

THE ACQUISITION OF IMMUNITY TO *HISTOPHILUS OVIS* BY SHEEP IN NATURE

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ABSTRACT

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It was demonstrated that skin wound infection with *Histophilus ovis* elicits an immune response which can protect a ram against a challenge injection of the same organism into its epididymis.

INTRODUCTION

The occurrence in nature of antibodies in animals has long been known. Gibson (1930) found antibodies against *Proteus* X 19, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B, *S. enteritidis*, *Shigella flexneri*, *Sh. dysenteriae*, *Proteus morgani*, *Klebsiella pneumoniae*, *Escherichia coli* and *Vibrio cholerae* in sera from the ox, rabbit, guinea pig, horse, sheep, pig, rat, cat and man. Lovell (1932, 1934) found flagellar agglutinins against different types of *Salmonella* in swine, cattle, sheep and horses. He also found somatic agglutinins against some *Salmonella* types in the sera of pigs, cattle, sheep and horses.

The antibodies reported on by the above 2 authors were not induced by immunization, but were the result of contact by the animals with the bacteria in their environment.

Natural exposure of animals to infective material may have an immunizing effect on them. Cunningham (1977) states that non-pregnant heifers, exposed to infection in nature by the conjunctival or oral route to *Brucella abortus*, usually show a temporary antibody response, but about 90 per cent of them become immune. Only a small percentage progress to active infection and persisting positive titres.

In this article evidence is presented to show that *Histophilus ovis*, entering the body of rams through skin wounds, can elicit an immune response which protects them against a challenge injection of the same organism into the epididymis.

MATERIALS AND METHODS

Experimental animals

Fifteen-month-old Merino rams, kept intensively on regularly cleaned floors, were tested for freedom from bacterial infection of their genitalia with semen obtained through electro-ejaculation in as sterile a manner as possible. Cultures were prepared from the semen, and the rams that were found free from bacterial infection of their genital tract were used for the experiment.

The controls were selected from a group of 12 rams, 6 of which had been subjected to Mules' operation as lambs. At that stage no connection between Mules' operation and resistance to *H. ovis* infection had been surmised.

Artificial infection of rams

H. ovis (Strain 0782*) was grown on 10 % horse blood tryptose-agar** for 48 h in 10 % CO₂ at 37 °C, whereafter the growth was washed off with sterile Hank's balanced salt solution to give a milky white suspension.

The wool was shorn from a patch of skin on the side of the body of the rams. Five cuts 50 mm long were made close to each other through the bare skin. The bacterial suspension was lightly rubbed into the cuts and the rams

were left for 3 weeks until challenged. Four rams were infected while 2 were kept as controls in a widely separated camp to avoid contact with the infective organisms.

Challenge procedure

The bacteria from a 24 h culture of *H. ovis* were washed from the surface of the medium in Petri dishes, using Hank's balanced salt solution, and the suspension was diluted so that a challenge dose contained about 0.5×10^6 organisms in 0.05 ml.

The rams were challenged by injecting the challenge dose directly into the tissue of the cauda epididymidis through the scrotal skin (Jansen & Hayes, 1984). Ten days after challenge the rams were killed and their entire genital tract was removed and transferred to the laboratory for the preparation of bacterial cultures from the component parts.

RESULTS

Immunity in rams previously subjected to Mules' operation

When 2 of the rams that had been subjected to Mules' operation were used as controls, neither developed epididymitis. As a result of this finding the remaining 4 in this category were also challenged with the same results.

Subsequently 2 of the 6 in the group that had not had the Mules' operation were challenged. Both developed severe epididymitis and orchitis.

Immunity subsequent to artificial infection

Of the 4 rams artificially infected 3 weeks prior to challenge, 3 did not show any clinical reaction to the challenge, and their tissues appeared normal at necropsy. No *H. ovis* could be cultured from their genitalia. One of the 4 and the 2 further control rams from the group that had not had the Mules' operation showed severe purulent epididymitis and orchitis from which *H. ovis* could be cultured.

DISCUSSION

H. ovis is an organism frequently associated with sheep and is responsible for a variety of pathological processes such as acute mastitis (Roberts, 1956), epididymitis in rams (Dodd & Hartley, 1955; Claxton & Everett, 1966; Ekdahl, Money & Martin, 1968; Webb, 1983), systemic or localized infections in young lambs (Kater, Marshall & Hartley, 1962; Hughes, Hartley, Haughey & McFarlane, 1964; Dennis, 1974; Rahaley & White, 1977; Webb, 1983) and mastitis and abortion in ewes (Webb, 1983).

No definite information is available on where the organism is harboured in its association with sheep, but it must be assumed that any skin wound will be exposed to contamination with *H. ovis*. The ability of *H. ovis* to survive for up to 4 days in wet manure or pen floor debris (Jansen, 1983) increases the likelihood of fresh wounds becoming infected while sheep are lying down. Woolled sheep are subjected to wounding during castration, docking, shearing and the performance of Mules' operation, and during all these procedures they are collected in pens. It seems reasonable, therefore, to assume that the

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wounds frequently become contaminated with *H. ovis* under such conditions. The experiments reported on in this publication have demonstrated that such contamination can render rams immune to challenge with this pathogen. Why some sheep should become immune and others develop systemic disease as a result of their contact with *H. ovis* is not yet clear.

The fact that woolled sheep are exposed to more wounding than non-woolled sheep could explain Van Tonder's (1979) observation that lesions of the scrotal contents caused by *Actinobacillus seminis* (which includes *H. ovis*) occurred in 5,7 % of Dorper rams as against 2,1 % of Merino rams in the sheep population examined by him.

The possibility that sheep can be rendered immune to an infection by *H. ovis* in the manner carried out in these experiments lends support to efforts at immunizing sheep with antigens prepared from this organism.

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