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Ventilation in the adults of *Amblyomma hebraeum* and *A. marmoreum* (Acarina, Ixodidae), vectors of heartwater in Southern Africa

FIELDEN (L.J.), DUNCAN (F.D.), LIGHTON (J.R.B.), RECHAV (Y.). Ventilation chez les adultes d'*Amblyomma hebraeum* et *A. marmoreum* (Acarina, Ixodidae), vecteurs de la cowdriose en Afrique australe. *Revue Élev. Méd. vét. pays trop.*, 1993, **46** (1-2) : 335-338

Cette étude avait pour but de déterminer les caractéristiques principales de l'échange des gaz respiratoires chez les adultes à jeun des tiques *Amblyomma hebraeum* et *A.marmoreum*, vecteurs de la cowdriose en Afrique australe. L'émission de dioxyde de carbone a été mesurée à 25 °C par respirométrie afin de déterminer le taux de métabolisme standard (TMS) et l'évolution dans le temps de l'émission gazeuse. Le TMS était extrêmement bas pour les deux espèces et environ 100 fois moindre que celui prévisible pour un insecte d'une masse corporelle équivalente. La ventilation chez des tiques inactives était discontinue et caractérisée par des éruptions périodiques de CO_2 lors de l'ouverture des spiracles. L'avantage sélectif principal de ce type de ventilation serait une réduction de la perte d'eau respiratoire. La périodicité des émissions de CO_2 était moins fréquente chez *A.marmoreum* (toutes les 2,5 h) que chez *A.hebraeum* (toutes les 1,5 h), ce qui indiquerait que *A.marmoreum* est plus efficace pour limiter la perte d'eau respiratoire. Il est suggéré que des recherches futures sur la physiologie de l'équilibre d'eau de tiques devraient se pencher sur le rôle des profils de ventilation pour déterminer la survie des stades non-parasitaires et les associations des tiques avec leurs habitats.

Mots clés : Cowdriose - Tique - *Amblyomma hebraeum* - *Amblyomma marmoreum* - Échange gazeux - Métabolisme hydrique.

INTRODUCTION

The ability of ticks to survive for extended periods whilst of the host has important implications for the transmission of diseases. Long off-host survival not only increases the chances of finding a suitable host but also may result in ticks being the long-term reservoirs of disease in nature rather than the hosts they feed upon (6).

The long survival of ticks between blood meals is largely due to stringent use of energy and water. The mechanisms that ticks employ for water maintenance are diverse and include an integument which can dramatically restrict transpirational water loss as well as the ability to actively absorb water vapour from unsaturated air (2).

Control of spiracular opening is also important in restricting water loss. Ticks possess only one pair of spiracles which in unfed adult ticks have been shown to open on an intermittent basis (2). These findings provide strong evidence for the occurrence of discontinuous ventilation in ticks (*i.e.* ventilation involving periodic bursts or emissions of CO_2 and intermittent uptake of O_2).

Discontinuous ventilation has been found to be wide spread in insects and its chief selective advantage is believed to lie in a reduction of respiratory water loss as a result of the loss of water vapour being restricted to discrete cyclic events (5). In ticks, however, the existence of discontinuous ventilation awaits experimental confirmation.

In this paper we confirm the existence of discontinuous ventilation in two species of ticks by direct measurement of respiratory gas exchange ; we compare the ventilatory patterns observed to those typical of insects and finally we discuss the significance of discontinuous ventilation with respect to the ability of ticks to survive for extended periods whilst off the host.

We investigated the dynamics of respiratory gas exchange in the unfed adult stages of the South African bont tick *Amblyomma hebraeum* Koch and the South African tortoise tick *Amblyomma marmoreum*. In Southern Africa, *A. hebraeum* is the main vector of the disease heartwater. *Amblyomma marmoreum* is also capable of transmitting the rickettsia but the significance of this species in the epidemiology of the disease heartwater remains unclear (9).

MATERIALS AND METHODS

Adult ticks (six-week-old) used in the experiments were from colonies maintained in the laboratory (25 °C, 85 % RH, ambient photoperiod).

Measurements of CO_2 emission were made at 25 °C using a flow through respirometry system described elsewhere (3). In brief, the system employed a Licor CO_2 analyser, respirometers of 3 ml volume, a computerized data acquisition system and flow rates of 50-100 ml. min^{-1} STPD. The incurrent air stream was scrubbed of H_2O and CO_2 by a Drierite/Ascarite column. Unfed ticks were

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monitored for 8-16 h at a temporal resolution of 5-10 s. Respiratory quotients (RQ) were determined by a closed system technique (4).

RESULTS

Respiratory gas exchange in unfed adults of both species (fig. 1) was characterized by elevated and erratic CO_2 emission rates during activity and discontinuous ventilation during inactivity. Discontinuous ventilation consisted of short but clearly defined bursts of CO_2 emission (B phase) followed by much longer interburst phases where CO_2 emission was marginally above base line levels. Characteristics of discontinuous ventilation in *A. hebraeum* flat adults are summarized in table I and compared to those in *A. marmoreum*. For both species rates of burst CO_2 emission (burst VCO_2) and burst length were similar. Rate of interburst CO_2 emission in *A. marmoreum* accounted for approximately 11 % of total CO_2 emission during each discontinuous ventilation cycle (DVC). In *A. hebraeum*, CO_2 emission during the interburst period was seldom distinguishable from base line levels and hence was not measured. The major difference in ventilatory characteristics between the two species was the duration of the DVC, approximately 2.5 h in *A. marmoreum* compared to 1.5 h in *A. hebraeum*.

Standard VCO_2 ($sVCO_2$) of unfed ticks was calculated from the mean VCO_2 of the animal whilst exhibiting regular discontinuous ventilation. Measurements of $sVCO_2$ were converted to sVO_2 after determination of respiratory quotients (RQ's). Unfed adult *A. hebraeum* had a considerably lower SMR than *A. marmoreum* (table I).

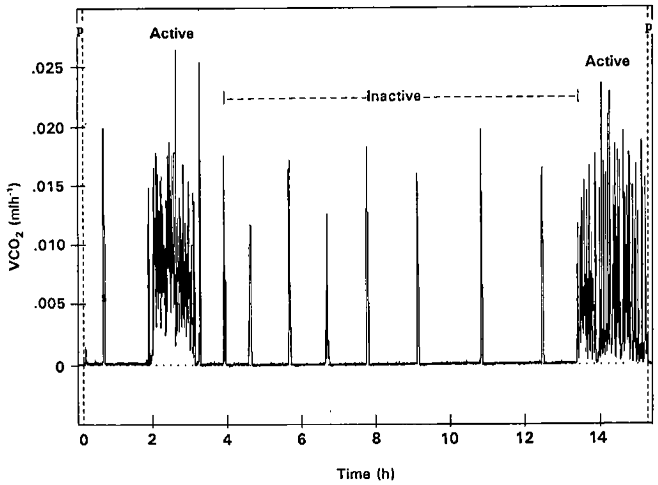

Figure 1 : CO_2 emission recording for male A. hebraeum *showing erratic and discontinuous ventilatory patterns. Baseline recording delineated by P.*

TABLE I Characteristics of the discontinuous ventilatory cycle in the unfed adults of two species of ticks.

Species	*A. hebraeum*	*A. marmoreum*[1]
Mass (mg)	31.3 ± 9.9	70.2 ± 1.9
N	12 (6 M, 6 F)	5 (F)
VCO_2 (µl h^{-1})	0.416 ± 0.269	1.334 ± 0.228
VCO_2 (µl mg^{-1} h^{-1})	0.0136 ± 0.0085	0.0190 ± 0.0033
Burst VCO_2 (µl mg^{-1})	0.0262 ± 0.0104	0.0502 ± 0.0015
Burst VCO_2 (µl mg^{-1} h^{-1})	0.3288 ± 0.1701	0.419 ± 0.107
Interburst VCO_2 (µl mg^{-1})	**	0.0055 ± 0.0017
Burst length (min)	5.16 ± 1.03	6.78 ± 1.06
Burst frequency (h^{-1})	0.74 ± 0.26	0.49 ± 0.26
RQ	0.82	0.83
VO_2 (µl mg^{-1} h^{-1})	0.0158 ± 0.0097	0.0230 ± 0.0040

[1] *Values taken from Table 3 (In 4).*
** *CO_2 emission undistinguishable from base line levels.*

DISCUSSION

In brief, the ventilatory cycle in insects consists of three stages : A closed phase (C phase) in which the spiracles are closed thus preventing any gas exchange or respiratory water loss. This stage is followed by a flutter phase (F phase) during which slight opening of the spiracles, on an intermittent basis, allows a slow ingress of O_2 but little egress of CO_2 or water vapour. Finally, in the open or burst phase (B phase), the accumulation of CO_2 from respiring tissues triggers some or all of the spiracles to open widely resulting in the rapid release of CO_2 and water vapour to the outside (5).

A continuous very low inter-burst emission of CO_2 does occur in ticks, similar to that described in insects during the constricted-spiracle (closed phase). However, unlike in insects there is no clearly defined increase in VCO_2 corresponding to the F phase occurring prior to the burst phase. This suggests that if an F phase exists in ticks, it must be characterized by extremely low rates of gaseous exchange because it cannot be clearly distinguished via diffusive loss of CO_2 through the spiracles.

It has recently been suggested (4) that ticks probably do not exhibit a fluttering-spiracle (F) phase of the kind observed in insects but instead may rely primarily on diffusive uptake of O_2 through the cuticle between burst phases. This diffusive uptake of O_2 through the cuticle appears sufficient for non-active respiration for prolonged periods, although regular release of CO_2 is still necessary. If this is the case then the standard metabolic rate of ticks can be expected to be very low compared to that of insects which are not thought to make significant use of cuticular O_2 ingress.

The allometric equation describing the relationship between insect body mass and standard metabolic rate is given below :

$$MR = 11.3M^{0.67} \ (10)$$

where MR is metabolic rate (jh^{-1}), and M is body mass (g).

The metabolic rates of the unfed stages of ticks are much lower than that predicted on the basis of body mass from data on insects. Metabolic rates of *A. hebraeum* and *A. marmoreum* are only 0.8 and 1.4 % (2.3 %) respectively of that predicted for insects of an equivalent body mass. Considering that metabolic rates of the unfed stages of ticks are much lower than that predicted for insects of a similar size, the role of transcuticular diffusion may indeed play an important role in ixodid tick gas exchange. It should also be noted that unfed adult ixodids, due to dorso-ventral flattening, must have a large surface area to volume ratio compared to most insects of similar body size. Although this feature makes ticks more prone to desiccation, it would presumably increase their capacity for diffusive gas exchange across the integument. However, we still lack the required information on actual tick surface areas and cuticular O_2 permeabilities to test the hypothesis that transcuticular diffusion may play an important role in ixodid respiratory gas exchange.

There is little doubt that the discontinuous ventilation in ticks is important in reducing respiratory water loss during the period between moulting and feeding. Previous workers have demonstrated dramatic increases in water loss in various species of ticks in which the spiracles have been forced to remain open by exposure to high CO_2 concentrations (1,2).

Since respiratory water loss is minimal during spiracle closure (interburst phase), but high when the spiracles are open (burst phase), one can postulate that burst frequencies in any particular species of tick may give some indication as to its ability to reduce respiratory water loss. During regular discontinuous ventilation cycles, the burst frequency of *A. hebraeum* was on average once every 1.5 h which is much higher than the burst frequencies of once every 2.5 h found for *A. marmoreum*. Such low rates of spiracular opening in *A. marmoreum* would suggest that this species can more effectively reduce respiratory water loss than *A. hebraeum*.

CONCLUSION

Previous reviews of the physiological ecology of ticks have emphasized that survival between blood meals is a function of maintaining a balance between energy and water use with the maintenance of water homeostasis being considered the most critical survical factor (7). Furthermore it has been suggested that integumental permeability to water flux may be of greater value to maintenance of water balance of ixodid ticks than the critical equilibrium activity (2). In the light of our findings, we suggest that ventilatory patterns in ticks and the role they play in reducing respiratory water loss should be given more attention when trying to interpret survival potential and habitat associations of ixodid ticks. The two species under consideration in this study, *A. hebraeum* and *A. marmoreum*, are largely sympatric in their distribution in Southern Africa although A. marmoreum is found in drier areas than *A. hebraeum* (8). The apparent superior ability of *A. marmoreum* to reduce respiratory water loss may be of significance in explaining its tolerance to drier habitats than *A. hebraeum*.

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The objective of this study was to establish the major features of respiratory gas exchange in unfed adults of the ticks *Amblyomma hebraeum* and *A. marmoreum*, both vectors of heartwater in Southern Africa. Carbon dioxide emission of ticks was measured at 25 °C using flow-through respirometry in order to determine standard metabolic rate (SMR) and the temporal pattern of gaseous emission. For both species, SMR was extremely low and approximately 100 fold less than that predicted for an insect of equivalent body mass. Ventilation in inactive ticks was discontinuous and characterized by periodic bursts of CO_2 emissions during spiracular opening. The main selective advantage of this type of ventilation is believed to lie in a reduction of respiratory water loss. The periodicity of CO_2 bursts was less frequent in *A. marmoreum* (every 2.5 h) compared to *A. hebraeum* (every 1.5 h) suggesting that *A. marmoreum* is more efficient at conserving respiratory water loss. It is suggested that future research into water balance physiology of ticks should address the role of ventilatory patterns in determining off-host survival and habitat associations.

Key words : Heartwater - Tick - *Amblyomma hebraeum* - *Amblyomma marmoreum* - Gas exchange - Water metabolism.

FIELDEN (L.J.), DUNCAN (F.D.), LIGHTON (J.R.B.), RECHAV (Y.). Ventilación en los adultos de *Amblyomma hebraeum* y *A.marmoreum* (Acarina, Ixodidae), vectores de la cowdriosis en Africa del sur. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 335-338

El objetivo del presente estudio fue el establecimiento de los principales patrones respiratorios de intercambio gaseoso en adultos no alimentados de garrapatas de *A.mblyomma hebraeum* y *A. marmoreum*, ambos vectores de la cowdriosis en Africa del sur. La emisión de dióxido de carbono por parte de las garrapatas se midió a 25 °C, haciendo pasar el flujo por un respirómetro, con el fin de determinar la tasa metabólica estandard (SMR) y el patrón temporal de emisión gaseosa. En ambas especies la SMR fue extremadamente baja, aproximadamente 100 veces menor que la esperada en un insecto de masa corporal equivalente. La ventilación en las garrapatas inactivas fue discontinua y se caracterizó por picos periódicos de emisión de CO_2 durante la apertura estigmática. Se cree que la principal ventaja selectiva de este tipo de ventilación reside en una reducción de la pérdida de agua mediante la respiración. La periodicidad de los picos de CO_2 fue menos frecuente en *A. marmoreum* (cada 2,5) que en *A. hebraeum* (cada 1,5), lo que sugiere una mayor eficiencia de la primera en la conservación respiratoria de agua. Se recomienda que las investigaciones futuras sobre el balance fisiológico del agua en las garrapatas se orienten hacia el posible papel de los patrones de ventilación en la determinación de la supervivencia fuera del huesped y de las asociaciones con el medio ambiente.

Palabras claves : Cowdriosis - Garrapata - *Amblyomma hebraeum* - *Amblyomma marmoreum* - Intercambio de gases - Metabolisma del aguá.