Onderstepoort J. vet. Res., 54, 291–294 (1987)

DIAGNOSIS OF HEARTWATER IN THE LIVE ANIMAL: EXPERIENCES WITH GOATS IN GUADELOUPE

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ABSTRACT

CAMUS E. & BARRÉ N., 1987. Diagnosis of heartwater in the live animal. *Onderstepoort Journal of Veterinary Research*, 54, 291–294 (1987)

A presumptive diagnosis of heartwater in the living animal can be based on clinical and epidemiologic observations. In Guadeloupe, heartwater can be confused with haemonchosis in goats or cerebral babesiosis in cattle. Confirmation of the clinical diagnosis by brain biopsy is useful in experimental infections but is hardly applicable in the field. Positive results were obtained from 92 % of animals 16 to 18 days after experimental infection. In febrile animals the best results were obtained between the 3rd and 6th days of the thermal response.

Diagnosis can also be supported by serological tests. These are useful for monitoring experimental infections and for checking recovered animals in the field. Nineteen goats out of 27 were negative on Day 1 of the febrile reaction but positive a week later. The remaining 8 goats were positive on Day 1 and had greatly increased antibody titres a week later.

Confirmation of a diagnosis can also be achieved by subinoculating into susceptible animals either blood or suspensions of ticks collected from suspect animals and then homogenized. Ticks that have engorged on a suspect animal can be allowed to moult and then fed on a susceptible animal to test their infectivity.

These methods are time consuming but useful for heartwater surveys.

INTRODUCTION

The diagnosis of heartwater is often difficult in the live animal because clinical signs of the disease are not pathognomonic and the course of the disease is fast for early treatment. Diagnosis is nevertheless necessary. Also, it is important to know if a non-lethal reaction in experimental animals, inoculated with tick homogenate from a country where heartwater has not yet been reported, corresponds to heartwater.

The diagnosis can be based on classical (that is, epidemiological or/and clinical) grounds. But other methods that have been used more or less successfully include subinoculation of blood or ticks collected from a suspect animal, homogenized and then inoculated into a susceptible sheep or goat; brain biopsies; pulmonary washing, and serology.

Details will be given of the methods used in Guadeloupe and the results obtained. Other available methods will be discussed.

MATERIALS AND METHODS

1. *Epidemiological and clinical diagnosis*

Since 1982 records of all clinical cases of heartwater in Guadeloupe have been kept, together with their characteristics: locality, age, sex and breed of animals affected, presence of *Amblyomma variegatum*, clinical signs observed, and number of deaths (Camus & Barré, 1987).

By inoculating numerous sheep and goats during a survey on heartwater in the Caribbean (Barré, Camus, Birnie & Uilenberg, 1984), we were able to observe the clinical signs and susceptibility of animals to the Gardel strain, which was isolated in Guadeloupe in 1982.

2. *Experimental diagnosis*

Subinoculation of blood. In our laboratory, when the reaction of goats or sheep inoculated with tick homogenate was not typical, blood was taken during the fever period or between 16 to 24 days post-inoculation, and either directly inoculated by the i.v. route into a susceptible goat (2–3 mℓ) or cryopreserved in liquid nitrogen with 10 parts per 100 dimethyl-sulphoxide (DMSO) and inoculated later. The subinoculated animal was then monitored for *Cowdria ruminantium* infection by recording daily rectal temperatures.

We also studied the minimum infective dose of blood. Blood was taken on the 2nd day of fever from a reacting goat, diluted and subinoculated into susceptible goats.

Infection by ticks. In an experiment to study the reservoir status of goats recovered from heartwater (Barré & Camus, 1987) batches of larvae were fed during the subsequent fever reaction on 9 goats that had been inoculated with the Gardel strain. Engorged larvae were allowed to moult in an incubator and the nymphae were then fed on 8 susceptible receiver goats.

Brain biopsies. Following Synge's method (1978) we made numerous brain biopsies in our laboratory from goats inoculated with tick homogenates. The fragment of brain collected (gray matter) is crushed between 2 slides, fixed in methanol and stained with Giemsa. Biopsies were made several days before or after the thermal reaction to determine the moment when *Cowdria* are more numerous in the endothelial cells of capillaries.

Pulmonary washing. Ilemobade (1976) and Da Graça (1966) stated that *C. ruminantium* occurred in the bronchial epithelium of a sheep that had died from heartwater. Using the Fuentes & Pedroso method (1986) for pigs, we tried to make pulmonary washings from 10 goats inoculated with the Gardel strain, on the 2nd and 4th days of the thermal reaction (temperature > 40 °C). We injected 50 mℓ of phosphate buffered saline (PBS) into the lungs, gently massaged the ribs and aspirated about 20 mℓ of PBS. Pulmonary macrophages were then washed 3 times in fresh PBS at low speed and the final pellet of cells was spread over 2 slides. One slide was fixed and stained with Giemsa, the other was used as antigen to make an IFAT with a hyperpositive serum.

3. *Laboratory diagnosis (serology)*

The IFAT with the Kümm strain described by Du Plessis (1981), and slightly modified, is a specific method for detecting antibodies in goats (Camus & Barré, 1987).

Antibodies appear about 2 weeks after experimental infection, i.e. when the thermal reaction occurs, and increase quickly (Du Plessis, 1981; Camus & Barré, 1987). Taking sera from inoculated goats on the 1st day

Received 14 May 1987—Editor

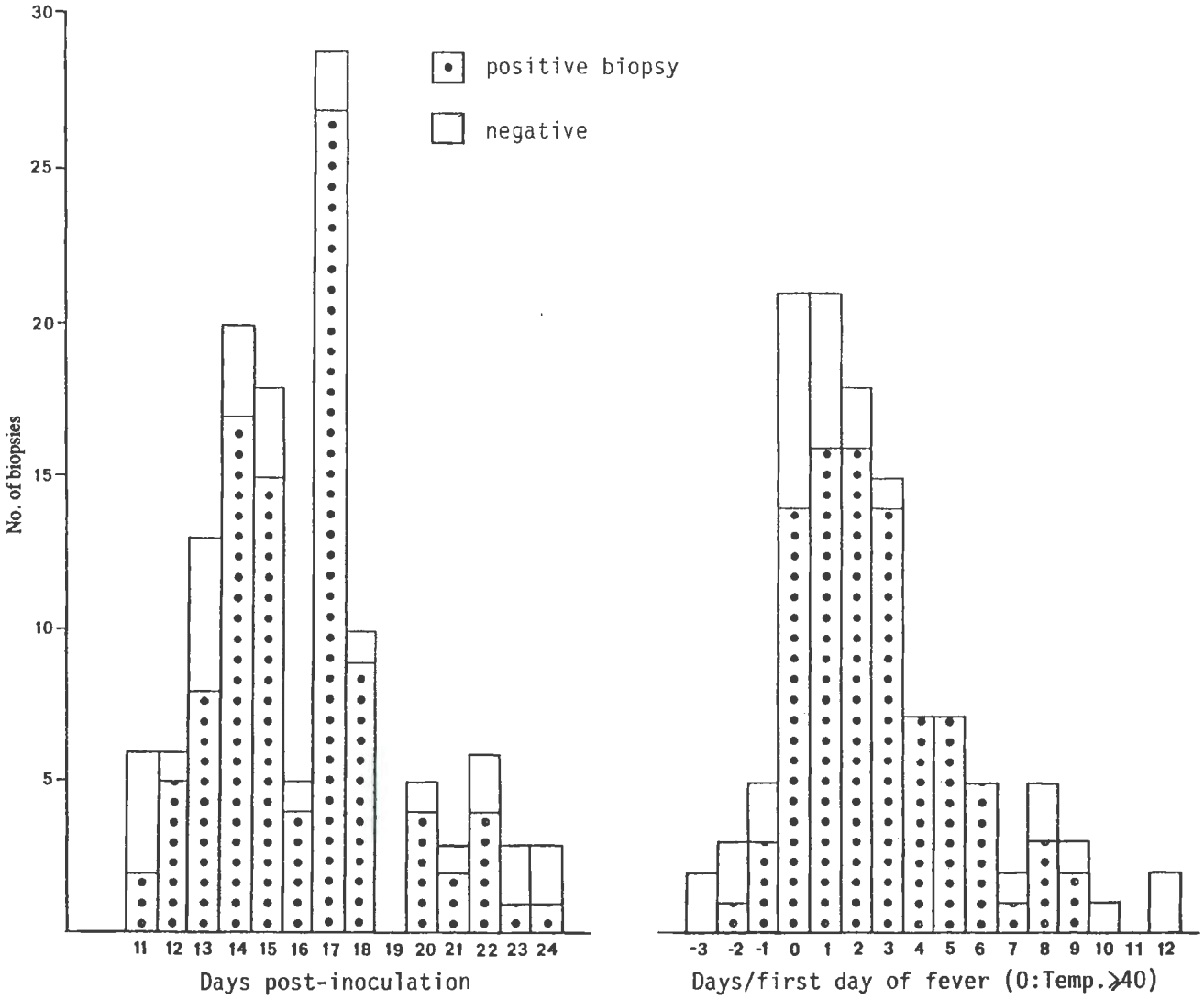

FIG. 1 Results of brain biopsies on experimentally infected animals

of the thermal reaction and 7 days later (if the goat survived) we looked for an increase in antibodies between the 2 samples.

RESULTS

1. *Epidemiological and clinical diagnosis*

In Guadeloupe heartwater occurs all over the island throughout the year (Camus & Barré, 1987).

Heartwater in Creole cattle is uncommon (1 case only recorded during 4 years in Guadeloupe) but occurs from time to time in purebred cattle when there is a break in dipping. Clinical cases are also rare in Creole sheep (1 case recorded).

Heartwater is, however, one of the main pathological problems in goats (besides haemonchosis) and must be suspected immediately.

Municipalities in Grande-Terre (St Francois, Ste Anne, Gosier, Petit Canal) are especially infected with the disease. Heartwater can induce small epizootics where several animals (from 2 to 17) are ill at the same time or can induce sporadic cases (11 small epizootics and 26 sporadic outbreaks in which a single animal only became sick).

In cattle, clinical signs of heartwater cannot be distinguished from cerebral babesiosis caused by *Babesia bovis*. The only differential diagnosis is by specific therapy in the live animal. Of 21 fatal cases in cattle, 9 were diagnosed at post mortem examination as due to heartwater and 6 to *B. bovis*.

In Guadeloupe, heartwater in sheep and goats has to be distinguished mainly from haemonchosis, but the thermal reaction (when present) allows a differential diagnosis to be made.

2. *Experimental diagnosis*

Subinoculation of blood

Blood: Goat No. 41, inoculated with a tick homogenate, developed an atypical reaction, with a temperature > 40 °C on Day 20 only. Blood was taken before and after this reaction, on Days 16 and 24, pooled and inoculated by the i.v. route into a susceptible sheep. This sheep failed to react but resisted a challenge with a virulent strain; so the subinoculum was infective and goat No. 41 had therefore been infected with heartwater.

Study of the minimum infective dose: In the 1st experiment, 2 susceptible goats were each inoculated with 2 mℓ of the following dilutions of infected blood: 1/1; 10^{-1}; 10^{-2}; 10^{-3}; 10^{-4}. All goats inoculated with blood diluted from 1/1 to 10^{-2} died from heartwater, but those injected with dilutions of 10^{-3} and 10^{-4} did not react. In the 2nd experiment, 4 goats were inoculated with the same (deepfrozen) blood diluted as follows: 10^{-2}; $10^{-2,33}$; $10^{-2,66}$, and 10^{-3}. Only the first 2 died from heartwater, the other 2 did not react.

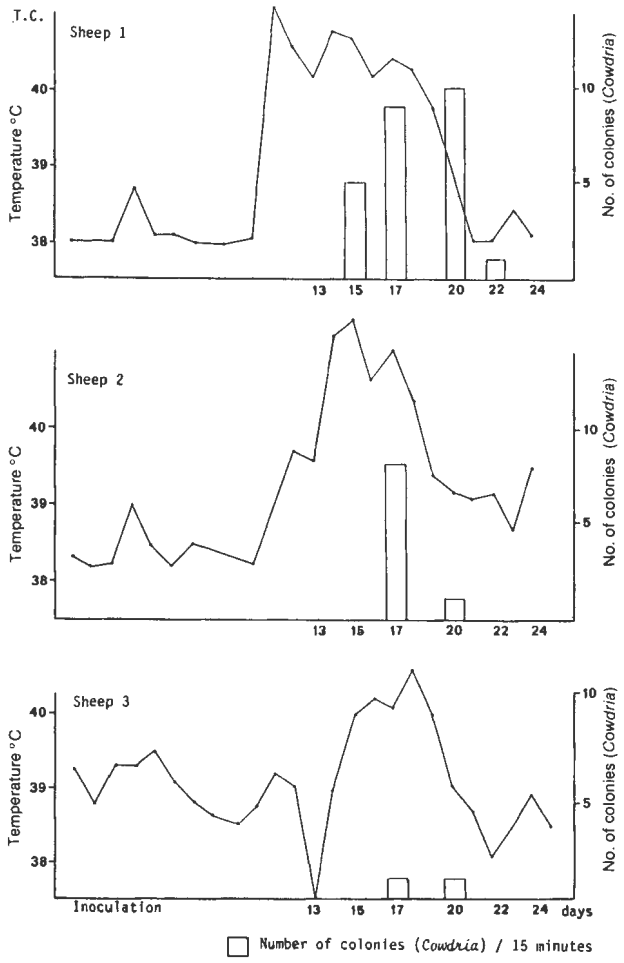

FIG. 2 Number of colonies (*Cowdria*) observed on biopsy smears examined during 15 min from 13th to 24th days post experimental infection

Hence a very small quantity of blood sampled during the fever reaction is enough to induce heartwater when subinoculated i.v. into a susceptible animal.

Infection by ticks. The 8 receiver goats infested with nymphs reacted with typical heartwater. The ticks were, therefore, infected. Ticks can thus be used to make the diagnosis.

Brain biopsies. A total of 350 brain biopsies were made from sheep and goats without mortality or other problems. We made as many as 8 biopsies over 11 days from the same sheep without any apparent ill effect on this animal.

The results obtained from the infected animals are shown in Fig. 1 and 2. Fig. 2 shows the number of *Cowdria* colonies counted during 15 min in biopsy smears made every other day from 3 infected sheep on the basis of their rectal temperatures and the number of days post infection. The optimal time for making a biopsy is from the 16th to the 18th day p.i.; colonies of *Cowdria* are more numerous and 92 % of the infections can be detected. During a thermal reaction, the best time is from the 3rd to the 6th day after the beginning of the fever.

The biopsy should not be too much delayed because colonies of *C. ruminantium* disappear quickly after the rectal temperature becomes normal.

In 36 non-fatal cases of heartwater, biopsy confirmed the diagnosis. Seven of these 36 cases had no fever or very little fever.

Pulmonary washing. In the 10 infected goats, we were unable to find any colonies of *Cowdria* in the pulmonary macrophages, either in those stained by Giemsa or those used as antigen in the IFAT.

3. *Laboratory diagnosis*

Serology is often negative on the 1st day of fever, and becomes positive (1:80) 1 week later: 19/27 goats (70 %). In such cases it is not necessary to test several dilutions of the serum.

Sometimes serum is already positive the 1st day of fever and antibodies increase during the following week (passing from 1:80 or 1:160 to 1:1280 or 1:2560); 8/27 goats (30 %). There are only about 2 % of false positive and 2 % of false negative reactions.

Epidemiological diagnosis

In Guadeloupe small epizootics or sporadic outbreaks of disease, mainly in goats in Grande-Terre, with *A. variegatum* present in the flock throughout the year, must evoke suspicion of heartwater.

In cattle, heartwater is observed only in improved breeds when there is a break in the dipping.

In Africa, heartwater occurs sporadically during the rainy season; in the distribution area of *Amblyomma* after a break in the dipping (Norval, 1978), or after the reappearance of ticks following a long drought (Gueye, Mbengue & Diouf, 1984). Anyway, epidemiology can act only as an indication.

Discussion

Clinical diagnosis

In Guadeloupe there are few diseases which can be confused with heartwater (haemonchosis for goats and cerebral babesiosis for cattle). But in Africa, heartwater has to be differentiated from tetanus, rabies, strychnine poisoning, poisoning by acaricides or plants, anthrax, pasteurellosis, ovine and bovine ehrlichiosis, and trypanosomiasis (Camus & Barré, 1982) and clinical diagnosis can only be a suspicion.

Experimental diagnosis

Subinoculation of blood, infection by ticks collected from sick animals, or brain biopsy allow a certain diagnosis. The first 2 methods, unfortunately, do not give quick results and are not useful methods for individual diagnosis in the field. But, in addition to the inoculation of tick homogenate, they can be used to demonstrate the existence of heartwater in a herd or in a region by using pools inoculated into susceptible goats (Barré *et al.*, 1984).

We have no experience with brain biopsies in cattle but the method is derived from the diagnosis of cerebral babesiosis so it can also be applied to cattle. In sheep and goats brain biopsy is easy and quick to perform. In some cases examination is lengthy because colonies are not numerous, but biopsies can be repeated at 1-day intervals if the first is negative or doubtful. The number of colonies increases after a few days of fever reaction.

In general, biopsies allow a diagnosis to be made on a live animal, even when its reaction is not typical. The technique can therefore be very useful, not only for experimental animals but also in the field if the stock owners accept the method.

Colonies of *Cowdria* in lungs have only been reported in Angola (Da Graça, 1966). Pulmonary washing should perhaps be attempted again when respiratory signs occur in heartwater.

Laboratory diagnosis

Serology works well on experimental animals and we use it more and more instead of biopsies, because it is a

"cleaner" method. In the field it is difficult to wait for a week, as the sick animal may die in the interval. But if heartwater is suspected on clinical and epidemiological grounds treatment can be started and the disease confirmed by a titration of antibodies a week later.

Other laboratory methods that have been described are haematological changes as follows:

Eosinophilic granulocytes decrease considerably in the circulating blood (Clark, 1962; Owen, Littlejohn, Kruger & Erasmus, 1973; Ilemobade, 1976).

Leucopenia (Ilemobade, 1976).

Important neutrophilia (Perreau, 1981).

These 3 factors are not specific for heartwater but can be of help in diagnosis.

Orange pigmentation of the blood plasma of Angora goats, not due to bilirubinemia (Clark, 1962; Grüss, 1981), but this phenomenon may not be a general one. It was not observed in Dutch goats (Uilenberg, 1983).

CONCLUSIONS

The diagnosis of heartwater in a specific area, utilizing live animals, can be:

A suspicion based on clinical and epidemiological observations.

A certainty based on brain biopsy, which is very useful in the case of experimental animals but hardly applicable in the field.

A near certainty based on serology. This is also very useful for experimental animals or to confirm a diagnosis in respect of treated animals in the field.

A certainty based on the subinoculation of blood or of a homogenate of ticks collected from suspected animals and inoculated into a susceptible sheep or goat; or by infection of a susceptible animal by ticks which have engorged on the suspected animal and then moulted. These methods are lengthy and can be used for a heartwater survey in a region by pooling samples.

Therefore, reliable methods can be used on experimental animals but cannot always be readily made in the field.

In the field, the most reliable method of diagnosis remains the examination of brain smears from the first animal that has died, followed by daily recording of the rectal temperatures of all animals and the treatment of those having a temperature of > 40 °C or of those whose physical condition is deteriorating.

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Printed by the Government Printer, Private Bag X85, Pretoria, 0001