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DEMONSTRATION OF A CARRIER STATE FOR *COWDRIA RUMINANTIUM* IN WILD RUMINANTS FROM AFRICA

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ABSTRACT: Four wild African ruminants, eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), kudu (*Tragephalus strepsiceros strepsiceros*), and blue wildebeest (*Connochaetes taurinus*), were experimentally infected with the rickettsia *Cowdria ruminantium*, the tickborne agent causing heartwater in domestic ruminants. The infections were established, and *C. ruminantium* was transmitted to naive small ruminants by the vector *Amblyomma hebraeum* when transmission attempts were made at days 128 (eland and wildebeest), 85 (giraffe), and 24 (kudu) post infection. These wild ruminants, which are natural hosts for the tick vector, and which commonly occur within heartwater-endemic areas of Africa, are likely to play important roles in the epidemiology of heartwater as reservoirs of *C. ruminantium* infection. These findings also demonstrate that considerable risks are associated with the translocation of wild ruminants from heartwater-endemic areas to heartwater-free areas such as the northern and southern American mainlands, which have large populations of susceptible domestic and wild ruminant hosts and tick species that are capable of transmitting the disease.

Key words: *Amblyomma hebraeum*, *Cowdria ruminantium*, experimental transmission, heartwater, wild ruminants.

INTRODUCTION

Wild ruminants have long been suspected of playing an important role in the epidemiology of heartwater, an acute tickborne disease of domestic ruminants caused by the rickettsia *Cowdria ruminantium* (Cowdry, 1925a, b). However, research on heartwater has, understandably, focussed on domestic ruminants and, as a result, the significance of wildlife is not fully appreciated. In Africa, wild animals which are hosts for the *Amblyomma* spp. tick vectors of heartwater (Horak et al., 1983; Norval, 1983; Horak et al., 1987) frequently share pasture with domestic ruminants. If these species also carry *C. ruminantium* they may contribute to heartwater transmission dynamics. Although many wild species have been implicated as *C. ruminantium* hosts (Oberem and Bezuidenhout, 1987), few have been shown conclusively to be susceptible to infection. Experimental studies have demonstrated susceptibility in black wildebeest (*Connochaetes gnu*) (Neitz, 1935), blesbuck (*Damaliscus albifrons*) (Neitz, 1935; 1937), white-tailed deer (*Odocoileus virginianus*) (Dardiri et al., 1987), and African buffalo (*Syncerus caffer*) (Andrew and Norval, 1989). Other studies have produced less conclusive results due to inadequate diagnosis and may be misleading (Grosskopf, 1958; Hofmeyer, 1956; Gradwell, et al. 1976), or are based on reports of suspected natural cases (Mohan, 1968; Young and Basson, 1973; Poudelet et al., 1982; Oyejide and Olaleye, 1984; Okoh et al., 1986; Pandey et al., 1992; Okewole et al., 1993; Jackson and Andrew, 1994). While such reports provide useful preliminary information on species susceptibility, diagnosis may be compromised by similar, perhaps unknown, conditions and proof can only be acquired by experimental infection studies. Knowledge of the susceptibility of wild species to *C. ruminantium* infection is required to aid in the diagnosis of natural cases and to assess their role in heartwater epidemiology. Furthermore, through international conservation efforts and game ranching, wild animals from heartwater-endemic regions are being in-

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creasingly imported into heartwater-free areas, with substantial risk for the spread of the disease. The identification of reservoir species is therefore required for the development of rational regulatory procedures for the translocation and importation of wild animals. Herein, we describe studies on the susceptibility of four species of wild ungulates, blue wildebeest (*Connochaetes taurinus*), eland (*Taurotragus oryx*), giraffe (*Giraffa camelopardalis*), and kudu (*Tragephalus strepsiceros strepsiceros*), to *C. ruminantium* infection and their ability to act as reservoirs of infection.

MATERIALS AND METHODS

Four 12-mo-old wildebeest (#1–4) and four eland, consisting of a 5-yr-old bull (#900), and three 3-yr-old cows (#8, 11 and 899), originating from a heartwater and *Amblyomma* spp.-free farm in Darwendale (17°40′S, 30°18′E) in the highveld of Zimbabwe were used for experimental infections. Five kudu, represented by two 3-yr-old cows (#F1 and F2) and three 9-mo-old juveniles, one male (JM) and two females (#JF1 and JF2), originating from a second heartwater and *Amblyomma* spp.-free highveld farm in Bromley (18°04′S, 31°′21E) also were used. Finally, two 15-mo-old giraffe (#1 and 2) utilized in this study were captured on Nuanetsi Ranch (21°27′S, 30°53′E) in the heartwater endemic southern lowveld of Zimbabwe.

Infection trials on the eland, wildebeest, and kudu were conducted in a tick quarantine facility on Somerby Estate (17°50′S, 30°51′E), which is free of heartwater and its vectors, in Norton in the highveld of Zimbabwe. In this facility, the animals were kept on concrete in individual pens surrounded by a concrete water moat, approximately 20 cm wide and 15 cm deep. Studies on the giraffe were conducted in a quarantine facility on Wildlife Management Services Farm (20°59′S, 31°29′E) near Triangle in the southern lowveld. The giraffe were kept in a penned field for the duration of the study.

Supernates of bovine endothelial cell cultures, infected with either the Mbizi or Plumtree (Byrom et al., 1991; Smith et al., 1998) strains of *C. ruminantium*, were used for infecting the wild animal species. The Plumtree and Mbizi strains were used because in earlier laboratory trials they had been shown to induce a similar, pathogenic disease course and to establish a carrier state in domestic ruminants (S. Mahan and T. Peter, unpubl. obs.). Two different strains of *C. ruminantium* were used for infections to demonstrate that the ability to infect wild species was not specific to a single strain. The infected cultures were grown at the UF/USAID/SADC Heartwater Research Project in Harare, Zimbabwe, as previously described (Byrom and Yunker, 1990), and harvested when infection of cell monolayers was >75% and large numbers of *C. ruminantium* organisms were free in the culture supernate. To isolate organisms for inoculation, the supernates were centrifuged at 1,500 × g for 15 min to pellet cellular debris. The number of viable *C. ruminantium* organisms in each supernate was determined by staining a separate aliquot with carboxy-fluorescein diacetate and counting under UV fluorescence (Mishell and Shiigi, 1980).

Three of the four eland (#8, 899, 900) were inoculated intravenously with infected culture supernate containing 2.3×10^8 *C. ruminantium* (Mbizi strain) live organisms. Three of the five kudu (#AF1, AF2, JF1) were inoculated each with 8.8×10^8 organisms (Plumtree) and three of the four blue wildebeest (#2, 3, 4) received 2.3×10^8 organisms (Mbizi). One of the two giraffe (#1) received 5.1×10^8 organisms (Plumtree). The remaining individuals of each species were used for trials on infection by ticks.

The virulence of the cell culture supernates used for wildlife infections was confirmed by inoculating the same doses of each enumerated supernate intravenously into susceptible sheep. The sheep were monitored for clinical signs of heartwater, and infection was confirmed by brain biopsy (Synge, 1978) on the third day of febrile reaction (rectal temperature > 40.5 C), or by brain crush smear (Purchase, 1945) and post mortem examination on any sheep that died.

To prepare infected ticks for infections of wildlife, three sheep were each inoculated with 5 ml of virulent *C. ruminantium* blood stabilate, two with the Mbizi strain and the third with the Plumtree strain. Blood stabilate was used for these infections instead of cell culture to ensure a prolonged febrile period for tick feeding, as infections with cell culture material are usually rapid and acute (S. Mahan and T. Peter, unpubl. obs.). The sheep developed heartwater, becoming febrile between the 12th and 14th day after infection. Between the sixth and the tenth day after infection, 200 unfed uninfected, laboratory-reared *A. hebraeum* nymphs (Sengwe strain) were placed daily in bags attached to the shaved backs of the sheep and allowed to feed to repletion. This allowed engorgement of the ticks to take place during the febrile reaction, which is the period of maximum infectivity for ticks (Peter et al., 1995).

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Engorged ticks were collected, incubated at 26 C and 75% relative humidity, and allowed to molt to the adult stage after which they were used to infect the wildlife. The ticks from the Plumtree-infected sheep were designated Plumtree I, and the ticks from the two Mbizi-infected sheep were designated Mbizi I and Mbizi II. Two batches of Mbizi strain-infected ticks were prepared to provide sufficient ticks for the transmission trials.

Ticks from the Mbizi I batch were used to infect eland #11 and wildebeest #1, the Mbizi II batch for giraffe #2, and the Plumtree I batch for kudu #JM and JF2. Twenty males were allowed to feed in body bags on the back of each animal for 7 days before being forcibly detached with forceps. Whenever possible, the same *C. ruminantium* strain which was used to infect a species with cultured organisms also was used for infections by ticks.

To determine the *C. ruminantium* infection rate of the ticks used for infecting wild animals, 80 Plumtree I, 120 Mbizi I, and 100 Mbizi II adult ticks were analyzed for infection by PCR (Peter et al., 1995). Additionally to confirm their infectivity, 20 male ticks from each infected batch were fed on susceptible goats. Rectal temperatures of these animals were monitored daily, and brain crush smears were prepared by biopsy on the third day of febrile reaction and after death to confirm heartwater. In the case of the Plumtree I batch of infected ticks, an attempt also was made to isolate *C. ruminantium* in bovine endothelial cell cultures from the plasma of the recipient goat during the febrile reaction (Byrom et al., 1991).

General clinical observations of vitality and appetence were made for 3 to 4 wk after infection of the wild animals. The animals were not monitored for febrile reactions because this would have necessitated frequent anaesthetization. Attempts were made to transmit infection from each wild animal to small ruminants at least once after infection. Both intrastadial and interstadial tick transmissions were attempted. For interstadial transmission, 200 to 400 laboratory-reared unfed, uninfected *A. hebraeum* nymphs were allowed to feed to repletion over 7 to 10 days in bags attached to the backs of the animals. Engorged ticks were collected and allowed to molt as described above. Molted adult ticks were fed on susceptible sheep or goats, which were monitored for the development of signs of heartwater. For intrastadial transmission, 75 to 100 uninfected unfed adult male *A. hebraeum* were allowed to attach and to feed for 7 to 10 days in bags on the backs of each animal, after which they were forcibly detached. Within 3 to 24 hr of detachment, they were transferred to bags on the backs of susceptible sheep or goats and allowed to attach and feed. All small ruminants in transmission trials were monitored daily for clinical signs of heartwater infection. Transmission of heartwater was confirmed in one or more of the following ways. First, a brain biopsy was performed on the third or fourth day of febrile reaction (rectal temperature > 40.5 C). Second, isolation of *C. ruminantium* from plasma of the affected animal onto bovine endothelial cell culture was attempted between the second and fourth day of the febrile reaction (Byrom et al., 1991). Lastly, a necropsy and brain crush smear was performed on any recipient animal that died after tick feeding to determine if death was due to heartwater.

Ticks were fed on the eland for attempts at interstadial and intrastadial tick transmission of *C. ruminantium* at days 15, 128 and 234 post infection. An attempt at interstadial transmission alone was also performed on the eland at day 380 post infection. Attempts at interstadial and intrastadial tick transmission were performed on the wildebeest at days 15, 128 and 247 post infection. Only one interstadial transmission trial was performed on the kudu, at 24 days post infection. No intrastadial transmissions were attempted from the kudu. In the case of the giraffe, interstadial and intrastadial transmission trials were carried out before experimental infection, to determine if the giraffe had been naturally infected by exposure to ticks prior to capture. After experimental infection, interstadial and intrastadial transmission tick feeds on giraffe were performed at days 15 and 85 post infection.

Serum was collected from all wildlife prior to infection and again at 3 to 5 wk post infection. To detect the development of antibodies to *C. ruminantium*, all sera were tested by immunoblot at a 1:100 dilution, using Protein G and a protocol previously described for bovine sera (Mahan et al., 1993).

RESULTS

Clinical heartwater occurred in all small ruminants used as controls to confirm the infectivity of the cell culture inocula and the ticks used for wildlife infections. All sheep that received culture inoculum became febrile 9 to 12 days post infection, were brain smear positive for *C. ruminantium* by biopsy, and died of heartwater by day 15 post infection. The control goats on which the Mbizi I– and Mbizi II–infected ticks fed became febrile between 13 and 16 days after tick placement and suc-

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cumbed to infection by day 20. They were positive for heartwater by brain smears prepared post mortem. The goat infested with the Plumtree I ticks developed a 4 day febrile reaction by day 16 after tick placement and recovered. *Cowdria ruminantium* was isolated in culture from the plasma of this goat on the second day of the febrile reaction.

High *C. ruminantium* infection rates, as determined by PCR analysis, were demonstrated in the Mbizi I, Mbizi II, and Plumtree I tick batches, with infection rates of 93%, 96% and 97%, respectively.

No clinical signs typical of heartwater were observed in any of the infected wild animals during the first 3 to 4 wk after infection. Three eland (#8, #11 and #900) were immuoblot negative prior to infection (Table 1). These three eland seroconverted by day 24 post infection, demonstrating that they had been exposed to *C. ruminantium*. Intrastadial transmission to susceptible goats occurred at day 15 after infection from eland #899 and #900, which were both infected by culture (Table 1). The recipient goats died of heartwater, and infection was confirmed by brain smear and post mortem examination. Interstadial transmission trials were not done at day 15 post infection due to poor engorgement success of nymphs, and the resultant low numbers of molted adult ticks available for transmission. Poor engorgement of nymphs also reduced the numbers of ticks available from each eland for interstadial transmission at day 128 post infection. However, transmission using ticks combined from all four eland was successful at day 128. The recipient goat died of heartwater, and infection was confirmed by post mortem and brain smear examinations. At 380 days post infection, a high yield of engorged nymphs was obtained from eland #8, #11 and #899, but no transmission was demonstrated from these three eland (Table 1). Intrastadial transmissions attempted at days 128 and 234 post infection also failed.

All four wildebeest were seronegative before infection, and each seroconverted by day 24 post infection, demonstrating exposure to *C. ruminantium* (Table 1). Interstadial transmission attempts at all tick feeds were limited by the fact that nymphal attachment and feeding was very poor on the wildebeest, and engorgement success was typically <5%. However, intrastadial tick feeding was more successful as adult males were able to attach and feed effectively on this species. Intrastadial transmission at day 15 post infection was positive from wildebeest #3, which had been infected by culture. Infection was confirmed in the recipient goat by post mortem and brain smear examinations. Further intrastadial transmission attempts from all wildebeest at days 128 and 247 post infection were negative. However, at day 128 post infection, an interstadial transmission using ticks combined from all four wildebeest was successful. The recipient goat died, and *C. ruminantium* infection was demonstrated by post mortem and brain smear examinations.

Both giraffe #1 and #2 were seropositive prior to infection. However, the intrastadial and interstadial transmission attempts from both of the giraffe prior to experimental infection were negative (Table 1). Intrastadial transmissions attempted from both giraffe at days 15 and 85 post infection failed. Interstadial transmission at day 15 post infection was not performed due to loss of the ticks because of an incubator failure. However, interstadial transmission at day 85 post infection was successful from giraffe #2, which was infected by ticks (Table 1). Infection in the recipient goat was confirmed by isolation of *C. ruminantium* from plasma into culture, as well as by post mortem and brain smear examinations.

Four of the five kudu (#AF1, #AF2, #JF1 and #JF2) were seronegative prior to infection. Only one interstadial transmission trial was performed, at day 24 post infection, and transmission was demonstrated from kudu #AF2, infected by culture, and #JF2, infected by ticks (Table 1).

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TABLE 1. Infection of wild African ruminants with *Cowdria ruminantium* and subsequent transmission to domestic ruminants by *Amblyomma hebraeum*.

Animal	Mode of inoculation/ *C. ruminantium* strain	Serology[a]		Transmission attempt (days post infection/number of ticks used/result)				
		Pre[b]	Post[c]					
Eland 11	ticks/Mbizi	−	+	15/21[d]/−	128/26[d]/−	128/44[f,e]/+	234/26[d]/−	380/150[f]/−
Eland 899	culture/Mbizi	+	+	15/38[d]/+	128/55[d]/−		234/51[d]/−	380/150[f]/−
Eland 900	culture/Mbizi	−	+	15/31[d]/+	128/33[d]/−		234/43[d]/−	ND[g]
Eland 8	culture/Mbizi	−	+	15/17[d]/−	128/56[d]/−		234/56[d]/−	380/153[f]/−
Wildebeest 1	ticks/Mbizi	−	+	15/19[d]/−	128/47[d]/−	128/31[f,h]/+	247/13[d]/−	ND
Wildebeest 2	culture/Mbizi	−	+	15/46[d]/−	128/58[d]/−		247/29[d]/−	ND
Wildebeest 3	culture/Mbizi	−	+	15/43[d]/+	128/65[d]/−		247/55[d]/−	ND
Wildebeest 4	culture/Mbizi	−	+	15/31[d]/−	128/28[d]/−		247/23[d]/−	ND
Giraffe 1	culture/Plumtree	+	+	Pre/31[d]/−	Pre/27[f]/−	15/33[d]/−	85/38[d]/−	85/26[f]/−
Giraffe 2	ticks/Mbizi	+	+	Pre/55[d]/−	Pre/42[f]/−	15/29[d]/−	85/43[d]/−	85/60[f]/+
Kudu AF1	culture/Plumtree	−	−	24/37[f]/−	ND	ND	ND	ND
Kudu AF2	culture/Plumtree	−	+	24/64[f]/+	ND	ND	ND	ND
Kudu JM	ticks/Plumtree	+	+	24/52[f]/−	ND	ND	ND	ND
Kudu JF1	culture/Plumtree	−	−	24/123[f]/−	ND	ND	ND	ND
Kudu JF2	ticks/Plumtree	−	+	24/116[f]/+	ND	ND	ND	ND

[a] Presence of antibodies to *C. ruminantium* as determined by immunoblot.
[b] Pre-infection.
[c] Post-infection.
[d] Intrastadial transmission.
[e] Ticks combined from all four eland for transmission.
[f] Interstadial transmission.
[g] No transmission attempted.
[h] Ticks combined from all four wildebeest for transmission.

These were the only kudu that seroconverted during these trials. Intrastadial transmissions were not attempted.

DISCUSSION

Previous studies on heartwater in wildlife have produced few conclusive results. Inadequate knowledge of the status of *C. ruminantium* infection and immunity of animals prior to the initiation of infection trials has prevented conclusions on their susceptibility to clinical disease. In addition, poor characterization of infection inocula and the utilization of unreliable diagnostic tests has often resulted in failure to prove decisively whether animals became infected or are refractory to infection. Most studies also did not determine if infection could be transmitted to and from wild animals by the tick vector and if a long-term carrier state can be established. This is required to assess the importance of wildlife species in the maintenance and transmission of *C. ruminantium* infection. To avoid these problems in this study, animals were obtained from and maintained in heartwater-free areas wherever possible and infections were attempted with defined inocula of known infectivity, with both ticks and quantified culture-derived *C. ruminantium* organisms. Furthermore, infection was confirmed by tick transmission of the disease which is highly specific for heartwater as well as epidemiologically relevant.

The demonstration of *C. ruminantium* infection in eland, wildebeest, giraffe and kudu brings to eight the number of wild ruminant species that have been proven experimentally to be susceptible to this agent. This highlights the significance of these species, and probably of other wildlife, in heartwater epidemiology. Long-term carrier states were demonstrated in eland and wildebeest (at least 4 mo) and while infection in kudu was only demonstrated at day 24 post infection it is possible that this species can also develop a long-term carrier state. Transmission of *C. ruminantium* from giraffe was demonstrated at nearly 3 mo after experimental infection, though, because these animals were not kept isolated from natural *Amblyomma* spp. tick infestation during the study, the possibility of natural *C. ruminantium* infection during this period cannot be ruled out. Persistent infections have been demonstrated previously in African buffalo by tick transmission at day 161 post infection (Andrew and Norval, 1989), and in blesbuck (Neitz, 1937) and black wildebeest (Neitz, 1935) by blood transmission at days 62 and 30 post infection, respectively. In the present study, failure to transmit *C. ruminantium* from the eland and wildebeest at days 247 and 380, respectively, after infection may be an artefact of the small numbers of animals or ticks used in these trials. Alternately, it may suggest either eventual clearance of the organism or rickettsemia levels below the thresholds required for transmission. In heartwater-endemic areas, however, repeated exposure to *C. ruminantium* from natural infections may result in prolongation of the carrier state and higher levels of infectiousness for ticks.

Serological analysis of the wildlife prior to infection supported the *C. ruminantium*-naive status of these animals, with the exception of the giraffe, and one eland and kudu. The serological reactions of the eland and kudu, however, were probably false positive due to exposure to cross-reactive agents before the start of the study, because these animals originated from heartwater-free areas, and the immunoblot assay has been shown to detect antibodies to *Ehrlichia* spp. and other unknown agents common in heartwater-free areas (Du Plessis et al., 1993; Jongejan et al., 1993; Mahan et al., 1993). Because the giraffe originated from a heartwater-endemic area, their serological reactions may have been truly positive due to prior infection with *C. ruminantium*. Furthermore, while the pre-infection tick transmissions from these animals were negative, it cannot be concluded that the giraffe were uninfected prior to experimental infection

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because low numbers of ticks were available for these transmissions. Serological analysis of the wildlife post infection suggested that infection was established in at least three of the eland and in all of the wildebeest, despite failure to transmit infection from some of these animals. These failures may have been due to the low numbers of ticks available for transmission. Based on serology, infection failed to establish in two kudu inoculated with culture, suggesting either individual resistance or failure of intravenous inoculation.

Although the animals were not closely monitored for clinical signs of *C. ruminantium* infection, none of the infected animals died or developed obvious signs of heartwater, despite having received doses of *C. ruminantium* organisms, from culture or via ticks, that were lethal for the control sheep and goats. All animals except for the giraffe had been born and kept in heartwater-free areas, suggesting an innate resistance to clinical heartwater. Previous experimental infections of naive African ruminants (blesbuck, black wildebeest and buffalo) are also reported to have been sub-clinical (Neitz, 1935, 1937; Andrew and Norval, 1989), and it is probable that African ruminants have evolved a degree of resistance to *C. ruminantium* infection. Ruminants evolving outside the distribution of heartwater may have higher susceptibility to clinical disease, as supported by fatal experimental infections in the exotic white-tailed deer (Dardiri et al., 1987) and suspected heartwater deaths observed in Indian spotted deer, Java deer and water buffalo (Mohan, 1968; Poudelet et al., 1982; Oyejide and Olaleye, 1984). The evidence for this distinction is, however, not entirely consistent as suspected heartwater deaths have also been reported in species originating from heartwater-endemic areas, such as eland, black wildebeest, steenbok, sitatunga, and kafue lechwe (Young and Basson, 1973; Okoh et al., 1986; du Plessis, *in* Oberem and Bezuidenhout, 1987; Pande et al., 1992; Jackson and Andrew, 1994). Variations by individual in host susceptibility or in *C. ruminantium* strain virulence, therefore, may play a more significant role.

Differences exist in the importance of the four species in this study as hosts for *Amblyomma* spp. ticks, and therefore in their significance as reservoirs of *C. ruminantium* infection (Horak et al., 1983; Norval, 1983; Horak et al., 1987). Giraffe and eland frequently carry high burdens of *A. hebraeum* of all developmental stages, possibly due to their larger size. Wildebeest, however, carry few *Amblyomma* spp. ticks, while infestations on kudu are intermediate. These host differences are likely to affect the relative contribution of each species to *C. ruminantium* infection in *Amblyomma* spp. populations, as will the relative densities of these species. While limited information on the precise density and distribution of large ungulate species in Africa is available, the four species in this study are common in many heartwater-endemic regions, both within wildlife preserves and increasingly in association with domestic ruminants (Norval et al., 1994) and are thus all likely to play significant roles in heartwater epidemiology.

Cowdria ruminantium may be capable of infecting a wide range of ruminant and, possibly, non-ruminant species. This agent has been shown to infect cultured endothelial cells of buffalo, bushpig (*Potamochoerus porcus*), eland, giraffe, kudu, sable antelope (*Hippotragus niger*) (Smith et al., 1998), and human beings (Totte et al., 1993; Smith et al., 1998). However, while the organism may be relatively indiscriminate in cell culture, host-specific resistance factors operating beyond the level of cellular infection are likely to prevent establishment of infection in non-susceptible species. Nevertheless, a broad host range for *C. ruminantium* is apparent and may promote widespread transmission, which is likely to be an important factor in the establishment of endemic stability for heartwater in domestic ruminants.

Tick control on wildlife is not easily

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achieved; thus a reservoir of *C. ruminantium* infection in wildlife may make heartwater difficult to control in domestic stock, and perhaps impossible to eradicate. Because infection with *C. ruminantium* may be common in African ruminants, there is substantial risk of introducing the disease into uninfected areas through the movement of subclinical carriers. Wild ruminants in Africa, often not restricted by farm boundaries, may be responsible for local dissemination of vectors and infection. Long-distance translocation of infected wild species to heartwater-free regions, such as the north and south American mainlands, pose a risk of outbreaks of disease because these regions have large populations of both susceptible domestic and wild ruminants (e.g., white-tailed deer) and *Amblyomma* species with demonstrated vectorial capacity, i.e., *A. cajennense,* and *A. maculatum* (Uilenberg, 1982; Barre et al., 1987). A definitive demonstration of the risk of long-distance translocation would be provided by infecting wildlife with *A. hebraeum* or *A. variegatum* and testing for acquisition of infection by *A. variegatum* and the American *Amblyomma* tick species.

Further assessment of the significance of different wildlife hosts in heartwater epidemiology is needed and should focus on: first, identifying other susceptible species; second, quantifying the duration and magnitude of their infectivity for vectors; third, determining their relevance as hosts for the vector population; and finally, determining wildlife densities and the level of their interaction with domestic livestock. Such studies will allow improved assessment of the role of wildlife in *C. ruminantium* transmission and the development of sound regulations for animal translocation.

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