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RESEARCH NOTE

EXPERIMENTAL INTRASPINAL *TRYPANOSOMA EQUIPERDUM* INFECTION IN A HORSE

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ABSTRACT

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To establish the ability of *Trypanosoma equiperdum* to cross the blood-brain-barrier in the horse, a susceptible stallion was infected via the cerebrospinal fluid of the subarachnoid space by lumbosacral puncture. Cerebrospinal fluid with low detectable levels of trypanosomes removed from a dourine-infected mare by lumbosacral puncture was used for infecting the animal.

The parasite was detected in blood smears of the recipient 13 days after infection and the subsequent parasitaemia and clinical course of the disease followed that of naturally infected horses.

Résumé

L'INFECTION EXPÉRIMENTALE INTRA-RACHIDIENNE DU CHEVAL AVEC TRYPANOSOMA ÉQUIPERDUM

Dans le but de déterminer l'aptitude de Trypanosoma équiperdum *à traverser la barrière céphalo-sanguine chez le cheval, l' auteur a infecté un étalon réceptif à la dourine à travers le liquide céphalo-ranchidien de l'espace sous-arachnoïdien par ponction sacro-lombale. Du liquide céphalo-rachidien à un taux de trypanosomes bas prélevé par ponction sacro-lombale d'une jument atteinte de dourine, a servi comme inoculum.*

Treize jours après la ponction de l'étalon, on a pu déceler le parasite sur frottis préparés du sang. La parasitémie et le développement clinique de la maladie ont été tout proche de ceux qui paraissent chez le cheval infecté dans la nature.

INTRODUCTION

The results of previous studies (Barrowman, 1976) on natural *Trypanosoma equiperdum* infections in horses suggested that the development of neurological lesions is associated with the presence of the parasite in the cerebrospinal fluid (CSF) of the horse. It is not known whether the parasite can only penetrate the blood-brain-barrier in some cases, whether there is any significant barrier to its movement into CSF, or whether its presence in CSF results from developmental stages in the choroid plexus (Ormerod & Venkatesan, 1971).

Experimental intraspinal infections with African trypanosomes were recorded in the baboon (*Papio jubilaeus*) by Lavier, 1928, (cited by Bourguignon, Van den Berghe & Van Bogaert, 1936), by Regendanz, 1932, and by Bourguignon, Van den Berghe & Van Bogaert, 1936. The baboon was refractory to *Trypanosoma brucei* infections when injected intraspinally and via the blood stream, but in a number of cases *Trypanosoma gambiense* infections were established by intraspinal injection and resulted in lesions of the central nervous system (CNS). Regendanz (1932) found that, whereas baboons could not be infected with *T. gambiense* via the blood stream, infection was possible by injection into the CSF. Moreover, the parasite was confined to the CSF and not found in the blood, and clinical symptoms were restricted to the CNS. In these cases the animals died 1–2 years later.

EXPERIMENTAL DESIGN

The following experiment was performed to determine the ability of *T. equiperdum* to cross the blood-brain-barrier after its introduction into the subarachnoid space of a non-infected horse.

Experimental animals

The recipient was a 4-year-old stallion negative for dourine according to complement fixation tests.

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The *T. equiperdum* donor was a mare which had previously been infected by an intravenous injection of 250 ml of blood from a naturally infected stallion. By Day 175 after infection, the mare showed progressive emaciation and clinical signs of posterior paresis. CSF withdrawn by lumbosacral puncture (see below) on that day was found to contain low numbers of trypanosomes demonstrable in fixed thick smears stained with 10% Giemsa. Blood samples withdrawn from the jugular vein on the same day were negative when examined for trypanosomes by the centrifugation and sedimentation methods described previously by Barrowman (1976).

Inoculation by lumbosacral puncture

The technique employed for lumbosacral puncture in the horse was that described by Tufvesson (1963) and modified by Barrowman (1976).

Both donor and recipient horses were restrained in adjacent crush pens and needles inserted into the subarachnoid space of each by simultaneous lumbosacral puncture.

The first 5 ml of the CSF withdrawn from the donor mare was discarded, 9,5 ml was used for transfer, and a further 65 ml, after which no further fluid could be aspirated, was withdrawn for other purposes. Following the withdrawal of 10 ml of CSF from the recipient, the 9,5 ml sample obtained above was injected into its subarachnoid space. The syringe and needle were left *in situ* in the recipient for 4–5 min to allow circulation of the introduced sample in the leptomeningeal space away from the injection site.

RESULTS AND CONCLUSIONS

An examination of wet smears and Giemsa-stained thick smears of CSF withdrawn from the recipient on Day 4 after intraspinal inoculation proved negative for trypanosomes. Sedimented blood examination (Barrowman, 1976) from this animal was positive for the first time on Day 13 after transfer with an estimated 60 trypanosomes per ml of serum. CSF

obtained by lumbosacral puncture on the same day was negative for trypanosomes on examination of wet smears and stained thick smears. The stallion developed typical clinical signs of dourine, viz. scrotal oedema, paraphimosis and emaciation. Examinations of sedimented blood remained positive until Day 97, and thereafter became irregular with very low numbers of parasites. An examination of CSF on Day 77 was positive for trypanosomes but the sample was visibly contaminated with blood. The frequency with which lumbosacral puncture can be performed is limited, as damage to the interarcual ligament occurs each time and leads to contamination of the CSF. This difficulty could be overcome, however, by the insertion of a permanent, biologically inactive catheter under sterile conditions.

The transfer of infected CSF into the leptomeningeal space of a susceptible stallion was followed therefore by the invasion of the blood stream by the parasite. The subsequent course of the parasitaemia and the clinical manifestations were similar to those previously described for naturally infected horses (Barrowman, 1976). The delay in finding trypanosomes in blood until Day 13 is probably a reflection of the difficulty of finding low numbers of the parasite in a large volume of blood. The rapid appearance of the parasite in the blood stream and its absence from CSF suggest that the parasite has no difficulty in crossing the blood-brain-barrier, at least in the fully susceptible horse. Thus the intraspinal infection of susceptible horses would not provide a suitable experimental model for the reproduction of the neurological lesions of dourine or for testing the efficacy of trypanocidal drugs across the blood-brain-barrier. Further studies on the intraspinal introduction of *T. equiperdum* into infected horses whose blood is immunologically refractory may prove of value (*cf.* Regendanz, 1932).

The withdrawal of a large volume of CSF from the donor mare had no apparently adverse effect as she remained standing and trotted back to her pen following the operation. There was subsequently a marked improvement in her condition with resolution of the clinical signs of nerve involvement and an increase in body mass. The improvement in her clinical condition is worthy of note. Removal of the large volume of CSF may have resulted in the elimination of the low number of trypanosomes present. Re-invasion from the blood stream was unlikely as her blood, when examined by sedimentation and centrifugation, was negative for trypanosomes at the time CSF was removed.

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