An enzyme-linked immunosorbent assay for the epidemiological survey of *Dermatophilus congoensis* infection in camels (*Camelus dromedarius*)

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**Summary:** The breeding of camels (*Camelus dromedarius*) is especially important in arid and semi-arid areas of Africa, where drought and famine frequently occur. A number of diseases which impair camel production have recently been described, including dermatophilosis (caused by *Dermatophilus congoensis*). However, it is not possible to determine the prevalence of infection from clinical cases alone.

An enzyme-linked immunosorbent assay has therefore been developed to determine the epidemiological prevalence of *D. congoensis* infection in sera of camels. Whole-cell antigen was used on microplates and the test serum was added. Horseradish peroxidase-conjugated sheep antibodies against heavy and light chains of camel immunoglobulin (Ig)G were then added, followed by substrate. The test was used to trace the antibody profile of twelve experimentally-infected camels. Peak antibody levels in serum occurred within twenty-one days following infection.

It is planned to use this test to determine the epidemiological prevalence of *D. congoensis* infection in camels reared in a pastoral area of Kenya.

**KEYWORDS:** Camel – Dermatophilosis – ELISA – Experimental infection.

**INTRODUCTION**

Rearing camels is practised widely by pastoral communities in arid and semi-arid areas of Africa. In Kenya, although camel husbandry is widespread in northern and north-eastern areas, the total camel population of 0.7 million is low (10), despite the potential of the camel to withstand and even thrive during prolonged periods of drought (5). During these periods, camel milk is sometimes the only source of food for pastoralists. Disease is one of the main problems facing camel husbandry and its exact role has yet to be fully determined.

Camel dermatophilosis is a skin disease of camels which is caused by *Dermatophilus congoensis* (8). Although the initial clinical cases were found on one commercial ranch

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in a semi-arid area of Kenya, the disease was later found to occur in other camels in the semi-arid areas of Samburu district in northern Kenya, and therefore appears to be more widespread than originally thought.

Camels are reared in vast areas which are remote and inaccessible, especially during or after the rainy season when many clinical cases of dermatophilosis occur. For this reason, the development of a serological test to determine in the field whether camels had been in contact with this pathogen, would be of immense value.

The control of dermatophilosis by vaccination has been partially successful in sheep (17) and even more so in bovines (4, 14). An effective serological test to assess the presence of antibodies following contact with the pathogen would be of great value in the future development of a vaccine against camel dermatophilosis. In cattle, a number of serological tests have been proposed for the monitoring and control of exposure of animals to *D. congolensis* (6, 13, 15). In sheep and rabbits, the enzyme-linked immunosorbent assay (ELISA) has been used to determine the antibody profile of vaccinated and unvaccinated animals (17, 9); an ELISA was developed and used on sera from experimentally-infected camels and a number of sera collected in the field.

**MATERIALS AND METHODS**

A previously-isolated strain of *D. congolensis* (8) was inoculated into nutrient broth and incubated aerobically for 72 h. The suspension was then centrifuged at 1,000 g for 15 min, and the cells resuspended in sterile saline containing 0.15% glucose. The concentration of the cells was then adjusted to 10^7, 10^8 or 10^9 colony-forming units/ml (CFU/ml).

Screening was performed using sera from a herd of 150 camels which had no clinical history of dermatophilosis. These animals were located in the Turkana district, where dermatophilosis had not previously been detected. Serum was obtained from all the camels and the presence of antibodies to *D. congolensis* was tested by ELISA.

Twelve weaner camels from this herd were selected for experimental infection. The camels were infected using the method described by Gheradi and colleagues (7) and Abu-Samra and colleagues (2). Sites were chosen on the dorsal flanks of each camel. Three sites measuring 6 cm in diameter, equally spaced on the left and right flanks were clipped, although not too closely, allowing some hairs to remain. The sites were swabbed with diethyl ether and then scarified with grade 2 sandpaper until hyperaemia of *D. congolensis* occurred, without bleeding. Each of the sites on the left flank were swabbed with 3 ml of *D. congolensis* (at 10^7, 10^8 or 10^9 CFU/ml). The right side was swabbed with similar dilutions of bacteria-free nutrient broth. All the sites were then swabbed twice daily by taping wet swabs to the skin for seven days. The skin lesions were observed daily for seven weeks and flakes of skin scabs were removed for bacterial isolation after ten days. Blood was obtained from the camels prior to inoculation and then at weekly intervals for ten weeks, and serum samples were prepared.

**Enzyme-linked immunosorbent assay**

Immunoglobulins (Ig) were prepared from whole camel serum by precipitation with ammonium sulphate solution. The camel IgG was isolated on a column of diethylaminoethyl cellulose (DE 52), from which IgG was separated by elution using
0.01 M phosphate-buffered saline (PBS) at pH 7.4. The IgG was then concentrated using Aquacide II-A. A sheep was immunised with 2 mg camel IgG in Freund's complete adjuvant by intramuscular injection and a further 2 mg was administered in incomplete Freund's adjuvant two weeks later. The sheep was bled six weeks after the first inoculation and IgG separated from serum as described above. The IgG was then tested for specificity on 1% agar gel slides against normal camel serum. The anti-camel IgG was equilibrated to a protein concentration of 5 mg/kg and stored at −20°C. The two-step glutaraldehyde method (3) was used to couple the peroxidase to the anti-camel IgG.

An ELISA procedure was followed, based on the method described by Voller and colleagues (18). Whole cells of *D. congolensis* were suspended in a minimum volume of PBS at 10⁹ CFU/ml and killed by heating at 56°C for 30 min. The antigen concentration was then readjusted to 1.5 µg diluted in 0.1 M sodium bicarbonate (pH 9.6). Wells in polystyrene microtitre plates were coated with 100 µl of the antigen. Adjacent rows were coated with negative control antigen at the same protein concentration. The plates were then incubated overnight at 4°C and washed three times with PBS (pH 7.3) containing 0.1% Tween 20. Each of the twelve test sera, diluted 1:40 in PBS-Tween 20, was added to wells coated with positive and control antigen. After incubation for 1 h at 37°C, the plates were emptied and rinsed in tap water at least three times. The optimum dilution (1:1,100) of a peroxidase-conjugated sheep anti-camel IgG conjugate prepared in PBS-Tween 20 was determined by a checkerboard titration. The conjugate (100 µl) was added in the wells and the plates incubated for one hour. The wells were then washed three times with PBS-Tween 20. The substrate solution was prepared by dissolving O-phenylene dihydrochloride at a concentration of 0.1 mg/ml in 0.1 M acetic acid, 0.2 M Na₂PO₄ buffer (pH 4.8) and adding 0.2 µl/ml of 30% H₂O₂. At the end of incubation, the reaction was stopped with 3N H₂SO₄. The absorbance was read using a multiscan photometer at a wavelength of 492 nm. The value obtained by subtracting the absorbance of the control antigen from that of the viral antigen was taken as the ELISA value.

**RESULTS**

Infections began to develop in the infected sites on day 3 with swelling and hyperaemia. Many areas of inflammation, some of which were confluent, were observed. By the end of day 5, a serous exudate was evident in the affected areas. By day 10, thin flakes of scabs were observed and eventually a thick brown scab formed which was firmly adherent to the underlying skin. Removal of the scab was difficult and revealed raw hyperaemic areas of skin. The main scab was present up to day 32 and recovery was complete by day 48. The percentages of camels showing lesions after contact with the various doses of *D. congolensis* are shown in Table I. It appears that 10⁸ CFU/ml is optimal for infection, while lower doses are less efficient. Bacteriological examination revealed the presence of *D. congolensis* in 95% of the infected scabs. Scarified control areas showed swelling and hyperaemia by the second day and a thin brown scab by the third to fourth day which was shed completely six to seven days after scarification, leaving a clear healed area. Bacteriological examination from the control sites revealed no presence of *D. congolensis*. 
TABLE I

The percentage of twelve camels (Camelus dromedarius) experimentally exposed to Dermatophilus congolensis at varying concentrations and showing lesions from the third to the ninth day following exposure

<table>
<thead>
<tr>
<th>Concentration of D. congolensis (CFU/ml)</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^7$</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (16)</td>
<td>5 (41)</td>
<td>7 (58)</td>
<td>9 (75)</td>
</tr>
<tr>
<td>$10^8$</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>3 (25)</td>
<td>7 (58)</td>
<td>10 (83)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>$10^9$</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>4 (33)</td>
<td>7 (58)</td>
<td>10 (83)</td>
<td>11 (92)</td>
</tr>
</tbody>
</table>

Serology

The mean antibody optical density (OD) levels from the control herd were 0.06 with a standard deviation (SD) of 0.033. The results were normally distributed. To give an ample safety margin, ELISA values of 0.16 (>0.06 + 3 SD) were taken as negative. The mean antibody levels of all positively infected camels rose steadily after infection to a peak on day 21 at an OD of 1 and then fluctuated slightly. The results are shown in Figure 1.

![Graph showing mean optical density over weeks post-infection](image)

**FIG. 1**

The mean antibody response of twelve camels experimentally infected with *Dermatophilus congolensis* measured by enzyme-linked immunosorbent assay
DISCUSSION

The experimental infection of camels was similar to that described by Abu-Samra and colleagues (2) and to experimental infection of sheep as described by Gheradi and colleagues (7). However, in the latter cases the fleece was inoculated without clipping (7). In this study, hair was clipped (although not completely) and scarification performed. The hair which remained allowed the scabs to adhere to the skin.

Skin lesions were localised at the site of infection and not generalised. In naturally-infected animals, lesions are generalised over most parts of the body of the animal (8). Some workers have suggested that *D. congolensis* infection is opportunistic and produces clinical signs only when susceptibility is increased by other factors, such as trypanosomiasis in cattle (16), splenectomy in goats (11) and debility in cattle, horses and sheep (1). The same is very likely to be true in camels. Naturally-infected camels, in which lesions are generalised, are more likely to have higher antibody levels than observed in experimentally-infected camels. For this test to be used for screening of field sera, OD values of 1 ± 0.2 would be indicative of dermatophilosis. Serological surveys for antibodies to *D. congolensis* infection in goats and sheep have been performed in Nigeria (12). Current work involves identifying species of other organisms, such as *Norcadia asteroides* or *Actinomyces* spp. These organisms have not yet been described in camels but, if present, they may reduce the specificity of the ELISA test.

The exact impact of *D. congolensis* infection on camel husbandry is difficult to ascertain with the scant information presently available on the prevalence of the infection. The ELISA test offers an alternative source of information, by screening a large number of serum samples, which could be obtained and then submitted for testing by local veterinarians even in remote and inaccessible areas.

ACKNOWLEDGEMENT

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**UN TEST IMMUNO-ENZYMATIQUE POUR L’ÉTUDE ÉPIDÉMILOGIQUE DE L’INFECTION DES DROMADAIRES (CAMELUS DROMEDARIUS) PAR DERMATOPHILUS CONGOLENSIS.** – C.G. Gitao.

**Résumé**: L’élevage de dromadaires (Camelus dromedarius) est particulièrement important dans les zones arides et semi-arides d’Afrique, où sévissent fréquemment la sécheresse et la famine. Certaines maladies, qui compromettent la production de ce type d’élevage, ont été récemment décrites, notamment la dermatophilose (due à Dermatophilus congolensis). Cependant, il n’est pas possible de déterminer la prévalence de l’infection à partir des seuls cas cliniques.

Un test immuno-enzymatique ELISA a donc été mis au point pour évaluer la prévalence épidémiologique de l’infection par *D. congolensis* à partir de
sérum de dromadaires. Pour ce faire, on fixe sur des microplaques un antigène brut, auquel on ajoute le sérum à tester. On rajoute ensuite des anticorps d’origine ovine spécifiques des chaînes lourdes et légères de l’immunoglobuline (Ig)G de dromadaire, couplés à la peroxydase de raifort, puis le substrat. Ce test a permis de déterminer l’évolution des anticorps chez douze dromadaires soumis à une infection expérimentale. Les titres maxima d’anticorps dans le sérum ont été enregistrés dans les vingt et un jours suivant l’infection.

On envisage d’utiliser ce test pour déterminer la prévalence épidémiologique de l’infection par D. congolensis chez les dromadaires élevés en zone pastorale au Kenya.


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UNA PRUEBA INMUNOENZIMÁTICA PARA EL ESTUDIO EPIDEMIOLÓGICO DE LA INFECCIÓN DE LOS DROMEDARIOS (CAMELUS DROMEDARIUS) POR DERMATOPHILUS CONGOLENSIS. – C.G. Gitao.

Resumen: La cría de dromedarios (Camelus dromedarius) es particularmente importante en las zonas áridas y semiáridas de África, en donde a menudo causan estragos sequías y hambrunas. Algunas enfermedades que ponen en peligro este tipo de cría son de descripción reciente, como por ejemplo la dermatofilosis (causada por Dermatophilus congolensis). Pero no es posible determinar la prevalencia de la infección a partir solamente de los casos clínicos.

Se desarrolló por lo tanto una prueba inmunoenzimática para evaluar la prevalencia de la infección por D. congolensis a partir de sueros de dromedarios. El procedimiento consiste en fijar en microplacas un antígeno bruto, al que se le agrega el suero que se someterá a prueba. Se agregan a continuación los anticuerpos de origen ovino específicos de las cadenas pesadas y ligeras de inmunoglobulina (Ig)G de dromedario, aparejados con peroxidasa de rábano blanco, y luego el sustrato. Esta prueba ha permitido determinar la evolución de los anticuerpos en doce dromedarios que fueron sometidos a una infección experimental. Los títulos máximos de anticuerpos en el suero se registraron en los veintiún días siguientes a la infección.

Se proyecta utilizar la misma prueba para determinar la prevalencia de la infección por D. congolensis en dromedarios criados en una zona de pastoreo en Kenia.


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REFERENCES


