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HEARTWATER. THE ARTIFICIAL TRANSMISSION OF *COWDRIA RUMINANTIUM* IN DOMESTIC RUMINANTS AND MICE

L. PROZESKY, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

PROZESKY, L., 1987. Heartwater. The artificial transmission of *Cowdria ruminantium* in domestic ruminants and mice. *Onderstepoort Journal of Veterinary Research*, 54, 277–279 (1987).

The artificial transmission of *Cowdria ruminantium* with infected blood, organ homogenates, peritoneal macrophages, tick stabilate and tissue culture cells is discussed.

Organ homogenates prepared from the myocardium, spleen, kidneys and liver of diseased animals are commonly used to infect mice. The efficacy of organ homogenates as a source of *C. ruminantium* depends on factors such as the route of incolulation and the heartwater isolate used.

Heartwater is artificially transmitted with infected tick stabilate, haemocytes, rectal ampules and hypodermal homogenates. The infectivity of saliva collected from *Amblyomma hebraeum* female ticks was very low compared to the ground-up suspensions prepared from the same group of ticks.

INTRODUCTION

Cowdria ruminantium is artificially transmitted to susceptible animals by the intravenous (i.v.) administration of infected blood, organ homogenates, peritoneal macrophages, tick stabilate and cell culture suspensions (Alexander, 1931; Ilemobade & Blotkamp, 1978; Bezuidenhout, 1981; Du Plessis, 1982; Bezuidenhout, Paterson & Barnard, 1985). A very low percentage of animals contract the disease when inoculated subcutaneously (s.c.), intraperitoneally (i.p.) and intramuscularly (i.m.). The efficacy of the s.c., i.m. and i.p. routes can be increased by applying certain additives to the infective material (Bezuidenhout, Olivier, Gruss & Badenhorst, 1987).

Infectivity tests with blood revealed that *C. ruminantium* was located in the white cell fraction (Ilemobade, 1976), the red cell fraction (Alexander, 1931) and plasma (Ramisse, 1971 cited by Uilenberg, 1983). *C. ruminantium* antigen was detected in the plasma and serum on the 4th day after inoculation (Neitz, Viljoen, Bezuidenhout, Oberem, Visser & Vermeulen, 1986).

The tick *Amblyomma hebraeum* is considered to be the most important vector of heartwater in South Africa. Theiler & Du Toit (1928) found that *C. ruminantium* could be transmitted to susceptible ruminants by the i.v. administration of ground-up infected *A. hebraeum* nymphae. Subsequently an attempt was made to develop a heartwater vaccine with *A. hebraeum* nymphae as the infective source (Bezuidenhout, 1981).

Factors that might influence the infectivity of the inoculum include the time of collection of the material from diseased animals, the method of preservation of the inoculum, the routes of inoculation and the type of infective material (e.g. blood or tick stabilate) (Logan, 1987; Oberem & Bezuidenhout, 1987; Bezuidenhout *et al.*, 1987). In this report additional information is supplied on the artificial transmission of heartwater.

INFECTIVE MATERIAL THAT CAN BE USED FOR THE ARTIFICIAL TRANSMISSION OF *C. RUMINANTIUM*

Infected animals

Blood: Infective blood is frequently used to transmit *C. ruminantium* artificially. The disease can be disseminated i.v. with as little as 0,1 mℓ of blood, but larger quantities (*c.* 5 mℓ for small ruminants and 10 mℓ for bovines) is needed for consistent results (Alexander, 1931).

Conflicting opinions have been reported by various workers regarding the infectivity and localization of *C. ruminantium* in different blood fractions (Neitz *et al.*, 1986). By light and electron microscopy, colonies of heartwater organisms were seen in leucocytes and free in the blood of diseased animals (Jackson & Neitz, 1932; Logan, Quintero, Whyard & Mebus, 1985; Pienaar, 1970). *C. ruminantium* antigen was detected by means of an enzyme-linked immunosorbent assay (ELISA) method in different blood fractions of an artificially infected sheep. *Cowdria* antigen was perceived in the serum and plasma on postinoculation day 4, in the red blood cell fraction on day 6, and in the leucocyte fraction on day 8. The highest antigen concentration was associated with the red blood cell fraction 2 days after the animal developed a temperature and was undetectable 7 days after the sheep was specifically treated for heartwater (Neitz *et al.*, 1986).

C. ruminantium was transmitted to susceptible domestic ruminants by means of the i.v. injection of the following blood fractions: leucocytes (Alexander, 1931; Ilemobade, 1976; Ilemobade & Blotkamp, 1978; Logan *et al.*, 1985), red blood cells (Fawi, Karrar, Obeid & Campbell, 1969; Alexander, 1931), and plasma (Ilemobade & Blotkamp, 1978). Contrary to these findings, Ilemobade & Blotkamp (1978) failed to infect animals by the i.v. route with red blood cells, and Alexander (1931) unsuccessfully attempted to transmit the disease with serum or plasma. These conflicting results could possibly be attributed to the different methods used by the workers to separate the blood fractions. The s.c. administration of infective blood to sheep and cattle was reported to be much less reliable when compared with the i.v. route. Alexander (1931) claimed that not more than 25 % of the cattle, sheep and goats infected s.c. developed heartwater and only exceptionally did animals contract the disease when infected by the i.p. route. On the other hand, 4 out of 4 sheep became ill when inoculated s.c. with 10 mℓ of infective blood containing 5 % dimethyl sulphoxide (Bezuidenhout *et al.*, 1987).

Organ homogenates: Ilemobade & Blotkamp (1978) infected goats i.v. and s.c. with 2 mℓ of a brain homogenate prepared by suspending approximately one gram of cerebral cortex from a goat that had died of heartwater in 10 mℓ phosphate buffered saline (pH 7,3) at 4 °C, and filtering the homogenate through a muslin cloth. Surprisingly, only animals inoculated s.c. contracted the disease. Contrary to these results Uilenberg (1971) claimed that he transmitted heartwater to a goat by the i.v. administration of infected brain material.

Homogenates prepared from organs such as the liver, kidneys, spleen and myocardium of heartwater-infected mice is an effective method of transmitting *C. ruminantium* to mice (Du Plessis, 1982). In addition, suspensions of infected peritoneal macrophages are used mainly to disseminate the Kümm isolate of *C. ruminantium* in mice. Peritoneal macrophages are harvested from

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diseased mice by removing the abdominal skin and injecting 1,5 mℓ of Hanks tissue culture medium with (200 IU/mℓ) heparin i.p. After gentle massaging of the abdominal wall the fluid is withdrawn. Homogenates of individual organs are prepared in buffered lactose peptone on a 10 % mass per volume basis. Recipient animals are infected i.v. or i.p. with *c.* 0,2 mℓ of the prepared inocula (Du Plessis, 1982).

The efficacy of organ homogenates as a source of infective material depends on the heartwater isolate, immune status of the donor animal, concentration of parasites in, and the time of collection of the specific organ after infection, and the route of inoculation (Du Plessis, 1982). The highest concentration of organisms was present in the myocardium followed by the lung, spleen, liver peritoneal macrophages and brain 14 days after mice were inoculated i.p. with tissue homogenates infected with the Kümm isolate. Macrophage suspensions were infective on postinoculation Day one and liver homogenates 5 days after inoculation (Du Plessis, 1982).

The Kümm isolate of *C. ruminantium* is highly infective to mice when injected i.v. or i.p. with peritoneal macrophages or organ homogenates. On the other hand the Welgevonden isolate is highly infective to mice only if inoculated i.v. with organ homogenates or tick stabilate (Du Plessis, 1985). A mortality of 99 % was recorded in mice i.v. injected with a liver homogenate or blood from animals infected with the Kwanyanga isolate, whereas only a few mice died when infected by the i.p. route with the same inocula (Uilenberg, 1983).

Lung macrophages: Ilemobade & Blotkamp (1978) transmitted heartwater to goats with lung macrophages collected in cold (4 °C) phosphate buffered saline (pH 7,3) and centrifuged at 600 *g* for 10 min. Five of the 7 goats infected i.v. developed heartwater but none of the 3 animals injected s.c. contracted the disease.

Body fluids and milk: Information regarding the infectivity of body fluids and milk as a source of *C. ruminantium* is limited. Alexander (1931) reported that inconsistent results were obtained when attempting to transmit heartwater with fluids collected from the thoracic and abdominal cavities and pericardial sac of diseased animals. The route of inoculation, time of collection and the amount of fluids administered to susceptible animals are not mentioned. He attributed the occasional infectivity of the fluids to the presence of parasitized leucocytes. Spreull (1922) claimed that milk from diseased animals is infective but the available information on this aspect is too incomplete to draw any conclusions.

Infected ticks

In 1928 *C. ruminantium* was transmitted to sheep by the i.v. injection of heartwater-infected *A. hebraeum* nymphae stabilate (Theiler & Du Toit, 1928). Many years later the development of a new heartwater vaccine using *A. hebraeum* nymphae infected with *C. ruminantium* was investigated (Bezuidenhout, 1981). Infected blood and tick stabilate i.v. administered to domestic ruminants can cause a shock reaction and must therefore be injected cautiously (Alexander, 1931; Bezuidenhout, 1981; Oberem & Bezuidenhout, 1987).

Kocan, Bezuidenhout & Hart (1987), suggested that in *A. hebraeum* nymphae, heartwater organisms initially develop in the epithelial cells of the gut and subsequent stages invade and develop in acini cells of the salivary glands. This is followed by transsalivary transmission to the vertebrate host. The infectivity of *A. hebraeum* female saliva was found to be very low compared to ground-up suspensions prepared from the same group of ticks, Bezuidenhout (1981). Infectivity of tick material is not confined to the gut and salivary glands, but also includes haemocytes and rectal ampules of prefed adult ticks, and homogenates of hypodermal tissue (Du Plessis, 1985; J. D. Bezuidenhout, personal communication 1986).

Cultured endothelial cells

C. ruminantium was cultivated *in vitro* in a calf endothelial cell line and the disease transmitted by the i.v. inoculation of parasitized cells. Infected cells were released from the culture flasks using activated trypsine versene and the trypsine neutralized with a medium containing 10 % bovine serum. The detached cells and corresponding supernatant were mixed and injected i.v. into susceptible sheep which contracted the disease (Bezuidenhout *et al.*, 1985).

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