north Vietnam. Veterinary Parasitology 92:87-96.