

The Transmission of Heartwater to and from Blesbuck (*Damaliscus albifrons*) by means of the Bont-Tick (*Amblyomma hebraeum*).

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INTRODUCTION.

IN 1933 and 1935 the writer reported on the transmission of heartwater to two species of South African antelopes namely the blesbuck (*Damaliscus albifrons*) and the black wildebeest (*Conochaetes gnu*). No visible clinical symptoms could be detected in these animals, but the presence of the "virus" could be demonstrated by sub-inoculating blood into susceptible sheep. These results prompted further studies on the transmission of this disease to antelopes by the bont-tick (*Amblyomma hebraeum*). The outcome of this work is published because it is believed that the experiments illustrate that heartwater can exist under natural conditions in the absence of domestic ruminants. Furthermore, this knowledge is of extreme practical importance and must serve as a basis for the promulgation of any prophylactic measures which aim at the eradication of heartwater.

Through the kindness of the Provincial Administration of the Orange Free State this Institute obtained several blesbuck from the Summerville Game Reserve, an area where heartwater is not known to exist. The animals were kept in a camp comparatively free of ticks. When the experiments were started the blesbuck were wild and difficult to handle. The temperature of the first animal could therefore not be taken. Later on the animals became more docile and it was possible to take the temperature twice a day from the second blesbuck.

THE BREEDING AND FEEDING OF TICKS.

In the literature numerous references to this subject are given. The methods in these experiments have been modified to some extent with the object of reducing the period of hatching of the eggs and moulting of the various stages. For this purpose the *A. hebraeum* ticks were kept in a room of constant temperature of 26° C. and a relative humidity of 80 per cent. Under these conditions the ticks thrive well and their life cycle can be completed within six months.

Another improvement that was necessary is the method of handling and feeding, since during such operations one has to consider the danger of ticks escaping. To overcome this difficulty bags having the shape of an ear are made from thin but strong linen. With the aid of a funnel and by careful manipulation the ticks are shaken into the bottom of the bag, the tip of which is tied off with tape in order to confine them there. The bag is then drawn over the ear and the open end fixed to the base of the ear by means of adhesive paste. When the paste has dried the tape is removed and the ticks are thereby liberated into a confined space. As a further precaution larger stout calico bags fitted with several lengths of tape are placed on both ears, to prevent damage to the inner bag. (See Figs. 1, 2 and 3.)

The bags are left undisturbed for three days. When ticks have to be collected the outer bag is removed. An incision about 8 to 10 cms. is made in the inner bag and the ticks can be examined. After collection the incision is sewn up and the outer bag placed back into position.

This system has been found to be satisfactory and if carefully carried out one can account for practically every tick that has been placed on the animal.

TICK TRANSMISSION.

For the sake of convenience the details of the experiments are appended in Tables I-III.

Experiment No. 1 (S. 5527).

Object.—To infect *Amblyomma hebraeum* larvae with heartwater.

Method.—(a) Virulent heartwater blood was injected intravenously into a susceptible sheep 45733. (b) On the fourth day after injection the ears of the sheep were infested with three hundred larvae batch 1416A.

Result.—The ticks fed readily and 225 engorged larvae were collected from the sheep during the reaction. The rest of the ticks had not attached and were found dead in the bag.

Conclusion.—No satisfactory explanation can be given why a number of apparently healthy ticks die when they are placed on the ears of an animal. In some tick feeding experiments carried out at Onderstepoort up to 50 per cent. of larvae have been found dead twenty-four hours after infestation.

The engorged larvae were allowed to moult and were used in the subsequent experiments.

Experiment No. 2 (S. 6020).

Object.—To ascertain whether the nymphae that fed as larvae on sheep 45733 in experiment No. 1 are able to transmit heartwater to a sheep.



FIG. I.—Blesbuck 47081 illustrating the method of fixing inner tick bags to an ear. For protection larger loosely fitting calico bags are placed over the inner bag.



FIG. II.—Blesbuck 47081 roaming freely in the camp with outer bag for protection attached.



FIG. III.—Sheep with inner tick bag attached.

Method.—The ears of a susceptible sheep 45872 were infested with approximately 18 nymphae batch 1416Ab.

Result.—The sheep reacted to heartwater and died. All the ticks had dropped by the 8th day.

Conclusion.—The ticks picked up the infection as larvae and were able to transmit heartwater as nymphae. This batch of ticks is considered suitable for further transmission work.

Experiment No. 3.

Object.—To transmit heartwater to a blesbuck by infesting the animal with known infected ticks.

Method.—Approximately 50 infected *A. hebraeum* nymphae batch 1416Aa that fed as larvae on sheep 45733 in experiment No. 1 were allowed to feed on the left ear of blesbuck No. 47081.

Result.—The ticks attached readily, but the feeding period was considerably longer than in the case of sheep 45872 in experiment No. 2. Ten ticks had not attached and were found dead in the bag. Forty engorged specimens were collected between the 8th to the 20th day, whereas in sheep 45872 the ticks dropped from the 6th to the 8th day.

Conclusion.—The feeding in the case of the sheep was carried out in a stable whereas in the case of the blesbuck in a camp. No satisfactory explanation can be given why the ticks took longer to engorge on the latter animal. An important factor may be the temperature which is more constant in a stable than in a camp.

Experiment No. 4.

Object.—(a) To ascertain whether nymphae have transmitted heartwater to blesbuck 47081. (b) To ascertain whether ticks will infect themselves while feeding on this blesbuck.

Method.—(a) Blood from blesbuck 47081 was injected at various intervals after tick infestation into susceptible sheep. (b) 14 days after infesting the left ear 100 larvae batch 1416D and 20 clean nymphae batch 1396Aa were placed on the right ear of the blesbuck.

Result.—(a) It was possible to demonstrate heartwater "virus" by subinoculating blood into sheep from the 28th to the 62nd day after tick infestation, i.e. for a period of 35 days. Sheep injected before this period did not react to heartwater.

The blesbuck died 70 days after tick infestation. Blood was collected after death but did not produce heartwater when injected into two susceptible sheep. The post-mortem examination of the animal showed hydrothorax, hydropericard oedema of the lungs and a few *Haemonchus contortus* in the abomasum. *Rickettsia ruminantium* could not be demonstrated in the smears prepared from the intima of the jugular vein.

(b) (i) Eighty-seven engorged larvae detached from the 13th to the 20th day. The rest had died. (ii) Fifteen engorged nymphae were collected from the 14th to the 17th day, the others had not attached and died. These ticks were allowed to moult and fed in their next stage on sheep in order to establish their infectivity.

Conclusion.—The incubation period of heartwater in blesbuck 47081 was considerably longer (approximately 28 days) than that of sheep 45872 (12 days). This probably stands in relation to the longer period taken by the ticks to engorge on the former animal.

During the time that the clean ticks were feeding on the blesbuck the presence of heartwater virus could be demonstrated by blood subinoculation. The larvae and nymphae therefore had every opportunity of infecting themselves.

Experiment No. 5.

Object.—To ascertain whether the larvae and nymphae infected themselves while feeding on blesbuck 47081.

Method.—(a) The nymphae batch 1416Da were allowed to feed on sheep 45980. (b) The adults batch 1396Aa1 were allowed to feed on sheep 46069.

Result.—(a) Sheep 45980 reacted to heartwater and recovered. (b) Sheep 46069 reacted to heartwater and died.

Conclusion.—The larvae and nymphae picked up the infection while feeding on blesbuck 47081 and transmitted heartwater to sheep when fed as nymphae and adults respectively.

Experiment No. 6.

Object.—To ascertain whether adult ticks that transmitted heartwater as nymphae to blesbuck 47081 retain their infection.

Method.—Adult *A. hebraeum* ticks batch 1416Aa1 were allowed to feed on sheep 45874.

Result.—The male ticks attached readily but the females only three days later. They detached from the 11th to the 17th day. The sheep reacted to heartwater and was killed *in extremis*.

Conclusion.—The adult ticks retained their infection.

Experiment No. 7 (S. 6104).

This experiment was carried out with the object of obtaining further information on the transmission of heartwater to a blesbuck by means of ticks.

Object.—To transmit heartwater to a blesbuck by infesting the animal with known infected ticks.

TABLE I.
The Feeding of Amblyomma hebraeum on the Blesbuck.

D.O.B. No. of animal and date of infestation.	Infested with batch.	Stage.	History of ticks.	Object.	Ticks drop : (a) Number of days after infestation. (b) Number of ticks collected.	Result.
Bb. 47081 25/8/36	1416 Aa	Nymphae	Ticks fed as larvae on a heartwater reacting sheep 45733	To transmit heartwater to the blesbuck	(a) 8, 10, 13, 14, 16, 18, 20 (b) 4, 1, 20, 10, 2, 1, 1	Animal showed a gradual loss of condition for a period of 7 weeks and died 3/11/36. No Rickettsia could be found in the intima smears from the jugular vein.
Sh. 45872 25/8/36	1416 Ab	Nymphae	Ticks fed as larvae on a heartwater reacting sheep 45733	To show that the batch of ticks are infected with heartwater	(a) 6, 7, 8 (b) 7, 7, 2	Reacted to heartwater 12 days after infestation and died 15/9/36
Bb. 47081 8/9/36	1416 D	Larvae	The progeny of adults collected at Pretoria North on 7/1/36	To infect larvae with heartwater	(a) 13, 14, 15, 16, 17, 20 (b) 2, 30, 10, 10, 25, 10	During the time that the larvae were feeding, the presence of heartwater "virus" could be demonstrated by subinoculation into susceptible sheep.
Bb. 47081 8/9/36	1396 Aa	Nymphae	Larvae fed on a guinea pig	To infect nymphae with heartwater	(a) 14, 15, 16, 17 (b) 2, 5, 2, 6	During the time that the nymphae were feeding, the presence of heartwater "virus" could be demonstrated by subinoculation into susceptible sheep.
Sh. 45980 6/11/36	1416 Da	Nymphae	Fed as larvae on blesbuck 47081	To ascertain whether these ticks became infected with heartwater	(a) 5, 8, 11 (b) 1, 10, 2	Reacted to heartwater 12 days after tick infestation and recovered. On testing the immunity the sheep was found to be immune.
Sh. 46069 13/11/36	1396 Aa1	Adults	Fed as nymphae on blesbuck 47081	To ascertain whether these ticks became infected with heartwater	(a) 10, 13 (b) 1, 2	Reacted to heartwater 17 days after tick infestation and died 6/12/36.
Sh. 45874 13/11/36	1416 Aa1	Adults	Transmitted heartwater to the blesbuck 47081 in the nymphal stage	To ascertain whether these ticks will retain the infection and pass it off in the adult stage	(a) 11, 12, 13, 17 (b) 2, 2, 2, 1	Reacted to heartwater 15 days after tick infestation. Animal destroyed in extremis.
Bb. 47083 21/12/36	1416 Ac	Nymphae	Ticks fed as larvae on a heartwater reacting sheep 45733	To transmit heartwater to the blesbuck	(a) 7, 8, 10 (b) 5, 6, 3	Animal showed a gradual loss of condition for a period of 13 weeks and died 22/3/37. No Rickettsia could be found in the intima smears from the jugular vein. From the 17 to 26 day after tick infestation, heartwater "virus" could be demonstrated by subinoculation into sheep.

Bb. = Blesbuck.

Sh. = Sheep.

TABLE II.

The Demonstration of Heartwater "Virus" in Blesbuck 47081 by Subinoculation in Sheep.

D.O.B. No. of animal.	Dose of blood i.v.	Number of days after tick infestation.	Date.	Incubation period in days.	Result.
43761	25 c.c.	14	8/ 9/36	—	No heartwater reaction.
42735	10 c.c.	20	14/ 9/36	—	No heartwater reaction.
45813	10 c.c.	20	14/ 9/36	—	No heartwater reaction.
42837	10 c.c.	24	18/ 9/36	—	No heartwater reaction.
43659	10 c.c.	24	18/ 9/36	—	No heartwater reaction.
46020	10 c.c.	28	22/ 9/36	11	Reacted to heartwater and died 16/10/36.
46123	10 c.c.	28	22/ 9/36	13	Reacted to heartwater and died 13/10/36.
46041	10 c.c.	31	25/ 9/36	10	Reacted to heartwater and recovered. On testing the immunity found to be immune.
46092	10 c.c.	31	25/ 9/36	10	Reacted to heartwater and died 19/10/36.
46228	10 c.c.	42	6/10/36	9	Reacted to heartwater and died 28/10/36.
46434	10 c.c.	42	6/10/36	10	Reacted to heartwater and died 21/10/36.
45974	10 c.c.	52	16/10/36	13	Reacted to heartwater and recovered. On testing the immunity found to be immune.
46340	10 c.c.	52	16/10/36	8	Reacted to heartwater and died 4/11/36.
43433	10 c.c.	62	26/10/36	12	Reacted to heartwater and recovered. On testing the immunity found to be immune.
46085	10 c.c.	62	26/10/36	10	Reacted to heartwater and recovered. On testing the immunity found to be immune.
46353	10 c.c.	70	3/11/36	—	No heartwater reaction. On testing the immunity found to be susceptible.
46728	10 c.c.	70	3/11/36	—	No heartwater reaction. On testing the immunity found to be susceptible.

Method.—(a) Approximately 20 infected *A. hebraeum* nymphae batch 1416Ac collected from sheep in experiment No. 1 were allowed to feed on the ear of blesbuck 47083. This animal was docile and the temperature was taken twice daily. (b) Blood was injected at various intervals after tick infestation into susceptible sheep.

Result.—(a) The ticks fed readily and 14 specimens detached from the 7th to the 10th day.

The temperature did not show any abnormal variation. There was a gradual loss of condition in this animal which died eight weeks after the last day on which heartwater "virus" was demonstrated. At autopsy the blesbuck was found to be suffering from bronchopneumonia. In addition there was a light *Haemonchus* and *Dictyocaulus* infection.

(b) The presence of heartwater "virus" could be demonstrated from the 17th to the 26th day but not on the 31st day after tick infestation. Only one out of two sheep reacted in each group.

Conclusion.—It was possible to transmit heartwater to blesbuck 47081 by means of infected *A. hebraeum* nymphae. Attention is drawn to the fact that only three out of six sheep reacted when injected with blood during the time while the blesbuck harboured heartwater "virus". From this observation it is not clear what factors control the infectivity of blood.

The incubation period was approximately 17 days whereas in the previous blesbuck, 47083, it was approximately 28 days.

TABLE III.

The Demonstration of Heartwater "Virus" in Blesbuck 47083 by Subinoculation into Sheep.

D.O.B. No. of animal.	Dose of blood i.v.	Number of days after tick infestation.	Date.	Incubation period in days.	Result.
43556	10 c.c.	17	7/1/37	—	No heartwater reaction. On testing the immunity found to be susceptible.
43755	10 c.c.	17	7/1/37	11	Reacted to heartwater and recovered. On testing the immunity found to be immune.
45736	10 c.c.	23	13/1/37	12	Reacted to heartwater and died 1/2/37.
45747	10 c.c.	23	13/1/37	—	No heartwater reaction. On testing the immunity found to be susceptible.
45725	10 c.c.	26	16/1/37	11	Reacted to heartwater and died 3/2/37. <i>Rickettsia ruminantium</i> could be demonstrated in the smears prepared from the intima of the jugular smears.
45891	10 c.c.	26	16/1/37	—	No heartwater reaction. On testing the immunity found to be susceptible.
42832	10 c.c.	31	21/1/37	—	No heartwater reaction. On testing the immunity found to be susceptible.
44525	10 c.c.	31	21/1/37	—	No heartwater reaction. On testing the immunity found to be susceptible.

DISCUSSION.

Amblyomma hebraeum is widely distributed in the Union of South Africa and is found chiefly in the Lowveld, Northern and Eastern Transvaal, Natal and Eastern Cape Province. It has been recorded from Bechuanaland. Not only does this species of tick parasitize domestic stock, but Bedford (1932 and 1936) records its presence from several different species of antelopes mentioned in Table IV and also from other wild animals.

TABLE IV.

Species of Antelopes from which A. hebraeum has been recorded.

Zoological name.	Vernacular name.	Stage.	Locality.
<i>Gorgon taurinus</i>	Blue Wildebeest	Adults	Umfolosi Game Reserve, Natal.
<i>Nyala angasi</i>	Nyala	Adults	Ubombo Flats, Zululand.
<i>Syncerus caffer</i>	African Buffalo	Adults	Umfolosi Game Reserve, Natal.
<i>Sylviacapra grimmii</i>	Duiker	Adults & Nymphae	Umfolosi Game Reserve, Natal.
<i>Strepsiceros strepsiceros</i>	Koodoo	?	South Africa.
<i>Tragelaphus sylvaticus</i>	Bushbuck	?	South Africa.
<i>Aepyceros melampus</i>	Impala	Adults	Swaziland.

Since ticks that become infected with heartwater as larvae retain their infection until the adult stage it is believed that antelopes and possibly other wild animals are capable of spreading infected ticks over considerably large areas.

The experiments mentioned in this paper show conclusively that infected ticks can transmit heartwater to a blesbuck and that clean ticks in their turn are capable of infecting themselves when they are allowed to feed on such an infected animal. Under natural conditions therefore the same may happen.

From observations it is known that the bont tick (*A. hebraeum*) may take a very long time to complete its life cycle. When conditions are ideal the period from the larvae to the adult stage can be as short as six months and under less favourable conditions the period is extended to over two years. During this time several susceptible

animals can be born. It is therefore possible that in areas where there are no domestic animals and where infected ticks have to rely chiefly on antelopes and their susceptible progeny as hosts heartwater can be maintained.

Up to the present heartwater has not been demonstrated in antelopes living under natural conditions. During the investigations on trypanosomiasis in antelopes a large number of blood subinoculations was made from these animals into domestic ruminants. It would be interesting to know whether any of the workers observed cases of heartwater in the injected animals while carrying out their studies. Naturally if immune animals were used in their experiments heartwater would not have been found.

SUMMARY.

- (1) The transmission of heartwater to two blesbuck is discussed.
- (2) Infected *A. hebraeum* nymphae are capable of transmitting heartwater to the blesbuck.
- (3) Heartwater could be demonstrated by blood subinoculation into susceptible sheep for a period of 35 days in one blesbuck and for 9 days in another.
- (4) The virulence of heartwater virus did not change by passage.
- (5) Infective nymphae do not lose their infection but retain it to the adult stage.
- (6) Clean larvae will pick up infection for transmission as nymphae.
- (7) Clean nymphae will pick up infection for transmission as adults.
- (8) The technique of tick breeding and feeding is briefly discussed.
- (9) Both blesbuck died, one 13 and the other 7 weeks after tick infestation.
- (10) The significance of these experiments is discussed.

LITERATURE.

- BEDFORD, G. A. H. (1932). A synoptic check-list and host-list of the ectoparasites found on South African Mammalia, Aves, and Reptilia (Second Edition). *18th Rept. Div. Vet. Serv. and An. Ind.*, Union of South Africa, pp. 223-523.
- BEDFORD, G. A. H. (1936). A synoptic check-list and host-list of the ectoparasites found on South African Mammalia, Aves and Reptilia (Supplement No. 1). *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 7, No. 1, pp. 67-110.
- NEITZ, W. O. (1933). The Blesbuck (*Damaliscus albifrons*) as a carrier of heartwater and Blue Tongue. *Jnl. South African Vet. Med. Assoc.*, Vol. 4, No. 1, pp. 26-28.
- NEITZ, W. O. (1935). The Blesbuck (*Damaliscus albifrons*) and the Black-Wildebeest (*Conochaetes gnu*) as carriers of Heartwater. *Onderstepoort Jnl. of Vet. Sci. and An. Ind.*, Vol. 5, No. 1, pp. 35-40.