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THE PASSIVE PROTECTION OF LAMBS AGAINST *CLOSTRIDIUM PERFRINGENS* TYPE D WITH SEMI-PURIFIED HYPERIMMUNE SERUM

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ABSTRACT

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Weaned lambs, having a detectable level of maternal antibodies (1–2 units/mℓ) against *C. perfringens* type D, showed protective antitoxin levels lasting for 29 days after receiving a single parenteral dose of 200 units/kg hyperimmune serum. Lambs, having no maternal antibodies (<0,07 units/mℓ) to *C. perfringens* type D but receiving the same dose of hyperimmune serum, maintained protective antibody levels for only 21 days. Three weeks after the titres fell below the minimum protective level of 0,15 units/mℓ, both these groups were treated again in the same manner. The passive immunity conferred in both groups now lasted for 42 days. When the hyperimmune serum was administered to lambs already immunized by vaccination, a slight increase was noted in the antibody titre.

INTRODUCTION

When sheep are vaccinated against *Clostridium perfringens* type D for the prevention of enterotoxaemia, there is a lag period of between 1–2 weeks before circulating antibodies appear (Sutton, 1952; Smith & Marsh, 1953). In a feedlot system this poses a serious problem because some animals are fully susceptible to enterotoxaemia during this period, when dietary changes create ideal conditions for enterotoxaemia outbreaks.

In South Africa sheep are actively immunized with 2 types of *C. perfringens* type D vaccines, issued by the Veterinary Research Institute, Onderstepoort. They recommend that in adult sheep the oil adjuvant vaccine be used initially to stimulate the primary response, that this be followed by the alum-precipitated vaccine 1 month later and a booster injection 6–8 months thereafter to achieve uninterrupted immunity to enterotoxaemia[1]. The same applies to 2–3 month old lambs (Cameron, 1980). This gives rise to various levels of antibodies circulating in the bloodstream, the minimum being 0,15 units epsilon antitoxin/mℓ for sufficient protection against enterotoxaemia (Jansen, 1960).

Howard, according to Tengerdy, Meyer, Lauerman, Lueker & Nockels (1983), stated that 54 % of the predominant causes of death in feedlot sheep were digestive and 15 % respiratory. Enterotoxaemia caused by *C. perfringens* type D accounted for 40 % of the observed deaths caused by digestive system disturbances.

When purchased sheep are admitted to a feedlot system it is standard procedure to vaccinate all the animals against various infectious diseases, including enterotoxaemia. This occurs irrespective of the fact that they might have had previous exposure to the particular vaccine. As initial mortality can be considerable, it is reasonable to assume that a certain percentage of animals have had no previous exposure to enterotoxaemia vaccinations and that these animals are fully susceptible to the disease in the lag period prior to the primary immune response taking effect.

Previously, the emphasis on the prevention of this disease was placed on the induction of an active immunity, and the role of passive protection was overlooked. The role of passive protection against sheep enterotoxaemia is not a new concept (Jansen, 1965; Smith, 1974); and Van der Walt (1981) demonstrated that partly purified, hyperimmune serum against *C. perfringens* type D offered passive protection in 6-month-old lambs that lasted for 60–90 days.

The purpose of this study was to prepare hyperimmune sheep against *C. perfringens* type D, recover the hyperimmune plasma from these animals with a continuous plasmapheresis system, concentrate the gamma globulins and compare the duration and levels of the parenterally administered passive immunity both in post weaned lambs that have either none or some maternal antibodies and in animals that have been actively immunized with the Onderstepoort oil and alum-precipitated enterotoxaemia vaccines.

MATERIALS AND METHODS

Hyperimmune serum production

Three adult Merino sheep were initally vaccinated with 4 mℓ of the Onderstepoort oil adjuvant enterotoxaemia vaccine (Lf=100 units/mℓ) in 4 divided doses. This was followed 4 weeks later by a booster of 4 mℓ of alum-precipitated vaccine (Lf=100 units/mℓ). Four weeks later 10 mℓ of toxoid (Lf=325 units/mℓ) was given intramuscularly in the left and right prescapular and inguinal areas. Two weeks later an injection of toxic culture supernatant (Lf 250 units/mℓ) was repeated 4 times at 3 day intervals, with the dosage increasing from 2–8 mℓ. It was given subcutaneously in 4 divided doses.

Plasmapheresis

The animals were mechanically restrained in a specially designed crate, to ensure easy access to the jugular veins. After placing a 14 g catheter in the vein the catheter was coupled to a tube system on the plasmapheresis apparatus[2] that added 4 % sodium citrate[3] containing 0,9 % NaCl to the blood. One litre of plasma was obtained before the packed red blood cells were diluted in 5 % dextro-saline and infused back into the animal. Depending on the size of the sheep, the procedure was repeated 1–3 times and took 3–4 h to complete. During plasmapheresis 1–2 ℓ of plasma was withdrawn from each animal. The plasma was stored at −20 °C before being processed. Fifteen to 24 ℓ of plasma was collected from each animal during a 11-week period. Haematocrit, haemoglobin, and red and white blood cell counts were done before and after each procedure.

Antibody assays

The antibody assays on sheep sera were done by the Section of Bacteriology, Veterinary Research Institute, Onderstepoort, by using the L+ test as described by Jansen (1967). For the purpose of this study 0,15 units/mℓ was taken as the minimum protective level of antibody present in the serum (Jansen, 1960).

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[2] Veterinary Research Institute, Onderstepoort 0110

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[1] Onderstepoort Vaccines, issued by the Director, Veterinary Research Institute, Onderstepoort

[2] Haemonitics Inc., USA.

[3] Saarchem, P.O. Box 144, Muldersdrift 1747

Preparation of gammaglobulins

The plasma was twice subjected to precipitation with polyethylene glycol 6000[3] (Van der Walt, 1981), sterilized by filtration and stored at 6 °C.

Experimental groups

Twenty-four post weaned, 3-month-old Merino type, cross-bred lambs with no history of vaccination against enterotoxaemia were used. These animals were mass-measured, ear-tagged and dewormed upon arrival. They received lucerne hay *ad lib.* and a balanced commercial lamb pellet ration, and were all housed under one roof on a cement floor. After the first blood sampling, when antibody titres were established, it became evident that at least half of the animals had maternal antibodies to a varying extent. These animals were subsequently divided into 2 main groups. All those with an antitoxin titre of 0,1 units/mℓ and higher were grouped together as animals possessing passive immunity, whereas animals with a titre of <0,07 units/mℓ were considered as devoid of any passive immunity and susceptible to enterotoxaemia.

Each group was divided into 3 blocks consisting of 3 different treatments with 4 replicates in each block. The first main group thus consisted of 12 animals possessing maternal antibodies (> 0,1 units/mℓ). The first treatment was administered to 4 animals, the animals were each passively immunized with purified gammaglobulins containing 800 units epsilon antitoxin/mℓ with a dosage of 200 units/kg on Days 0 and 48. The 2nd treatment was administered to the next 4 animals on Days 0 and 27, when they were first actively immunized with 1 mℓ of oil and alum-precipitate adjuvant vaccine respectively. On Day 48 they were passively immunized with purified gammaglobulins, with a dosage of 200 units/kg. The 3rd block of 4 animals served as the controls and received only a placebo of 18 % polyethylene glycol[3].

The second main group also consisted of 12 animals but differed from the first in that they did not possess any maternal antibodies. These animals received exactly the same treatments, administered on the same day as those in the first group.

Collection of specimens

The 1st serum specimens, collected in vacuum glass tubes with a 20 g needle on Day 0 reflected the initial antibody titre. After the 1st round of treatment serum was taken on Days 1, 3 and 6 and weekly thereafter until Day 125. The blood was allowed to clot at room temperature for 1–2 h, stored at 4–8 °C overnight, and centrifuged at 1 500 × g for 10 min with a Sure-sept serum separator[4]. The supernatant serum was dispensed into 5 mℓ plastic test tubes (12 × 85 mm) fitted with a screw-cap, frozen and stored at −20 °C until titres were determined.

RESULTS

Blood parameters measured before and after plasmapheresis

The parameters that were measured during the whole period of 68–77 days before and after each process included the haematocrit, haemoglobin level, red and white blood cell counts. Sheep 2 yielded 22 ℓ; sheep 3, 14 ℓ, and 5, 24 ℓ of plasma. Sheep 3 was much smaller than the other two, and consequently yielded less plasma. The cumulative average values of the parameters were calculated and are presented in Table 1.

[4] General Diagnostics, P.O. Box 123, Johannesburg 2000

TABLE 1 The cumulative average values of blood parameters of hyperimmunized sheep before and after plasmapheresis during a period of 68–77 days

Sheep no	Haematocrit %		Haemoglobin (g/100 mℓ)		Red blood cells (10*6/mℓ)		White blood cells (10*3/mℓ)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
2	31,6	31,09	9,22	9,17	5.17	5,08	4,43	4,57
3	32	31,85	8,95	8,93	4,8	4,79	5,15	5,1
5	31,23	31,38	9,04	9,0	4,83	4,89	5,87	5,29

The level of immunity achieved in hyperimmunized sheep

Varying antibody responses to the hyperimmunization procedure were found. Sheep 3 gave the highest level of antibodies (>1000 units epsilon antitoxin/mℓ), and sheep 2 and 5 had 600 and 333 units epsilon antitoxin/mℓ respectively after 76 days.

The duration of passive immunity in lambs with and without maternal antibodies to enterotoxaemia

The geometric mean of the antitoxin titres from each group of lambs was calculated and used as an indication of the protective immunity. The group of lambs with an existing level of maternal antibodies showed an increase from 1,19 u/mℓ to 3,54 u/mℓ within 24 h after receiving the hyperimmune serum. This protective level was sustained for 29 days (Fig. 1).

The corresponding group of lambs with no maternal antibodies, showed an increase in antibody titres from 0,07 u/mℓ–1,88 u/mℓ within 24 h. This level of protection lasted only for 21 days (Fig. 2) (P<0,05).

After a lag period of 2–3 weeks the antibody titre dropped to 0,07 u/mℓ in both groups. A 2nd injection of hyperimmune serum was given and in both groups maximal titres of 2,5 u/mℓ were observed. Protective levels were thus maintained for 42 days.

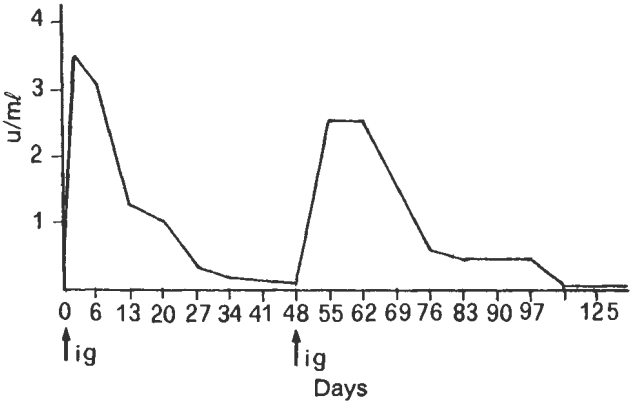

FIG. 1 The level of passive immunity conferred on lambs with maternal antibodies (ig = immune globulin)

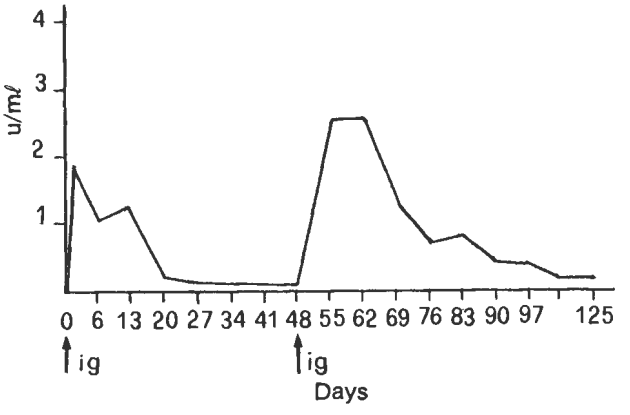

FIG. 2 The level of passive immunity conferred on lambs without any maternal antibodies (ig = immune globulin)

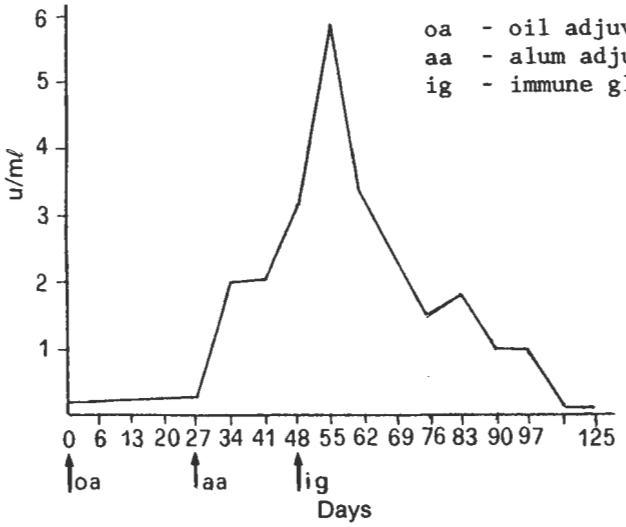

FIG. 3 The level of passive immunity conferred on lambs with maternal antibodies after active immunization

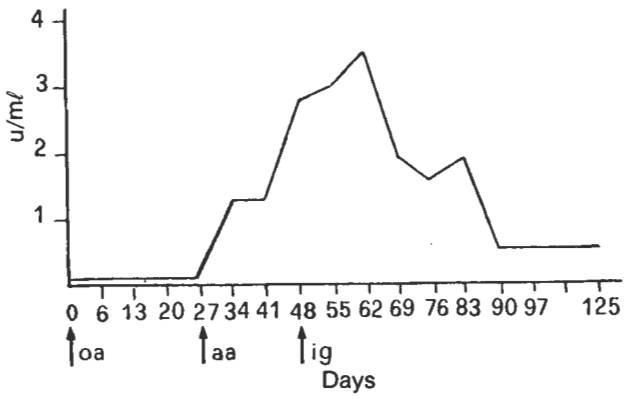

FIG. 4 The level of passive immunity conferred on lambs without any maternal antibodies after active immunization

The duration of passive immunity in lambs with and without maternal antibodies after active immunization against enterotoxaemia

The group of lambs that had a measurable level of maternal antibodies showed a slight titre increase on Day 13, after receiving the oil adjuvant vaccine on Day 0. The booster injection with the alum-precipitated vaccine increased the titre from a geometric mean of 0,26 u/mℓ to 1,99 u/mℓ within a week after administration (Fig. 3). The administration of hyperimmune serum (immune globulin) on Day 48 increased the titre even further to 5,9 u/mℓ. The protective titre lasted for approximately 42 days before the animals became susceptible again.

The group of lambs without maternal antibodies remained susceptible until receiving the booster vaccination with the alum-precipitated vaccine, on Day 27 when a level of 2,81 u/mℓ was recorded.

After receiving hyperimmune serum on Day 48, the titre increased to 3,53 u/mℓ on Day 62. These titres decreased but remained protective at 0,5 u/mℓ till day 125 (Fig. 4).

The duration of passive immunity in the control animals

The group of lambs that had protective titres at Day 0, received a placebo of polyethylene glycol 6000, on day 0. Until Day 13 they had a protective level of 0,37 u/mℓ, which subsequently dropped to 0,09 u/mℓ on day 20. These animals remained susceptible until the last serum speciment was taken on Day 125.

The last group of lambs that had no maternal antibodies remained susceptible at 0,07 u/mℓ throughout the whole 125 days of the trial.

DISCUSSION

When a number of animals are hyperimmunized, some will respond more readily than others, because of biological variation. It is important therefore to select for those animals that show the highest titres for subsequent plasmapheresis to ensure that it remains a viable proposition. At this stage it is evident that plasmapheresis is a convenient method of obtaining hyperimmune serum without placing too much stress on the animal. It is also important to realise that the antibody titres to pulpy kidney should be monitored regularly to determine any decline in titres and to take corrective measures by administering the necessary boosters.

The polyethylene glycol fractionation technique is not the most ideal method available for the precipitation of the gammaglobulins from sheep plasma. Some definite disadvantages were demonstrated during these experiments. With each factionation step the volume doubled, the final solution became more viscid and difficult to administer parenterally and the final concentration of epsilon antitoxin units/mℓ, after fractionation, did not differ very much from the initial concentration.

A dosage of 200 units of epsilon antitoxin/kg hyperimmune serum, used throughout the trial, gave passive protection of the lambs for 21–29 days. Lambs with a protective maternal passive immunity, receiving the hyperimmune serum, showed a protective antitoxin level that lasted for 29 days. In the control group the passive immunity disappeared after 13 days, rendering the animals susceptible during the whole period of the trial. The group of lambs without any passive immunity, after receiving hyperimmune serum had a protective antibody titre lasting for only 21 days. This difference was statistically significant at a 95 % level of probability. In the other two groups of animals with or without maternal antibodies that received vaccine as well as hyperimmune serum, there was no significant difference between the duration of protective immunity. It is also important to note that there was no deleterious effect of the passive immunity on those animals with an existing active immunity conferred by vaccination.

If the dosage were to be increased to 1 000 units/kg, the period of protection would probably be longer. The great advantage of using hyperimmune serum for passive protection is the immediate response reflected in the increased circulating antibodies that appear within hours. As these animals were earmarked for slaughter, the problem of sensitization was not a great one. The fact that this hyperimmune antiserum was prepared from sheep might also result in a better tolerance of the product compared to that prepared from other animals.

At this stage it should be quite clear that hyperimmune serum should not be regarded as a substitute for enterotoxaemia vaccines but as an additional short term prophylactic treatment in animals with and without maternal antibodies. Its use is indicated wherever the risk of enterotoxaemia is elevated such as with new introductions into feedlots and sudden improvements in the level of nutrition. Used in conjunction with the Onderstepoort enterotoxaemia vaccines, it may help to bridge the gap

during the lag period between the 1st and 2nd injection in young lambs and may help to prevent unnecessary mortalities from *C. perfringens* type D.

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