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APPLICATION OF PCR IN HEARTWATER EPIDEMIOLOGY

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Les tests basés sur la biologie moléculaire en vue de détecter et d'identifier les agents pathogènes sont de plus en plus utilisés en épidémiologie vétérinaire. Cet article décrit l'utilisation de la technique PCR pour diagnostiquer l'infection à Cowdria ruminantium afin de répondre à des questions essentielles de l'épidémiologie de cette maladie. Ces questions comprennent la détermination : (i) des taux d'infection des vecteurs, (ii) la vitesse de diminution du pouvoir infectieux des animaux pour les tiques entre la phase des signes cliniques et le portage sain, et (iii) la comparaison des niveaux d'infection se développant dans la nymphe et chez l'adulte vecteur, pour leur importance relative dans la transmission de C. ruminantium. Ces paramètres sont des facteurs importants régulant l'efficacité et la fréquence de transmission de C. ruminantium, et peuvent être utilisés pour le développement d'un modèle de transmission dynamique de cette maladie.
Leurs niveaux permettent de déterminer la stabilité de l'endémie, et sont critiques pour l'évaluation de la vaccination en tant que stratégie de contrôle de la maladie.

INTRODUCTION

Heartwater is a severe tick-borne, non-contagious disease of ruminant livestock caused by the rickettsia *Cowdria ruminantium*, which is endemic in much of sub-Saharan Africa and in the Caribbean . Transmission occurs via ticks of the *Amblyomma* genus, primarily *A. variegatum* and *A. hebraeum*. Intense research efforts are presently directed towards developing new vaccines for heartwater which will provide safe, effective and less expensive control options to acaricide treatment and the blood-based vaccine currently in use (Mahan et al., 1995; Martinez et al., 1993). Essential to the successful implementation of these vaccines is a thorough understanding of heartwater epidemiology, in particular disease transmission dynamics in host and vector populations. Past studies on heartwater epidemiology have been limited by the difficulty in detecting *C. ruminantium* infection or exposure. The few antigen detection tests described, brain biopsy, ELISA, and xenodiagnosis with blood or ticks, cannot reliably detect infection past the clinical stage of disease, and are too cumbersome and lengthy for large scale use in field and experimental epidemiologic studies. Similarly, heartwater serological tests currently available lack the sensitivity and specificity required to be useful epidemiologic tools. The recent development of a polymerase chain reaction (PCR) *C. ruminantium* detection assay of high sensitivity and specificity has permitted more rigorous analysis of infection dynamics (Mahan et al., 1992; Peter et al., 1995). The PCR assay can be applied quickly and inexpensively to large numbers of samples. However, while PCR performs well on infected ticks, it is not yet sensitive enough for single test detection of chronically infected ruminants, due to the very low levels of circulating rickettsemia in such animals. Nevertheless, analysis of tick infections has allowed reasonably detailed investigation of transmission dynamics through the quantification of key variables such as field tick infection rate and the relative importance of adult and nymphal tick transmission. In addition, the importance of hosts as sources of infection at different stages of infection can be studied indirectly by analysis of infections established in ticks that feed on them. These analyses can provide valuable estimates of parameters required for mathematical models of heartwater transmission dynamics that evaluate the impact of current and alternate disease control strategies (O'Callagahan et al., 1997).

FIELD TICK INFECTION RATES

Previous studies on *C. ruminantium* infection in natural *Amblyomma* populations have yielded a wide range of prevalence estimates. The prevalence of infection in adult *A. variegatum* has been estimated at 0.2-4% in the Caribbean (Camus and Barre, 1992) and 1.1-1.2% in Senegal (Gueye et al., 1993a,b). In heartwater endemic regions of South Africa and Zimbabwe, estimates of infection prevalence in *A. hebraeum* range between 1.6-45% in adults and 0-14% in nymphs (Du Plessis and Malan, 1987; Norval et al., 1990). These studies utilized xenodiagnosis by tick feeding or by inoculation of homogenised tick material into susceptible mice, or small ruminants. However, as the reliability of these tests have never been evaluated and small sample sizes were used, the accuracy of these estimates is questionable. To apply the PCR assay to determination of vector infection rates, collections were made using sentinel cattle of wild *A. hebraeum* adults and nymphs on a beef ranch in a heartwater-endemic region of the southern lowveld of Zimbabwe. DNA was extracted from the ticks individually and tested for the presence of *C. ruminantium* by PCR. This analysis yielded average infection rates of 11.48% in adult ticks and 3.16% in nymphal ticks (Table I). These results confirm the existence of relatively high *A. hebraeum* infection rates in heartwater-endemic areas and, in view of the frequently high tick burdens frequently found in such areas, demonstrate the potential ease of establishment of endemic stability for this disease.

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Table I
Infection rates by PCR of *A. hebraeum* collected in a heartwater-endemic region of Zimbabwe

Instar	Number analysed	Percentage positive
Adult males	200	10.5
Adult females	241	12.45
Nymphs	95	3.16

ADULT VS NYMPHAL TRANSMISSION

It has long been assumed that adult ticks are the primarily responsible for transmission of *C. ruminantium*, and that nymphs play a relatively minor role in disease transmission. This was borne out of the belief that infection rates in nymphs are lower than in adults and the observation that adults are more numerous on livestock. However, nymphs may not be less numerous than adults. Adult male *Amblyomma* remain attached to their hosts for extended periods of time (Norval et al., 1991), during which time they feed intermittently and mate repeatedly. The result is an accumulation of males on hosts over time, which gives the impression of higher attachment rates for this instar. Nymphs on the other hand complete feeding within 6-9 days and thus by having a higher turnover may actually have higher attachment rates. Investigations are currently in progress to examine this aspect. Studies on ticks collected in heartwater-endemic areas of Zimbabwe demonstrate that nymphs have lower infection rates (Norval et al., 1990). While this observation has been confirmed by recent PCR analysis of nymphs from an endemic region of Zimbabwe (see above), the infection rate observed is still relatively high, approximately one third of the adult infection rate, and might be compensated for by high nymphal attachment rates. The significance of nymphal transmission lies in the potential for milder disease, due to the lower levels of rickettsia that they may carry compared to adults ticks. Non-fatal disease transmission, if common, would be one mechanism promoting the establishment and maintenance of endemic stability for heartwater.

TICK INFECTIVITY DECAY

PCR analysis of *C. ruminantium* infection in *Amblyomma* ticks fed as nymphs on animals during the acute, febrile phase of heartwater has demonstrated that 80-100% of the resultant moulted adults become infected (Peter et al., 1995). Conversely, ticks fed on chronically infected animals, several months after recovery from the clinical phase, develop infection rates ranging from 10% to less than 1%. The duration and rate of decay of the highly infective stage of hosts is important in heartwater transmission dynamics as it helps to determine vector infection rate. To measure this, the PCR assay was used to determine the prevalence of infection in batches of ticks fed on groups of experimentally infected cattle at intervals during the clinical phase of heartwater and after recovery. The preliminary results show that the prevalence of infection in ticks by PCR drops from approximately 80% at 3 weeks after infection to 64% at 5 weeks and then to 43% by 9 weeks. While, in the absence of re-infection the infectivity of these animals is likely to eventually fall to low levels, it is clear that cattle remain higly infective for ticks for at least 4-6 weeks after recovery. Further analyses will be done to confirm this pattern and to determine the rate of infectivity decay after 9 weeks.

CONCLUSION

The studies presented here demonstrate that PCR is a powerful molecular tool for tracing the transmission of disease agents in host and vector populations. It's application in heartwater epidemiology permits the refining of key parameters in heartwater transmission dynamics. This is integral to the process of constructing accurate mathematical models of disease transmission whose outputs can be reliably used to evaluate the efficacy and impact of current and alternate control strategies, such as vaccination, for heartwater.

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