SUMMARY

Serum samples were collected from sheep prior to, during, and after recovery from cutaneous myiasis (body strike). Serum proteins were separated electrophoretically into albumin, alpha-1, alpha-2, beta, and gamma globulins. The percentage of albumin in the serum decreased significantly \((P<0.01)\) from pre-infestation levels of 55.26% to 39% during infestation, but returned to 45.46% twelve days after treatment. Total plasma proteins did not vary significantly (range 9.7 to 10 gm/100 ml) during the disease, or after recovery. The gamma globulin fraction of the serum increased significantly from 23.98% pre-infestation to 32.44% at point of treatment, and 36.93% after 5 days recovery. In sheep which had recovered from cutaneous myiasis, the shape of the gamma globulin absorption curve indicated that antibody production was against a number of antigens. (Keywords: serum proteins, cutaneous myiasis, Lucilia cuprina, body strike).

INTRODUCTION

Cutaneous myiasis of sheep is an important cause of economic loss in the Australian Wool Industry (Hart 1984). A large proportion of this loss is due to deaths in affected sheep; 2.8 million sheep were estimated to have died in the 1975/6 summer season (Brideoake 1979). Although the cause of death has not been completely elucidated, the clinical, pathological, and physiological changes which accompany cutaneous myiasis have been reported (Broadmeadow et al. 1984; Gibson et al. 1984). These workers described pathological changes consistent with a toxemia.

The susceptibility to cutaneous myiasis is more common in young animals (Belschner 1937). This age effect suggests that an immune process may be involved but O'Donnell et al. (1980) were not able to demonstrate any protective action for antibodies to Lucilia cuprina larvae. More recently, Sandeman et al. (1985) reported that sheep immunized by repeated larval infestation had significantly smaller cutaneous myiasis lesions than sheep which had received no pre-infection.

This paper reports on the serum changes which occurred in a group of sheep affected with natural cutaneous myiasis, after artificial induction of fleece rot.

MATERIALS AND METHODS

Experimental animals

Nine Merino ewe hoggets with seven months growth of wool were placed in a rain simulator (Copland 1992; Copland and Chenoweth 1984) where they were subjected to a total of 100 mm of rain over 4 hours on each of 7 successive days. All nine sheep developed fleece rot lesions, and four became naturally infested with L. cuprina larvae on the sixth or seventh day of wetting. The cutaneous myiasis was detected at the first instar larval stage during the twice daily inspections of the sheep. The cutaneous myiasis lesions were located between 10 cm and 30 cm posterior to the dorsum of the scapulae, on the midline, extending laterally. These lesions were disturbed as little as possible, so as to simulate natural

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conditions, and the number of larvae was not counted. The infestations were treated nine days after detection by removal of the wool and the application of a topical insecticide (Diazinon, Bayer). The area of the skin lesion was measured after removal of the wool at treatment, by tracing the shape of the lesion onto graph paper.

Samples

Jugular blood samples were collected 4 times before infestation, at time of treatment, and after 5, and 12 days of recovery. Samples were allowed to clot, and the serum poured off and frozen so all measurements could be conducted at the same time. Electrophoretic separation of the individual serum proteins was obtained using cellulose acetate plates and a Tris-barbital-sodium barbital buffer, pH 8.6 after subjection to a current of 180 V for 15 minutes. The cleared plates were scanned in a Quick Scan Jr (Helena) spectrodensitomitor using a 525 nm filter. The proteins were resolved into five distinct fractions; albumin and four globulins, named alpha-l, alpha-2, beta-1 and gamma. The relative percentages of the serum protein fractions were calculated by integration.

RESULTS

The relative percentages of the various serum protein fractions are presented in Table 1. During the course of the disease, the albumin fraction decreased significantly, while the globulins (particularly gamma globulin) increased significantly. This change was evident in the albumin, globulin ratio, which had a preinfestation level of 1.23, but decreased to 0.66 at the time of treatment. However, the total plasma proteins did not vary appreciably during the course of the cutaneous myiasis; from a minimum of 9.7 gm/100 ml (sd 1.4) prior to infestation to a maximum of 10.0 gm/100 ml (sd 1.62) at time of treatment.

Table 1 Serum protein fractions for 4 sheep affected with cutaneous myiasis; means and standard deviations for the electrophoretically separated fractions are expressed as percentages of total serum protein.

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin%</th>
<th>Globulins%</th>
<th>alpha-1</th>
<th>alpha-2</th>
<th>beta-1</th>
<th>gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line observations</td>
<td>55.26</td>
<td>4.48</td>
<td>10.30</td>
<td>6.00</td>
<td>23.98</td>
<td></td>
</tr>
<tr>
<td>mean of four samplings</td>
<td>(6.07)</td>
<td>(1.92)</td>
<td>(1.51)</td>
<td>(1.58)</td>
<td>(4.79)</td>
<td></td>
</tr>
<tr>
<td>At time of treatment</td>
<td>39.72**</td>
<td>6.25ns</td>
<td>10.84ns</td>
<td>10.71**</td>
<td>32.44**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8.71)</td>
<td>(1.20)</td>
<td>(3.99)</td>
<td>(4.31)</td>
<td>(5.29)</td>
<td></td>
</tr>
<tr>
<td>Five days post treatment</td>
<td>39.71**</td>
<td>5.54ns</td>
<td>9.83ns</td>
<td>7.94ns</td>
<td>36.93**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10.48)</td>
<td>(1.18)</td>
<td>(2.26)</td>
<td>(3.04)</td>
<td>(7.13)</td>
<td></td>
</tr>
<tr>
<td>Twelve days post treatment</td>
<td>45.46*</td>
<td>3.84ns</td>
<td>10.63ns</td>
<td>4.83ns</td>
<td>35.20**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12.72)</td>
<td>(0.85)</td>
<td>(1.83)</td>
<td>(0.40)</td>
<td>(10.7)</td>
<td></td>
</tr>
</tbody>
</table>

Legend: **, P<0.01 when compared to base line readings.

The area of the-cutaneous myiasis lesion varied from 153 cm² to 586 cm², with a mean of 300 cm² (sd 72). At the time of treatment, the area of the skin lesion was significantly related to the albumin, globulin ratio (r=0.64).

Typical traces of the electrophoresis patterns are presented in Fig. 1a, lb and lc for preinfested, affected, and recovering (12 days post treatment) sheep respectively.
Fig. la. Typical electrophoresis traces for serum proteins from sheep prior to development of cutaneous myiasis. Key as per Fig. lc.

Fig. lb. Typical electrophoresis traces for serum proteins from sheep after development of a cutaneous myiasis lesion. Key as per Fig. lc.

Fig. lc. Typical electrophoresis traces for serum proteins from sheep 12 days after recovery from cutaneous myiasis.

Key: Alb=albumin, Al=alpha-1 globulin, A2=alpha-2 globulin, B=beta-1 globulin, Gamma=gamma globulin. Vertical lines indicate divisions between protein fractions.

DISCUSSION

The leakage of proteins onto the skin, as described by Watts and Merritt (1981) for fleece rot, may be the reason for a decrease in the albumin fraction early in the course of infestation. This loss of albumin was accompanied by production of globulins, especially gamma globulins as the disease progressed. A loss of albumin was also reported by Gibson et al. (1984) but this occurred as sheep approached death. The barrier function of the skin could also be disrupted by the cutaneous myiasis lesion, permitting entry of toxins from bacteria and larval metabolism. These products may act as antigens for a rapid production of gamma globulins (Elliot et al. 1980, Sandeman et al. 1985). The rise in gamma globulins. These gamma globulins could be produced in response to a wide range of antigens associated with the cutaneous myiasis lesion, rather than reaginic antibodies, as measured by Sandeman et al. (1985).

The first defence against infestation of the sheep by L. cuprina is postulated to be the skin and fleece. As sheep grow, characteristics such as
yield and staple length change (Brown et al. 1966). These factors, together with others such as nutrition or testosterone production may affect resistance to fleece rot and cutaneous myiasis (Copland and Chenoweth 1984). If this first line of defence fails and fleece rot develops, oviposition takes place, and larvae come in contact with the skin.

The second level of defence may be immunity of the skin to L. cuprina larvae. The role of previous experience in this immunity is not clear. The hypersensitivity reaction which occurs could assist larval establishment (O'Donnell et al. 1980), but Sandeman et al. (1985) reported smaller lesions in immune sheep although immunity had no significant effect on larval survival.

The third level of resistance concerns the survival of sheep with large cutaneous myiasis lesions. If death is caused by a toxin, its origin and precise nature is not known. The broad gamma globulin response found in this study indicates that a group of antigens challenge the host immune system. Some of these antigens may be associated with deaths of the sheep, while others are incidental. This production of gamma globulins was principally responsible for the fall in the albumin to globulin ratio as the disease progresses.

Further work needs to be done on the causes of mortality of sheep with cutaneous myiasis and the toxins involved in deaths of affected sheep. If a small number of specific toxins are implicated then protection from death by vaccination may be possible.

REFERENCES


