

The systemic effect of adult and immature *Amblyomma variegatum* ticks on the pathogenesis of dermatophilosis

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LLOYD (C.M.), WALKER (A.R.). L'effet systémique des adultes et des nymphes d'*Amblyomma variegatum* sur la pathogénie de la dermatophilose. *Revue Élev. Méd. vét. Pays trop.*, 1993, 46 (1-2) : 313-316

L'effet systémique des adultes et nymphes d'*Amblyomma variegatum* sur la pathogénie d'infections expérimentales avec *Dermatophilus congolensis* a été étudié. Trois groupes de 4 moutons ont été utilisés, tous infectés avec des doses titrées identiques de *D. congolensis*. Un des groupes a été infesté simultanément par des adultes d'*A. variegatum*, le deuxième groupe par des nymphes, et le troisième comprenait les témoins non-infestés. L'évaluation des infections indique que l'effet systémique de la tique est limité aux adultes. Des tests cutanés et sérologiques utilisant d'autres antigènes ont montré une réduction significative des réponses immunitaires cellulaires et humorales uniquement chez les moutons infestés par les tiques adultes.

Mots clés : Ovin - Dermatophilose - *Dermatophilus congolensis* - Tique - *Amblyomma variegatum* - Maladie systémique - Test ELISA - Test cutané.

INTRODUCTION

Amblyomma variegatum ticks have long been associated with chronic dermatophilosis lesions on cattle in the wet tropics (4). Until recently the association of these ticks chronic with dermatophilosis lesions has been based on field observations alone. The present report compares the effect of adult and nymphal ticks on the severity and duration of dermatophilosis on sheep. Adult ticks have been implicated as an important factor in the formation of chronic dermatophilosis lesions (5, 6). However other evidence suggests that the inflammatory and hypersensitive reactions to immature ticks and other haematophagous arthropods may predispose to dermatophilosis (1,3).

MATERIALS AND METHODS

Experimental animals

Three groups of four sheep were used with equal numbers of Blackface x Suffolk and Blackface in each group. All the sheep were kept in constant conditions throughout the experiment, 18-20 °C with 12 h light/12 dark.

Tick infestations

The ticks used in the experiment were adult or nymphal *A. variegatum* from an uninfected laboratory colony, held at 16 °C with 14 h light/10 dark at 85 % relative humidity. One group of sheep (group A) were infested with 20 adult ticks each, the second group (group B) were infested with 600 nymphal ticks and the third group (group C) were not exposed to any ticks. Equivalent numbers of adult and nymphal ticks were calculated by counting the number and size of salivary acini found in salivary glands of both life-stages.

All of the infestations were applied to cloth bags glued to the wool on the shoulders of the sheep. The wool was shaved from inside the bags and the whole area cleaned with alcohol and ether. The adult infestations consisted of 10 males and 10 females with the males being applied 7 to 10 days before the females. The nymphal infestations were applied in three batches of 200, at weekly intervals with the final batch being applied 7 days after the adult females.

Dermatophilus congolensis infections

One day after the final batch of nymphs were applied all of the sheep were experimentally infected with *D. congolensis*. The *D. congolensis* was taken from a large batch of stabulate previously cultured and frozen at -20 °C at a concentration of 1.2×10^7 cocci/ μ l. This stabulate was diluted to the required concentration of 1×10^7 cocci/ μ l in Hank's balanced salt solution with pig gelatin at 0.5 % w/v.

All 12 of the sheep were infected with identical titrated doses of *D. congolensis* consisting of 100 μ l doses of seven ten-fold dilutions starting at a concentration of 1×10^7 cocci/ μ l applied to seven areas, 2 x 4 cm on the left flank of each sheep.

Before the application of the *D. congolensis* the wool was removed from the application sites and the whole area was cleaned using alcohol and ether. Each of the infection sites were marked using an indelible pen and the skin fold of each area was recorded. The *D. congolensis* was then applied, without scarification, using a bent pipette tip.

The resulting infections were assessed using a ranking system of 0 to 4 for : skin fold thickness ; percentage of each area showing signs of infection ; the severity of the

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scab, ranging from erythema to thick layers of dead, flaking epidermis; extent of exposed dermis at the infection sites. The progression of the dermatophilosis was monitored every 3 to 4 days for 4 weeks and then weekly for another fortnight.

After an interval of several weeks the entire procedure of tick infestations and *D. congolensis* infections were repeated using the same sheep. In previous experiments differences in the infections produced on the test and control sheep were more pronounced at the second infection.

Skin testing

All 12 of the sheep were inoculated with chicken egg ovalbumin, a T lymphocyte activator. Two B lymphocyte activators were used with six of the sheep being inoculated with a polyamino acid, poly-D-glutamate-D-lysine and the remaining sheep being inoculated with freeze-dried *Brucella abortus* (Central Veterinary Laboratory, Weybridge).

Initial sensitising doses of 2.5 mg of each antigen were injected intramuscularly in an equal mixture of one T and B lymphocyte activator suspended anhydrously in Freund's incomplete adjuvant approximately one week prior to first *D. congolensis* infection. A booster dose of 1.25 mg of each antigen was injected in the same way one week before the start of the second infection.

The challenge injections of the individual antigens in phosphate buffered saline (PBS) were applied intradermally in five titrated doses to sites previously shaved and cleaned on the rump of each sheep. The ovalbumin and *B. abortus* were applied in five 100 µl doses of five-fold dilutions starting at a concentration of 2.5 mg/100 µl. The polyamino acid was also applied in 100 µl doses of five-fold dilutions, but the starting concentration was 1 mg/100 µl due to excessive viscosity of higher concentrations.

The skin test reactions were assessed 24 and 48 h after challenge by measuring the skin fold thickness and average diameter of the reactions at each of the five sites. The results were analysed using the median values obtained for the reactions at each of the challenge sites.

Serological tests

Enzyme linked immunosorbent assay (ELISA) was used to measure the humoral antibody response of the sheep to the T and B lymphocyte activating antigens. Aseptic serum was collected from all 12 sheep prior to the start of the experiment and at weekly or fortnightly intervals throughout the entire procedure. For each serum sample collected, duplicate series of five doubling dilutions, starting at 1:1,000 and 1:2,000 were tested for antibodies to ovalbumin and *B. abortus*, respectively.

The tests were carried out using 96 well ELISA plates (Immulon 1, Dynatech Laboratories). Each well was coated with 0.25 µg and 0.051 µg of ovalbumin or *B. abortus* respectively. RASH/IgG(H+L)/PO anti-ovine conjugate (Nordic Immunology-Immunoconjugate) and 3,3', 5,5'-tetramethyl-benzidine dihydrochloride in phosphate citrate buffer were used to complete the ELISA. To stop the substrate reaction 50 µl of 2M H₂SO₄ was added to each well, the optical density of each well at 450 nm was then recorded using an optical density scanner (Titertek Multiskan, Labsystems).

RESULTS

D. congolensis infections

Kruskal-Wallis test was used to compare the ranked scores of the dermatophilosis on the individual sheep in the three groups at each of the assessment days. During the first infection a significant difference between the groups was recorded only on day 27 with $P < 0.05$. The median ranked scores of the three groups on this day were 13.5 for the group infested with adult ticks and 1.5 for the sheep infested with nymphs or not exposed to ticks.

Using the Kruskal-Wallis test on the scores obtained on individual assessment days during the second infection demonstrated a very significant difference ($P < 0.01$) developing between the severity of the dermatophilosis lesions at day 27 which was maintained up to day 41 when the experiment was terminated (fig. 1).

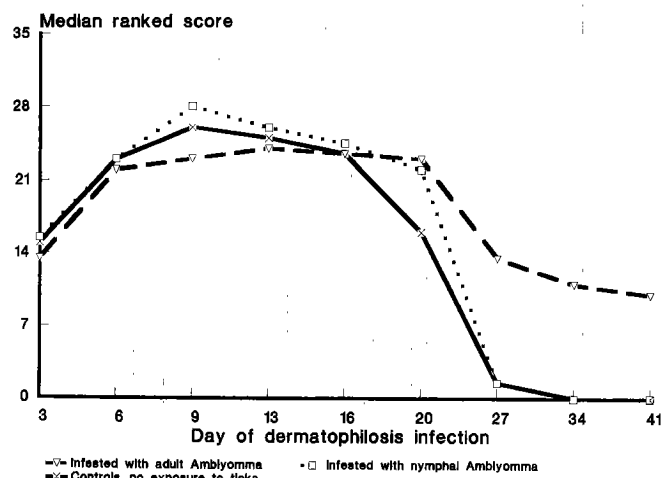


Figure 1 : Changes in the median ranked scores of secondary dermatophilosis lesions on sheep. Comparison of the effect of simultaneous infestations of adult or nymphal *A. variegatum*.

Skin test

Using Friedman's test on the median reactions to ovalbumin and *B. abortus* at the five individual skin test sites a very significant difference, $P < 0.01$ and $P = 0.01$ respectively, was observed between the reactions of the three groups of sheep to the antigens. Table I shows the median reactions of the three groups to both antigens.

TABLE I Skin test reactions of sheep in response to ovalbumin and *B. abortus*.

Ovalbumin

Median reactions (Skin fold x average diameter mm)			
Amount of antigen ($\mu\text{g}/100\mu\text{l}$)	Infested with adults	Infested with nymphs	Not tick infested.
2500	102.8	369.92	246.43
500	74.64	224.57	168.74
100	25.95	91.52	64.65
20	6.6	8.2	7.65
4	4.75	6.4	5.5

B. Abortus

Median reactions (Skin fold x average diameter mm)			
Amount of antigen ($\mu\text{g}/100\mu\text{l}$)	Infested with adults	Infested with nymphs	Not tick infested
2500	257.02	489.56	420.99
500	142.45	516.72	516.33
100	175.38	420.97	320.16
20	105.09	303.93	229.52
4	12.31	76.48	167.1

ELISA

Figures 2 and 3 show Log_{10} of 1/highest positive serum dilution of the serum samples when tested against ovalbumin and *B. abortus* antigens, respectively. Using samples collected from all 12 sheep from day thirteen after initial sensitisation until 49 days after the booster inoculation.

Using Kruskal-Wallis a significant difference, $P < 0.01$, was recorded between the immune response of the three groups of sheep to ovalbumin. Using Mann-Whitney the responses of the three groups were shown to divide into 2 very significant classes ($P < 0.01$), with the sheep infested with nymphs and the controls in one class and the sheep infested with adult ticks in another class producing a significantly lower response.

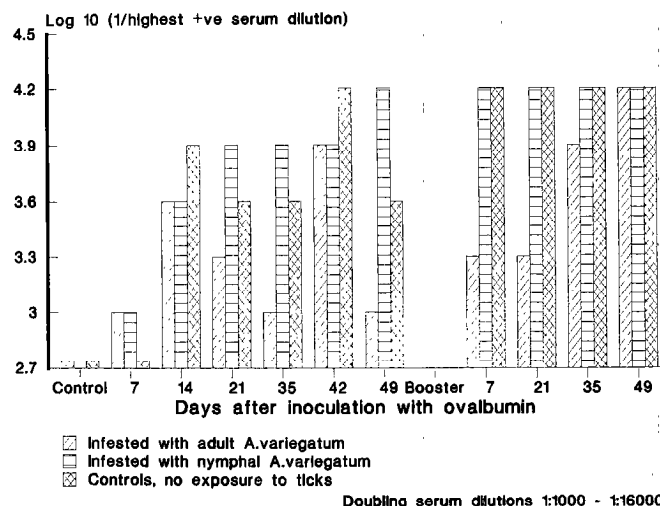


Figure 2 : Comparison of the effect of adult and nymphal *A. variegatum* infestations on the antibody levels of sheep in response to ovalbumin, using ELISA.

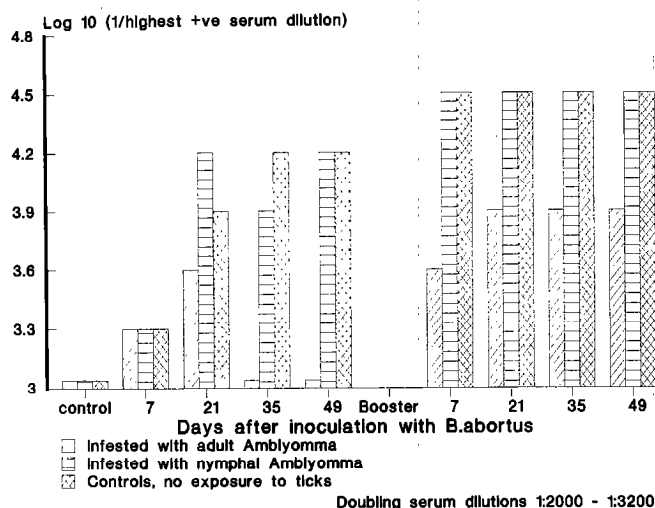


Figure 3 : Comparison of the effect of adult and nymphal *A. variegatum* infestations on the antibody levels of sheep in response to *B. abortus*, using ELISA.

Using the same analyses on the results obtained using *B. abortus* the immune responses again divided into the same significant classes as above.

DISCUSSION

This comparison of the effect of adult and nymphal *A. variegatum* follows previous work demonstrating the significant systemic effect of adult *A. variegatum* on the progression of experimental dermatophilosis on sheep (6).

In this investigation moderate chronic dermatophilosis lesions were reproduced only on sheep simultaneously infected with adult *A. variegatum* with nymphal tick feeding having no significant effect on the progression of the disease.

Evidence from the assessment of the clinical dermatophilosis of reduced immune response in the sheep infested by adult *A. variegatum* has been supported by the results obtained from the skin and serological tests.

B. abortus has been used as a sensitising antigen in previous studies to demonstrate antibody responses in sheep infected with *D. congolensis* (2). The method of application of antigen in PBS was used previously by ELLIS and SUTHERLAND (2).

Due to the experimental protocol the sheep infested with adult ticks were subjected to prolonged *D. congolensis* infections with the remaining sheep subjected to acute infections. It is possible that the significant difference in the immune reactions of the sheep may have been caused by the different levels of exposure to *D. congolensis*. However it has been concluded that the reduced immunological reactions recorded were caused by the tick feeding, this role of tick feeding causing immunosuppression in the host is well documented (4, 7).

In conclusion, the assessment of clinical dermatophilosis infections on sheep indicates that the systemic effect of *A. variegatum* is confined to the adults. A significant reduction in both the cell mediated and the humoral immune response of sheep infested with adult *A. variegatum* has been demonstrated by skin and serological testing.

LLOYD (C.M.), WALKER (A.R.). The systemic effect of adult and immature *Amblyomma variegatum* ticks on the pathogenesis of dermatophilosis. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 313-316

The systemic effect of adult and nymphal *Amblyomma variegatum* on the pathogenesis of experimental infections of *Dermatophilus congolensis* was investigated. Three groups of four sheep were used with all 12 sheep being infected with identical titrated doses of *D. congolensis*. One group of sheep was simultaneously infested with adult *A. variegatum* the second with nymphal *A. variegatum* and the third group were the controls, with no exposure to ticks. Assessment of the resulting infections indicate that the systemic effect of this tick is confined to the adults. Skin and serological tests using foreign antigens showed significantly reduced cell mediated and humoral immune response only in the sheep infested with adult *A. variegatum*.

Key words : Sheep - Dermatophilosis - *Dermatophilus congolensis* - Tick - *Amblyomma variegatum* - Systemic disease - ELISA test - Skin test.

ACKNOWLEDGEMENTS

The author would like to thank the Central Veterinary Laboratory, Weybridge for the supply of freeze-dried *B. abortus* used during this study. This project was supported by the Overseas Development Association of Great Britain.

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LLOYD (C.M.), WALKER (A.R.). Efecto sistémico de estados adultos e inmaduros de garrapatas *Amblyomma variegatum* sobre la patogenicidad de la dermatofilia. *Revue Élev. Méd. vét. Pays trop.*, 1993, **46** (1-2) : 313-316

Se estudió el efecto sistémico de los adultos y las ninfas de *Amblyomma variegatum* sobre la patogenicidad de las infecciones experimentales de *Dermatophilus congolensis*. Se trabajó con tres grupos de cuatro ovejas cada uno. Todas las ovejas se infectaron con dosis idénticas de *D. congolensis*. Uno de los grupos se infectó simultáneamente con adultos de *A. variegatum*, el segundo con estados ninfales de *A. variegatum* y el tercer grupo se utilizó como control, por lo que no fue expuesto a ninguna garrapata. La verificación de las infecciones resultantes indica que el efecto sistémico de esta garrapata se limita a los adultos. Tests serológicos y dermatológicos, para los cuáles se utilizaron antígenos extranjeros, mostraron una respuesta inmune reducida, tanto celular como humoral, pero solamente en las ovejas infestadas con adultos de *A. variegatum*.

Palabras claves : Ovino - Dermatofilia - *Dermatophilus congolensis* - Garrapata - *Amblyomma variegatum* - Enfermedad sistémica - Test ELISA - Prueba cutánea.