Immunity to experimental dermatophilosis in rabbits and cattle following immunisation with a live whole cell vaccine

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La protection conférée contre la dermatophi-lose expérimentale par un vaccin vivant préparé avec Dermatophilus congolensis a été évaluée chez des lapins et des bovins. Les lapins furent vaccinés par voie intradermique ou sous-cutanée 7 jours avant l'épreuve avec la souche homologue de D. congolensis. Les animaux vaccinés par voie intradermique furent revaccinés et les deux groupes, ainsi qu'un troisième groupe témoin non vacciné, firent l'objet d'une épreuve identique. Les bovins furent vaccinés par voie intradermique par deux fois et l'épreuve, à l'aide de la souche homologue, fut effectuée 14 jours après chaque vaccination en même temps qu'une épreuve sur des animaux de contrôle non vaccinés. Une troisième épreuve fut également tentée sur tous les bovins, à l'aide de la souche homologue et d'une souche hétérologue pour évaluer l'immunité croisée entre les différentes souches de D. congolensis.

Une diminution significative de la gravité et de la durée des lésions fut observée après vaccination aussi bien chez les lapins que chez les bovins. Une diminution identique fut constatée après infection seule. Cet effet fut moins prononcé après une épreuve d'infection hétérologue, ce qui suggère une absence d'immunité croisée entre les différentes souches de D. congolensis. Aucune corrélation n'a pu être établie entre les taux sériques d'IgG anti-D. congolensis et la gravité des lésions. Mots clés : Bovin - Lapin - Dermatophilose - Dermatophilus congolensis - Infection expérimentale - Immunisation - Vaccin - Immunité.

MATERIALS AND METHODS

Rabbit study protocol

The rabbit study protocol is summarised in table I. Sixteen adult female New Zealand white rabbits (approximately 3 kg body weight and previously unex-posed to D. congolensis) were housed at a controlled temperature of 20 °C in separate cages. Six animals (group IDVAC) were vaccinated on two occasions intradermally on either side of the neck, the first vaccination (day 0) consisted of two 0.1 ml volumes and the second vaccination (day 28) of two 0.5 ml volumes. Another 6 animals (group SCVAC) were vaccinated subcutaneously in the neck region, each receiving two 1.0 ml volumes on day 0. All the vaccina- ted animals together with 4 unvaccinated animals (group UNVAC) were challenged as described below 1 week (day 7) after the first vaccination of IDVAC and SCVAC animals and 2 weeks (day 42) after the second vaccination of IDVAC animals. Following each chal-lenge the severity of any lesions which developed was assessed as described below.

Bovine study protocol

The protocol for the bovine study is summarised in

INTRODUCTION

Dermatophilosis (streptothricosis) is an exudative dermatitis caused by the bacterium Dermatophilus congolensis. The disease affects many animal species and is of particular importance in cattle in the humid and tropical regions where it causes high morbidity and mortality. It results in severe economic loss by reduc-ing meat and milk production and causing the downgrading of affected hides (5, 6).

Attempts have been made to control the disease by vaccination. A formalin-killed whole cell vaccine used in field trials in Senegal failed to reduce the incidence of the disease (14) and a similar vaccine did not protect rabbits from experimental infection (2). Howev-er a live whole cell vaccine produced complete protection against experimental challenge in rabbits (2) and when used to immunise cattle significantly reduced the incidence of natural infection in Chad, Cameroon and the Central African Republic (3, 4). BLANCOU (1) used a similar vaccine to immunise cattle in Madagascar and failed to reduce the inci-dence of disease but reported a decrease in its severity. Although vaccination remains the most pro-mising prospect for the control of this disease (8), no further immunisation trials have been reported. The incidence of the disease has not abated in Africa and it has become a serious problem in the Caribbean (17). In view of the conflicting evidence of vaccine efficacy the rabbit immunisation studies of CHAMOISEAU and LEFEVRE (2) were re-evaluated. The protection offered against experimental infection of cattle by a live intradermal vaccine was also tested.

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### TABLE I Summary of the rabbit study experimental protocol, including viable counts for D. congolensis challenge preparations.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Rabbit vaccination study</th>
<th>Bovine vaccination study</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Number of rabbits)</td>
<td>1st vaccination strain &amp; route</td>
<td>1st challenge strain, count, site treatment</td>
</tr>
<tr>
<td>IDVAC (6)</td>
<td>SS18C Intradermal</td>
<td>SS18C 1.5 x 10^9/ml Scarified ether</td>
</tr>
<tr>
<td>SCVAC (6)</td>
<td>SS18C Subcutaneous</td>
<td>As above</td>
</tr>
<tr>
<td>UNVAC (4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Days</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

### TABLE II Summary of the bovine study experimental protocol, including viable counts for D. congolensis challenge preparation.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Bovine vaccination study</th>
<th>Bovine vaccination study</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Number of calves)</td>
<td>1st vaccination strain &amp; route</td>
<td>1st challenge strain, count, site treatment</td>
</tr>
<tr>
<td>VACC (4)</td>
<td>SS18C Intradermal</td>
<td>SS18C 6 x 10^8/ml Scarified ether and clipped</td>
</tr>
<tr>
<td>UNVAC1 (4)</td>
<td>—</td>
<td>As above</td>
</tr>
<tr>
<td>UNVAC2 (2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Days</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

Table II. Ten Hereford calves aged 8 months and having shown no clinical signs of *Dermatophilus* infection were penned in pairs in a well ventilated unheated barn. Two pairs of animals (group VACC) were vaccinated intradermally, with two 0.5 ml volumes of vaccine on each of two occasions (day 0 and day 28) at separate sites on each side of the lower third of the neck. They were challenged 2 weeks after each vaccination. Two other pairs of calves (group UNVAC 1) were also challenged at the same time. The remaining calves (group UNVAC 2) were challenged only after the second vaccination. All 3 groups of animals were then subjected to a third challenge 7 weeks after the second vaccination of VACC animals. After each challenge the severity of any lesions which developed were assessed at intervals as described below. The mean temperatures on the challenge days were -6.0°C, +5.1°C and +4.3°C for the 1st, 2nd and 3rd challenge procedures respectively.

### Bacterial strains

A Scottish ovine (SS18C) and a Caribbean bovine isolate (FD11) were stored in the lyophilised state and cultured and maintained as required on blood agar (7 p. 100 defibrinated horse blood in Oxoid blood agar base No. 2) at 37°C.

### Vaccine preparation

Broth cultures of strain SS18C were obtained by inoculating 50 ml volumes of nutrient broth (Oxoid No. 2) in 100 ml conical flasks with 1 ml of a *D. congolensis* suspension prepared from a 48-hour blood agar culture. The flasks were sealed with cotton wool bungs and incubated for 72 hours at 37°C. Aliquots were examined by phase contrast microscopy to ensure purity and presence of both motile zoospo-
res and filaments. The bacterial growth was pelleted by centrifugation (3,000 g for 20 mins) and resuspended in half the culture supernatant to provide the intradermal vaccine, complete Freund’s adjuvant was added to give a final concentration of 10 p. 100 v/v before using subcutaneously.

**Challenge preparation**

Zoospore suspensions were prepared by suspending the growth from 48-hour blood agar cultures of either strain in peptone water (1 p. 100 w/v). These suspensions were left to stand for 15 minutes at room temperature to allow release of the zoospores. The liquid phase containing the motile zoospores was then decanted and diluted in peptone water to give a concentration of 10^8 to 10^9 zoospores/ml. A viable count was carried out on each challenge preparation by the spread plate method.

**Treatment of challenge sites**

Sites were prepared for challenge on both rabbits and cattle by clipping the back of the animal immediately before use to expose the skin. The sites were then inoculated without treatment (clipped sites), swabbed with cotton wool soaked in ether (ether sites) or lightly scarified avoiding haemorrhage using a 19 gauge needle in a 15 by 15 criss-cross pattern (scarified sites).

**Challenge procedure**

In the rabbit study 3 cm² sites were inoculated by immersing a sterile cotton wool swab into a separate 1 ml aliquot of the challenge preparation and rolling it over the surface of the site for 1 minute. The first challenge was carried out on 2 scarified sites, 1 either side of the midline, and the second challenge on 2 new scarified sites and 2 ether sites, 1 of each either side of the midline. In the bovine study separate sites were inoculated as described by LLOYD and JENKINSON (10) using 1.5 ml aliquots of the challenge preparation. The first challenge procedures were carried out on 2 clipped, scarified and ether sites on each animal, 1 of each either side of the midline, in groups VACC and UNVAC1. The second challenge was carried out as above on all animals, including group UNVAC2, and on new sites. The third challenge was also carried out on all animals on 4 new ether sites, 2 either side of the midline. Two sites on each animal were inoculated with strain SS18C and two with strain FD11. The viable counts for all the above challenge procedures are given in tables I and II.

**Assessment of infection**

In the rabbit study challenge sites were examined 6, 8, 13 and 15 days after the first challenge and 3, 5 and 7 days after the second challenge. Lesions were scored on an overall severity scale from 0 (unaffected) to 4 (severe confluent lesions) according to the degree of erythema, oedema, and scab formation. In the cattle study challenge sites were evaluated 7 and 14 days after each challenge procedure. The lesion score for each site was calculated from the sum of individual 0-4+ scores for erythema, oedema, scab formation, and overall severity multiplied by the percentage area affected. In both studies the mean scores were calculated for each type of site on each observation day and the means compared by unpaired Student’s t tests.

**Serological studies**

Serum samples were obtained in the rabbit study on the day before each vaccination and challenge, 1 week after the first challenge and 2 weeks after the second challenge. In the bovine study serum samples were obtained on the day before each vaccination and challenge and 2 weeks after the third challenge. Serum antibody titres to *D. congolensis* whole cell antigen were measured using an indirect microplate enzyme linked immunosorbent assay as described by LLOYD (7) with the difference that a goat anti-rabbit IgG alkaline phosphatase conjugate was used as the second antibody for the rabbit sera assays.

**RESULTS**

**Rabbit study**

Following the first vaccination all IDVAC animals developed intradermal nodules approximately 4 mm in diameter. These had either dispersed or sloughed from the vaccination site of all but 1 of the animals by 5 days after challenge. In contrast, in SCVAC animals large subcutaneous nodules (approximately 1 cm diameter) developed which remained throughout the challenge procedures. Large intradermal nodules (8 mm in diameter) developed following revaccination of IDVAC animals, which either dispersed or sloughed in 4 out of the 6 animals 7 days following the second challenge.

After the first challenge the SCVAC mean lesion scores were significantly (p < 0.001) less severe then the UNVAC scores on all the observation days (Fig. 1). The IDVAC lesions however were not significantly different from the UNVAC lesions on days 6 and 8 but on days 13 and 15 the lesion severity had decreased to
Fig. 1: Mean lesion scores of scarified sites inoculated with D. congolensis strain SS10C 6, 8, 13 and 15 days after rabbit challenge procedure 1. Rabbits were vaccinated 7 days prior to challenge intradermally (IDVAC) or subcutaneously (SCVAC). An unvaccinated (UNVAC) control group was also challenged.

N. B.: In all figures, *, ** and *** indicate significant differences between groups on the same day at the 5p. 100, 1 p. 100 and 0.1 p. 100 levels respectively.

Fig. 2: Mean lesion scores of scarified or ether swabbed sites inoculated with D. congolensis strain SS10C 3 and 7 days after rabbit challenge procedure 2. IDVAC animals were revaccinated 2 weeks prior to challenge 2.

The second challenge involved sites prepared by scarification or by ether swabbing. Between groups SCVAC and UNVAC, following the second challenge, there was no significant difference in the mean lesion scores at scarified sites (Fig. 2). The mean lesion scores for scarified sites however were significantly greater than for ether site scores (p < 0.01) on the third day after the second challenge (day 3 [2]). Seven days after the second challenge (day 7 [2]) mean lesion scores on both types of site and in all groups were not significantly different (Fig. 2).

Between groups the only difference seen was 3 days after challenge when the IDVAC mean lesion score was significantly lower than the UNVAC score (p < 0.001). By day 7 there was no difference between any of the groups, and the lesions had begun to heal.

Fig. 3: Mean ELISA absorbance titres (405 nm) of serum samples from IDVAC, SCVAC and UNVAC rabbits to D. congolensis whole cell antigen. = vaccination 1, 7 days before challenge 1; *= vaccination 2, 14 days before challenge 2.

The mean ELISA absorbance titres (405 nm) for each group, a measure of the serum IgG levels against D. congolensis antigens, are represented graphically in figure 3. Seven days after vaccination (day 0 [1]) there was little change in the mean titre of any of the groups and only a slight increase was seen 7 days after the first challenge (day 7 [1]). Twenty-one days after challenge much greater levels of antibody were detected in all groups. Following the second vaccination of IDVAC animals and following the second challenge an increase was seen in the mean titre. Statistical analysis failed to demonstrate any significant differences in titre between the groups.
Bovine study

After both vaccinations intradermal nodules between 1.0-1.5 cm in diameter developed which could be palpated for up to 28 days. First vaccination nodules were still present when the second vaccination took place but were of reduced size (0.5-0.75 cm) and 2 weeks later were only just palpable.

Seven days after challenge 1 the mean lesion score for VACC sites was significantly lower (p < 0.001) than for UNVAC1 sites (Fig. 4) and 7 days later (day 14 [1]) had decreased whereas there had been an increase in the UNVAC1 lesion score. The second challenge included UNVAC2 animals as positive controls. Seven and 14 days after the second challenge the mean lesion scores for UNVAC2 animals were significantly (p < 0.001) greater than those on VACC and UNVAC1 animals. As seen following the first challenge, the peak of infection was later and more severe in the naïve UNVAC2 animals compared to the vaccinated cattle. However, vaccination afforded no increased protection following the second challenge compared with the effect of previous challenge alone; after challenge 2 there was no difference between VACC and UNVAC1 lesion severity. In both these groups the lesions following the second challenge were much less severe than those following the first challenge.

Of the 3 treatments, ether swabbing resulted in the most severe lesions in terms of mean scores (Fig. 5) with scarification (Fig. 6) and clipping (Fig. 7) approxi-
mately equal. The response after the second challenge however was different between site treatments. At the scarified sites the mean lesion scores for UNVAC1 following the first challenge and for UNVAC2 following the second challenge were equivalent in severity. In contrast, at ether swabbed and at clipped sites less severe lesions were observed in UNVAC2 animals following the second challenge procedure.

After the third challenge no differences were detected between the groups in terms of their overall response towards the challenge (Fig. 5). The response to the 2 challenge strains was however significantly different (p < 0.001). The mean score for all groups on SS18C inoculated sites was 46.1, compared with 104.4 on all FD11 inoculated sites. The most severe lesions were seen seven days after challenge and both SS18C and FD11 lesions had decreased in severity 14 days later.

A substantial pre-existing level of IgG antibody to Dermatophilus antigen was detected in all experimental groups using the ELISA technique (Fig. 8). Two weeks following vaccination of VACC animals (day 0) a marked increase in the mean IgG titre was detected and following the second vaccination of this group (day 0) a further increase occurred. The mean IgG titres for VACC animals 2 weeks after each challenge procedure were not any higher than those detected 2 weeks after each vaccination. In UNVAC1 animals the first and third challenge procedures resulted in increases in the mean IgG titre detectable 2 weeks later. Two weeks after the first challenge of UNVAC2 animals an increase in mean IgG titre was detected to a level equal to that of UNVAC1 animals. Two weeks after the second vaccination of this group a further increase was detected again equal to that seen in UNVAC1 animals.

DISCUSSION

Both the rabbit and bovine studies reported here demonstrated partial but significant protection to experimental D. congolensis infection following immunisation with a live whole cell vaccine. The overall effects of vaccination were to decrease lesion severity and shorten the time course of infection. In both studies challenge alone also provided protection to a second challenge equal to that of previous vaccination and challenge.

In the rabbit study the route and type of vaccination, intradermal or subcutaneous with 10 p. 100 CFA, did not affect the degree of protection afforded towards the first challenge. A second intradermal vaccination increased the level of protection towards a second challenge but did not affect the rate of recovery compared with the other groups. In the bovine study a second vaccination gave no increased protection against a second challenge compared with previous challenge alone.

Lesions following the second challenge in both studies were less severe and developed and healed more rapidly compared with the first challenge. The viable counts on each challenge preparation were of the same order and any differences in lesion severity were therefore unlikely to be due to variability in the potency of challenge. Only on scarified sites were the lesions of equivalent severity following challenge procedures 1 and 2 on group UNVAC1 and UNVAC2 animals respectively. On ether swabbed and clipped sites the lesions on naive animals were significantly less severe following the second challenge. In cattle clipping of skin removes the top 5-8 layers of the stratum corneum but leaves layers of corneum and intercellular lipid intact whereas clipping followed by ether swabbing removes additional layers of corneum and all the intercellular lipid (9). Following these treatments influx of lipid from the hair follicles may act to re-establish the skin's barrier to infection (16). In scarified skin however the damage is more severe and penetrates through the stratum corneum and into the living epidermis. Such damage cannot be repaired solely by lipid replacement. The first bovine challenge took place in sub-zero conditions, whereas during the second challenge, one month later, the temperature was relatively warmer. It is possible that following the first challenge the low ambient temperature decreased the flow of lipid on the skin surface thus impeding the repair of the corneum and facilitating D. congolensis infection. On scarified sites the temperature would not be expected to influence the severity of infection due to the increased damage caused to the epidermis by this treatment. Further experiments in controlled climatic conditions are required to confirm this interpretation of our findings. LLOYD and JENKINSON (10)
carried out Dermatophilus infection studies in controlled climatic conditions of temperature and humidity but failed to demonstrate any differences between climate and severity of infection. However, the lowest temperature used was 15°C, at which lipid influx would still be expected to occur.

Overall the results from the rabbit study do not agree with those of CHAMOISEAU and LEFEVRE (2) who reported complete protection of rabbits from experimental infection afforded by vaccination with a live whole cell preparation. This discrepancy may have been due to the different strains of D. congolensis used or in the criteria for assessment of infection in the 2 studies. In addition, in this present study the first challenge was carried out 1 week after vaccination, compared with 3 weeks in the CHAMOISEAU and LEFEVRE (2) experiments. However in our studies lesions occurred in the rabbits even after repeated vaccination and challenge.

In the bovine study, challenge was carried out 2 weeks after vaccination, by which time a definite increase in D. congolensis-specific IgG was detected. This however was likely to have been a secondary response, not primary as in the rabbit study, as all animals had a pre-existing titre to D. congolensis. Following the second vaccination a further increase in IgG titre was detected which did not result in any increased protection. Between animals there was a large variation in antibody titres and in the severity of lesions but there was however no correlation between these factors. Overall serum IgG titre to D. congolensis had no relationship with severity of infection in either the rabbit or bovine study.

LLOYD and JENKINSON (11) demonstrated that intradermal vaccination of cattle with a live whole cell preparation significantly increases the levels of IgG, IgA and IgM to D. congolensis in skin washings. Serum levels in contrast showed increases in IgG, IgG, no change in IgM and a reduction in IgA. It is possible that local immunity in the form of surface antibody may play a part in the host's defence to D. congolensis infection. The CMI response is also probably involved. ROBERTS (15) demonstrated phagocytosis and subsequent killing of zoospores in the dermis of rabbits experimentally challenged with Dermatophilus and MAKINDE and WILKIE (12) used a lymphocyte transformation assay to further demonstrate a CMI response to the organism.

The bovine study demonstrated the lack of cross-immunity between D. congolensis strains, as shown by the increased severity of lesions on heterologous sites after the third challenge. Subsequent investigations in rabbits have shown this result was not due to a difference of pathogenicity between the two strains (HOW and LLOYD, unpublished). More work is required in order to identify strain and species specific antigens and determine their role in Dermatophilus infection and immunity. The work reported here strongly suggests that antigenic differences between D. congolensis strains play an important part in the immune response and must be investigated if progress towards an effective D. congolensis vaccine is to be made.

ACKNOWLEDGEMENTS

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The ability of a live whole cell Dermatophilus congolensis vaccine to protect against experimental Dermatophilus infection in rabbits and cattle was assessed. Rabbits were vaccinated intradermally or subcutaneously 7 days before challenge with the vaccine homologous strain of D. congolensis. Intradermally vaccinated animals were revaccinated and both groups together with a third unvaccinated control group were challenged as before.

The cattle were vaccinated intradermally on two occasions and challenged with the vaccine homologous strain 14 days after each vaccination together with control unvaccinated animals. A third challenge of all cattle using the vaccine homologous and a heterologous strain was also carried out to examine cross-immunity between different strains of D. congolensis.


Se investigó si una vacuna a base de células vivas de Dermatophilus congolensis protege contra la infección experimental con Dermatophilus en conejos y bovinos. Los conejos se vacunaron intradérmica o subcutáneamente 7 días antes de la infección con una cepa de D. congolensis homóloga a la de la vacuna. Los animales vacunados inadecuadamente fueron revacunados y ambos grupos junto a un tercer grupo control fueron infectados.

Los bovinos se vacunaron dos veces intradérmicamente y 14 días después de cada vacunación estos animales y un grupo control no vacunado se infectaron con la cepa homóloga de la vacuna. Se realizó una tercera prueba con todos los animales infectándose con una cepa homóloga y una cepa heteróloga de la vacuna, a fin de evaluar la inmunidad cruzada entre diferentes cepas de D. congolensis.
In both rabbits and cattle vaccination led to a significant reduction in lesion severity and duration following experimental infection. A similar reduction was observed following infection alone. This effect was less pronounced following heterologous challenge indicating a lack of cross-immunity between different *D. congolensis* strains. There was no correlation between levels of serum IgG antibody to *D. congolensis* and lesion severity. Key words: Cattle - Rabbit - Dermatophilosis - *Dermatophilus congolensis* - Experimental infection - Immunization - Vaccine - Immunity.

Tanto en conejos como en bovinos la vacunación condujo a una reducción significativa de la intensidad y duración de las lesiones producidas por la infección experimental. Una reducción similar se observó tras la infección sola. Este efecto fue menos pronunciado tras la aplicación de la cepa heteróloga, indicando la ausencia de inmunidad cruzada entre diferentes cepas de *D. congolensis*. No hubo correlación entre los niveles de IgG sérica anti-*D. congolensis* y la gravedad de las lesiones. Palabras claves: Bovino - Conejo - Dermatofilosis - *Dermatophilus congolensis* - Infección experimental - Inmunización - Vacuna - Inmunidad.

REFERENCES


