

THE EFFECT OF PASSIVE IMMUNIZATION ON ACTIVE IMMUNITY AGAINST *CLOSTRIDIUM PERFRINGENS* TYPE D IN LAMBS

M. W. ODENDAAL⁽¹⁾, J. J. VISSER⁽¹⁾, N. BERGH⁽¹⁾ and W. J. S. BOTHA⁽²⁾

ABSTRACT

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Lambs in different stages of development of active immunity against *Clostridium perfringens* type D were treated with partially purified immunoglobulin in an attempt to superimpose a passive immunity on an existing or developing active immunity. Three different studies were undertaken to determine the impact of partial purified immunoglobulins on these vaccinated animals. In 2 of the 3 studies, active immunity was induced by administering the normal routine enterotoxaemia vaccinations and allowing the basic immunity to become established, for a period ranging from 2 weeks for the animals in study 1 and 4 months for those in study 2, before passive immunization with the partially purified immunoglobulins took place. An increase in the epsilon antibody titre occurred in each of the 2 studies after the animals were passively immunized with immunoglobulin, though this increase was not statistically significant ($P > 0.05$). In the 3rd study, when the animals were given the initial vaccination of the Onderstepoort enterotoxaemia oil adjuvant vaccine together with the immunoglobulin, an immediate increase in the epsilon antitoxin titre occurred that was statistically significant ($P < 0.05$) 2-14 days after administration.

No negative effects were noted on the development of an initial active immunity or an existing active immunity against *Clostridium perfringens* type D when they were passively immunized with partially purified immunoglobulin.

INTRODUCTION

The use of immunoglobulin to treat various infections amongst domestic animals have been utilized to include a number of specific diseases like calf diarrhoea (Murakami, Hirano, Inoue, Tsuchiya, Chitose, Ono & Yanagihara, 1986), clostridial enterotoxaemia in piglets (Ostle & Welter, 1987) and enterotoxaemia in lambs (Van der Walt, 1981; Hoefler & Hallford, 1985; Odendaal, Visser, Botha & Prinsloo, 1988). Normal ewe's serum was given to newborn lambs during the first 4 days of life, and although it only contained non-specific antibodies, it increased their survival rate significantly (Heath, 1985). Rats were passively protected with immune serum against intraperitoneal challenge with *Salmonella dublin* (Collins, Parsons & Jones, 1988).

The importance of clostridial infections amongst cattle (Knott, Erwin & Classick, 1985) and sheep (Tengerdy, Meyer, Lauerman, Lueker & Nockels, 1983) in feedlot systems, and the immunoprophylactic precautions to be taken, is well documented. In spite of an effective and readily available vaccine, 0.1-0.5 % of lambs still die from pulpy kidney 2-3 weeks after being allowed into the feedlot (Jensen & Swift, 1982). In a large feedlot this could amount to a considerable number of animals. Sheep normally receive a series of vaccinations including *Clostridium perfringens* type D (pulpy kidney or enterotoxaemia), when brought into a feedlot. Due to the fact that the vaccination history of these animals to pulpy kidney is mostly uncertain or unknown, it is usually assumed that they are susceptible and are subsequently vaccinated again. When susceptible sheep are immunized against *Clostridium perfringens* type D, it takes 10-14 days for the primary immune response to take effect (Kennedy, Norris & Beckenhauer, 1977; Dhein & Gorham, 1986). A booster, given after 4 weeks results in an immunity lasting for 6-8 months before another booster injection is

required (Cameron, 1980). It is during the 10-14 day lag period after the initial vaccination that animals, vaccinated for the first time, are still susceptible to pulpy kidney.

When susceptible lambs are passively immunized with immunoglobulin against pulpy kidney, antibodies are detectable within 1 h in the peripheral blood circulation (Van der Walt, 1981), and this protective level of circulating antibodies (mainly IgG) is maintained for 19 days (Odendaal *et al.*, 1988).

The ideal situation would be to combine the advantages of both passive and active immunization to ensure that an immediate immunity occurs within a short period after administration and that it is sustained for the period the animal remains in the feedlot. Potential problems to be clarified before this approach could be utilized include the effect of passive immunization on the development of an active immunity when these were induced simultaneously, as well as the effects of maternal antibodies on passive immunization and the effect of passive immunization on an animal that already had an existing active immunity.

The effect of maternal antibodies on passive immunization has already been investigated (Odendaal *et al.*, 1988), but the paucity of information regarding the influence of passive immunization on an existing active immunity as well as on a developing active immunity prompted us to undertake this study.

MATERIALS AND METHODS

Hyperimmune plasma production

This was previously described (Odendaal *et al.*, 1988).

Antibody assays

The antibody assays on sheep sera were done by the Quality Control Section of the Vaccine production unit, Veterinary Research Institute, Onderstepoort, by using the L+ test as described by Jansen (1967). For the purpose of this study 0.15 units/ml

⁽¹⁾ Roodeplaat Research Laboratories, P.O. Box 13873, Sinoville 0129

⁽²⁾ Veterinary Research Institute, Onderstepoort 0110

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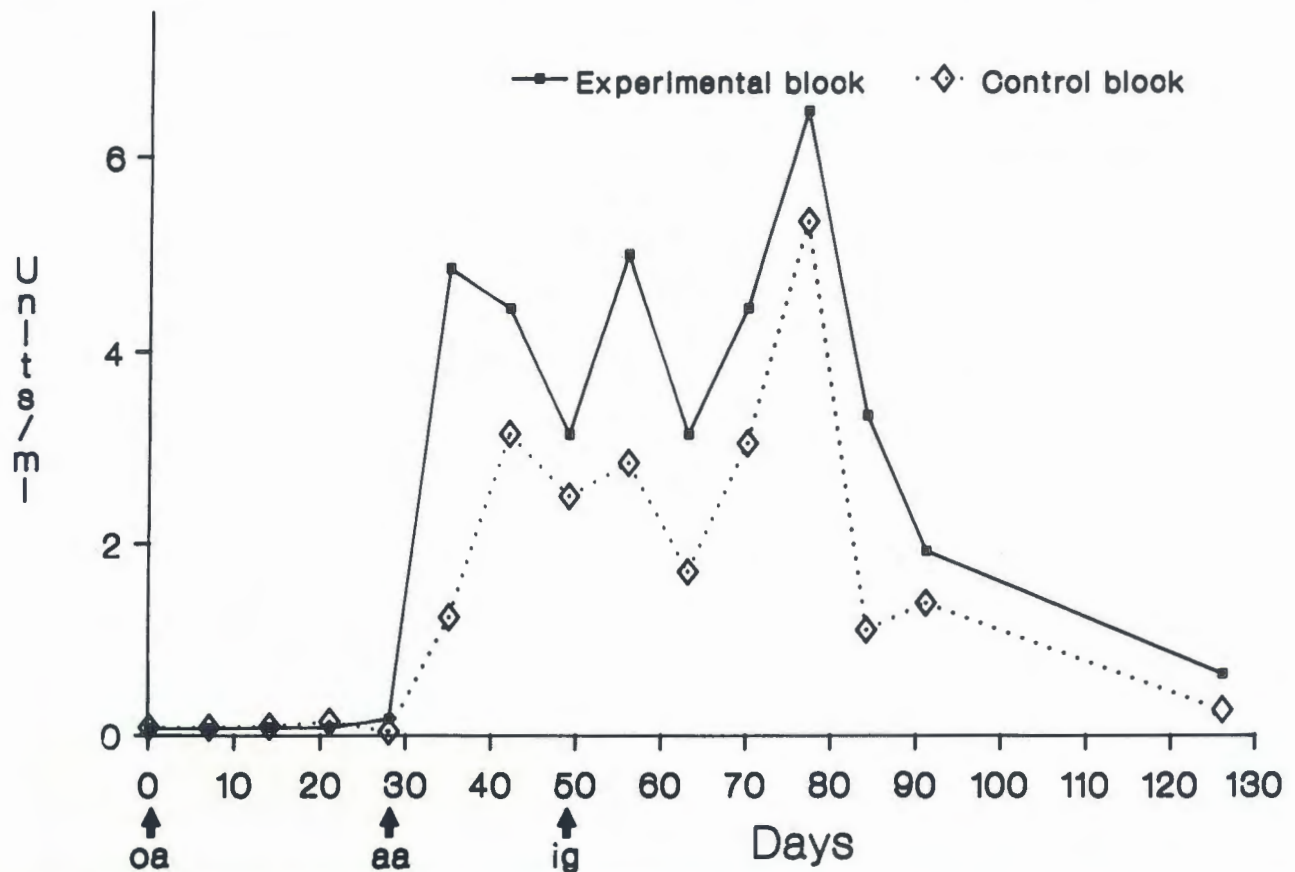


FIG. 1 The level of epsilon antitoxin titers in weaned lambs with a recently acquired active immunity with oil adjuvant (oa) and alum adjuvant (aa) enterotoxaemia vaccine. The experimental block was treated with immunoglobulins (ig) on day 49 ($P > 0.05$)

was taken as the minimum protective level of antibody present in the serum (Jansen, 1960).

Fractionation of hyperimmune plasma

The plasma was treated once with 20% and twice with 35% saturated ammonium sulphate¹. The final filtrate was reconstituted to the same as the original volume with physiological saline. Dialysis took place by means of ultrafiltration on a Millipore Pellicon² cassette system with a PTGC 10 000 membrane until there were no more ammonium sulphate ions present in the filtrate. The final product was filtered through a combination of Millipore filters and pre-filters² ranging from 1.2 to 0.22 μm and freeze dried in 5 ml quantities. The partly purified immunoglobulin had a final epsilon antitoxin concentration of 1 250 units/ml.

Plasma collected from animals with epsilon antibody titres of 0.07 uunits/ml and less were treated in the same way and used as a placebo.

Experimental design

Study 1: The influence of passive immunization in weaned lambs with recently acquired active immunity

In this group 8 post-weaned 3-month-old Merino type, cross-bred lambs were randomly assigned to the experimental and 7 lambs to the control block. These animals had no antitoxin titres to enterotoxaemia and were mass-measured, ear-tagged and dewormed upon arrival and housed in roofed pens with a cement floor. Lucerne hay and water was

given *ad lib.* in conjunction with a balanced commercial lamb pellet ration. On day 0 all the animals in the experimental and control blocks were vaccinated with the Onderstepoort enterotoxaemia oil adjuvant vaccine (> 30 Lf units/ml). This procedure was repeated on all animals on day 28 with the Onderstepoort alum precipitated vaccine (> 60 Lf units/ml). The animals in the experimental block were treated with the partially purified immunoglobulin (1 250 Lf units/ml) on day 49 at a dosage of 200 units/kg, whilst those in the control block received a placebo. Ten ml of serum was collected on day 0 and weekly thereafter until the 126th day. After collection it was allowed to coagulate at ambient temperature for 2–3 h then stored overnight at 10 °C before the serum was removed from the clot.

Study 2: The influence of passive immunization in sheep with a well established active immunity

The animals in this study consisted of eight 8-month-old Merino crossbred lambs that were equally divided and randomly assigned to the experimental and control blocks. These animals were housed under the same conditions as those in the first study and had already been vaccinated with the Onderstepoort oil adjuvant enterotoxaemia vaccine at 3 months and the alum precipitated vaccine at 4 months and had a basic protective active immunity. They were approximately 8 months old when they were given the second alum precipitated enterotoxaemia vaccine. After 14 days the experimental block was treated with partially purified immunoglobulin (1 250 epsilon antitoxin units/ml) at a dosage of 200 units/kg. The animals in the control block received a placebo. Ten ml of serum was collected on day 0 and weekly thereafter until the 126th day.

¹ Saarchem, P.O. Box 144, Muldersdrift 1747

² Millipore SA (Pty) Ltd, P.O. Box 391647, Bramley 2018

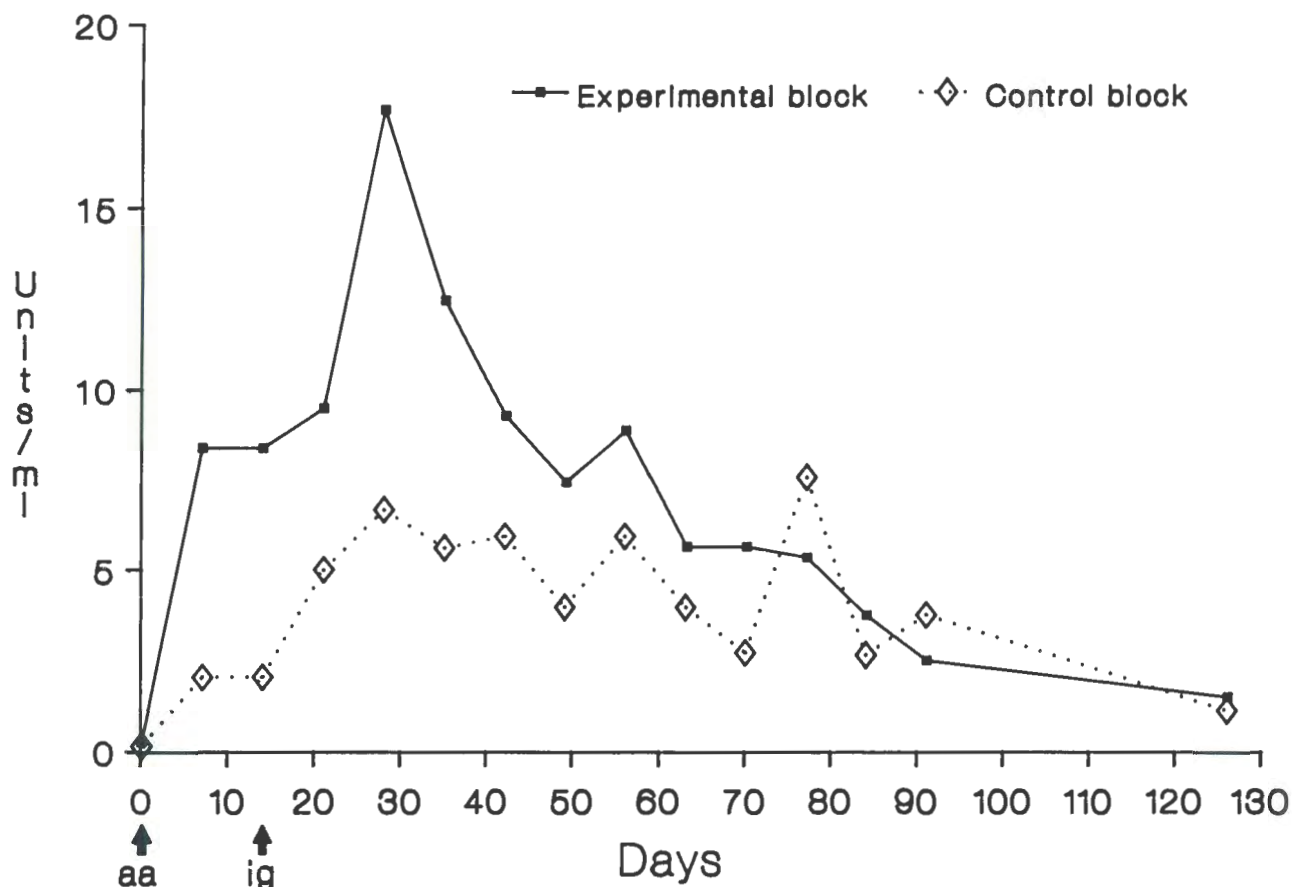


FIG. 2 The level of epsilon antitoxin titers in sheep with an existing active immunity that received the alum adjuvant vaccine (aa) on day 0. The experimental block received immunoglobulins (ig) on day 14 ($P > 0.05$)

The serum was treated in the same way as in the 1st study.

Study 3: The influence of passive immunization on the development of an active immunity in weaned lambs

A group of 10 post-weaned, 3-month-old Merino type cross-bred lambs were divided into 2 blocks with 5 replicates in each block and had no antitoxin titres to enterotoxaemia. The experimental block received the Onderstepoort oil adjuvant vaccine simultaneously with the immunoglobulin on day 0, whereas the control group only received the oil adjuvant. Both blocks were subsequently vaccinated on day 32 with the alum precipitated vaccine. Ten ml of serum was collected on day 0, and then after 3 and 4 day intervals till the 69th day. The serum specimens were collected and treated in the same way as those in the 1st and 2nd studies.

Statistical analysis

The geometric mean of the antitoxin titres collected from animals in the same replicate from each experimental block were calculated for each block from all 3 studies. These were statistically compared to those of the control blocks by means of the Mann-Whitney distribution free test.

RESULTS

Study 1: The influence of passive immunization in post-weaned lambs with recently acquired active immunity

Initially, these animals had no epsilon antibody

titres. In the lag phase, after receiving the primary immunization of oil adjuvant enterotoxaemia vaccine, both the experimental and control blocks demonstrated a lack of circulating antitoxins. It remained below the protective margin of 0.1 units/ml for 21 days. After receiving the alum precipitated vaccine booster the titres of the experimental and control blocks rose within 7 days to 4.86 and 1.23 epsilon antitoxin units/ml respectively (Fig. 1). When the partly purified immunoglobulin was given to the experimental block on day 49, the titre went up to 5.0 epsilon antitoxin units/ml. The corresponding antitoxin titres in the control block only reached 2.8 epsilon antitoxin units/ml. After 126 days both the experimental and control blocks experienced a drop in titres to 0.64 and 0.26 epsilon antitoxin units/ml, respectively. The statistical differences between the geometric mean of the epsilon antitoxin titres of the experimental and control blocks was not significant ($P > 0.05$) during the whole period.

Study 2: The influence of passive immunization in sheep with a well established active immunity

The animals in the experimental and control blocks had previously been vaccinated with the oil and alum precipitated enterotoxaemia vaccines and had titres > 0.15 antitoxin units/ml. On day 0 the antitoxin titre for the experimental block was 0.28 epsilon antitoxin units/ml and for the control block 0.16 epsilon antitoxin units/ml. After receiving the second alum precipitated booster the epsilon antitoxin titre of the experimental block rose to 8.4 units/ml whereas in the control block the cor-

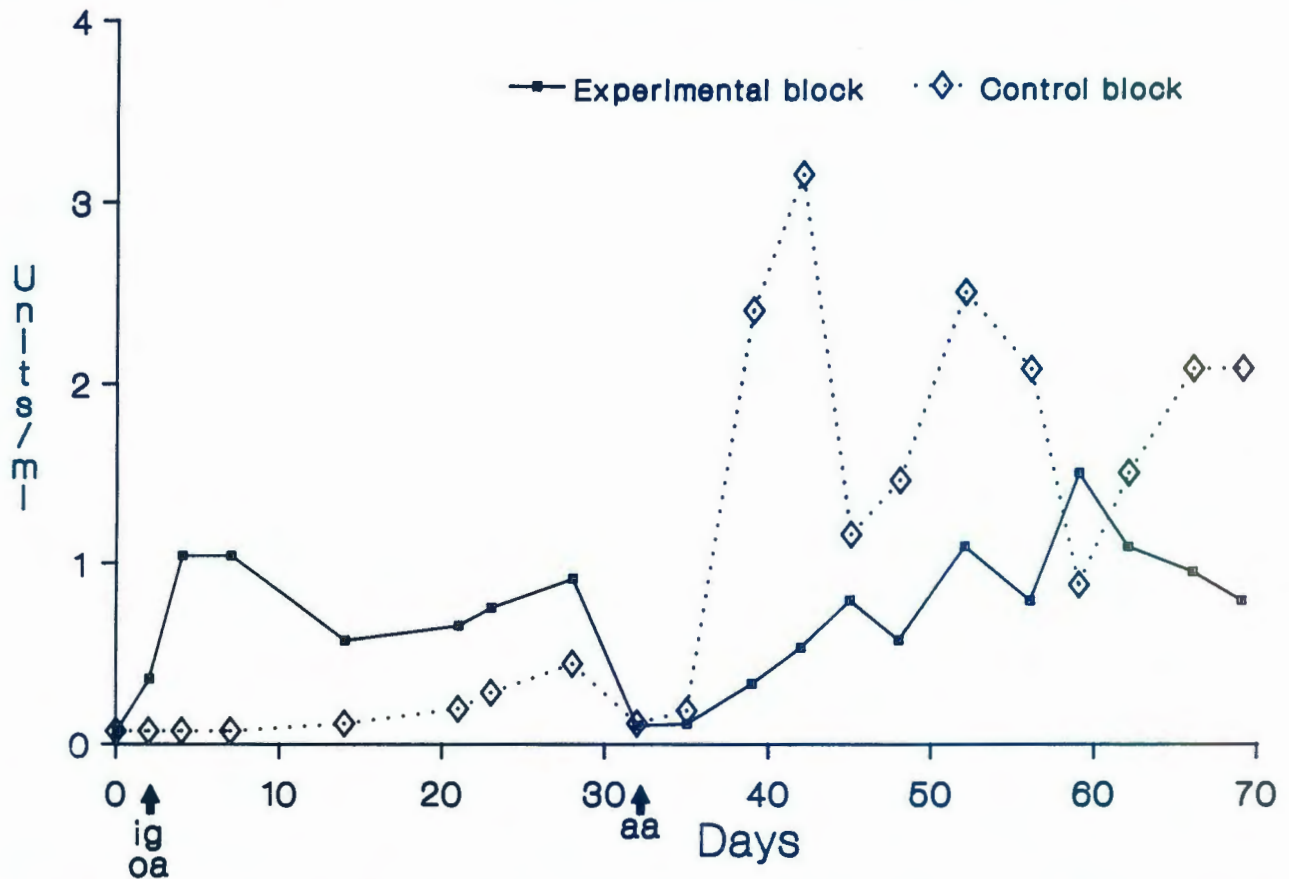


FIG. 3. The level of epsilon antitoxin titers in weaned lambs treated simultaneously on day 0 with oil adjuvant vaccine (oa) and immunoglobulins (ig) ($P < 0.05$) followed by alum precipitated (aa) adjuvant vaccine, 32 days later

responding titre was 2.04 units/ml (Fig. 2). Although the titre went up to 17.7 units/ml (day 28) for the experimental block, it only reached 6.68 units/ml in the control block. This difference was not statistically significant ($P > 0.05$). The antitoxin titres dropped slowly to reach a level of 1.49 epsilon antitoxin units/ml in the experimental block and 1.11 epsilon antitoxin units/ml in the control block after 126 days.

Study 3: The influence of passive immunization on the development of an active immunity in post weaned lambs

None of the animals in this group had been previously vaccinated against enterotoxaemia. The antitoxin titres in the experimental and control blocks were 0.07 epsilon antitoxin units/ml on day 0. Within 48 h after receiving 200 units/kg of the immunoglobulin subcutaneously, the antitoxin titres of the experimental block rose to 0.36 units/ml. It increased to 1.04 units/ml on day 4 (Fig. 3), went down to 0.57 units/ml (day 14) and then increased slightly to 0.91 units/ml on day 28, before it reached a low of 0.1 units/ml on day 32. The titres of the control block, representing the animals that only received the oil adjuvant vaccine, rose gradually to 0.11 units on day 11 and eventually to 0.28 units/ml on day 28. The statistical difference between the geometric means of the antitoxin titres between the experimental and control blocks, was significant ($P < 0.05$) during the first 2–14 days of the primary immune response. Hereafter the difference was not significant and remained so for the rest of the period.

The booster injection consisting of alum precipitated enterotoxaemia vaccine was given simultaneously to both blocks on day 32. The antitoxin titres from the animals in the control block went up to 3.15 units/ml (day 42) whilst the titres of the experimental group only reached 0.53 units/ml. The titres of the control block fell back to 1.46 units/ml (day 60) but improved slightly to reach 2.08 units/ml on day 69. The antitoxin titres of the experimental block showed a steady increase to reach 1.5 units/ml on day 59 before declining to 0.79 units/ml on day 69.

DISCUSSION

The fractionation of serum with ammonium sulphate was used to obtain partially purified immunoglobulins from the sera of various animals, including horses, sheep, rabbits and goats (Herbert, Pelham & Pittman, 1973), dogs (Halliwell & Longino, 1985), the mouse, hamster, guinea pig, monkey, chimpanzee, swine, chicken and cattle (Herbert, 1974). In a study that compared 6 different methods to purify immunoglobulin IgG, the salt precipitation procedure was recommended for routine use on grounds of its simplicity and minimal destruction caused to the immunoglobulin molecule during fractionation (Phillips, Martin & Horton, 1984). In the previous study polyethylene glycol 6 000 was used as the method of fractionation, but due to certain impracticalities (Odendaal *et al.*, 1988) its use was discontinued and the switch to fractionation with ammonium sulphate was made with these considerations in mind. The presence of fibrin in plasma

necessitates the initial fractionation of 20 % ammonium sulphate.

The animals in the experimental and control blocks of the 1st and 2nd studies were vaccinated with the Onderstepoort oil and alum precipitated enterotoxaemia vaccines. A predictable and normal active immunity developed in both these groups (Fig. 1 and 2) that was representative of animals exposed to the vaccine for the first time (Fig. 1) and animals that were already primed with previous exposure to this antigen (Fig. 2). The administration of partially purified immunoglobulin to the experimental blocks in studies 1 and 2, on day 49 and day 14 respectively, did not have any detrimental effect on the epsilon antitoxin levels at that stage. No significant increase or decrease in epsilon antibody titres were encountered when statistical comparisons were made to the titres of each control block. In a feedlot situation this would simulate the condition where the immune status of the animals concerned are not affected when treated with partly purified immunoglobulin against *Clostridium perfringens* type D.

In the 3rd study none of the animals had any previous exposure to the enterotoxaemia vaccine. The control block reacted as expected and did not show any epsilon antitoxin titres for the first 21 days. After the simultaneous administration of the immunoglobulin and the vaccine to the experimental block the epsilon antitoxin titre showed a dramatic increase which remained protective for the first 28 days. In this study the booster was given after 32 days, allowing the titre to fall below its required protective level. The interval between the primary and secondary injections should be restricted to 28 days. An interesting phenomenon occurred after the booster vaccination was given to both the experimental and control blocks. The reaction of the control block was much higher than that of the experimental block, though this difference was not statistically significant. Sufficient protection was still evident in both these blocks.

On the short term, the passive immunization of post-weaned lambs with partially purified immunoglobulin against *Clostridium perfringens* type D does not have a negative effect on an existing or developing active immunity. By using both the immunoglobulin and the Onderstepoort enterotoxaemia vaccine together, it was possible to bridge the 14-day gap that occurred after the primary vaccination and afford immediate protection to the animals concerned.

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