Optimum Time Interval between the First Vaccination and the Booster of Sheep for *Clostridium perfringens* Type D

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Abstract

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The goal of the present study was the determination of optimum time interval between the first and second vaccination of sheep against *Clostridium perfringens* type D with aluminium hydroxide-adjuvanted monovalent vaccine. Eight weeks after the first vaccination of 13 sheep aged 6 months, 12 animals had developed a higher and 1 animal a lower antibody titre than 0.1 IU/ml, which is protective under natural conditions. No correlation was found between the extent of the immune response and the length of the period between the immunizations in 24 sheep vaccinated twice at an interval of 2, 4 or 8 weeks. In spite of this, revaccination is suggested 8 weeks after the first immunization: because in this case most of the animals are protected continuously up to the time of the second vaccination, and the second antibody peak develops later. This vaccination schedule provides protective immune response for the longest period of time.

Enterotoxaemia, pulpy kidney disease, immune response, immunization, adjuvanted vaccine, antibody titre

Different authors suggest very different time intervals for the revaccination of sheep, even when similarly adjuvanted vaccines are used against *Clostridium perfringens* type D, the causative agent of enterotoxaemia. The proposed time interval between the first and second immunizations with aluminium hydroxide-adjuvanted vaccines ranges, for example, from 2 weeks (Kadymov 1975; Srinivasan et al. 2001) to 20 days (Pankratov et al. 1977), 4 weeks (Frerichs et al. 1975; Webster et al. 1985; Walker 1992), 2-6 weeks (Kennedy et al. 1977), and 6 weeks (Kerry et al. 1979).

Various data are available as concerns the effects of different time intervals between the first and second immunizations on the antibody level in the blood. Kennedy et al. (1977) found very small differences in the antibody responses of sheep revaccinated 2, 4 and 6 weeks after the first immunization. Their results did not prove the advantages of later revaccination, because the antibody levels of sheep vaccinated after the various time intervals were rather similar, though the level for the 4-week group was slightly lower. In spite of this observation, Géresi et al. (1984) found a significantly higher antibody response, when sheep were revaccinated after 3 or 6 weeks in comparison with those revaccinated 2 weeks after the first immunization. The aims of the present report were to determine the optimum interval between the first and the second vaccination of sheep with *Clostridium perfringens* ε-toxoid and aluminium hydroxide adjuvant-containing vaccine, and to examine whether the length of the interval has a definite effect on the level of the immune response.

Materials and Methods

The immune responses of 37 six-month-old, healthy, unvaccinated Merino sheep were checked. During the examination, the sheep were housed in a clean stable, and fed with hay. The subcutaneously administered vaccine

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was monovalent, containing *Clostridium perfringens* D ε-toxoid and aluminium hydroxide adjuvant; the dose for sheep immunization was 5 ml. The immune response of sheep was measured according to the European Pharmacopoeia (2002) using toxin neutralization test in mice.

The susceptibility of the sheep was proved individually before immunization: no *Clostridium perfringens* ε-antitoxin was found in the sera of the sheep by toxin neutralization test when the sensitivity of the system was 0.1 IU/ml. The *Clostridium perfringens* ε-antitoxin was an international standard, obtained from the Central Veterinary Laboratory (Weybridge, Surrey, UK); 0.1 L+ toxin was used. The mixtures of the serum dilutions and the test toxin were incubated for 30 minutes at room temperature. Two mice weighing 20 g were inoculated intravenously with 0.5 ml of the given mixture. The observation period was 72 hours.

Two sheep immunization tests were performed. In the first, the length of protective immunity was checked after a single vaccination. Thirteen sheep were immunized with the vaccine, and blood samples were taken 8 weeks after immunization to check the immune response.

In test two, the effect of the length of the period between the first and the second vaccination on the antitoxin level was investigated on 24 sheep. Twelve sheep each was immunized both in group A and B with the vaccine, 4-4 animals selected at random, and was revaccinated 2, 4 and 8 weeks later (Table 1). The sera of the four sheep in the same group were pooled for toxin neutralization test.

Table 1

Clostridium perfringens ε antitoxic values (IU/ml) of pooled sheep sera in case of different revaccination intervals

Time between 1st and 2nd immunization	Sheep groups	Prevaccination titres**	Time after the 1st immunization in weeks 2	4	6	8	10	12
2 weeks	A	< 0.1	0.1↓	3.0	2.3	2.6	1.9	nt
2 weeks	B	< 0.1	1.5↓	6.2	4.4	nt	1.2	nt
4 weeks	A	< 0.1	0.7	1.7↓	6.0	6.0	3.3	nt
4 weeks	B	< 0.1	0.1	0.5↓	1.7	1.7	0.7	nt
8 weeks	A	< 0.1	1.9	2.6	1.9	1.2↓	17.4	14.0
8 weeks	B	< 0.1	1.8	1.5	0.8	0.6↓	4.2	2.0

** = individual sheep sera were investigated
↓ = the time of 2nd immunization
nt = not tested

Results and Discussion

In test one, the *Clostridium perfringens* ε-antitoxin levels of 12 of the 13 sera eight weeks after immunization were 0.2-1.8 IU/ml, while the serum of the 13th sheep contained < 0.1 IU/ml antitoxin. The geometric mean of the antitoxin titres of the 12 sheep was 0.33 IU/ml. According to Thomson and Batty (1953) under natural conditions an antibody level of 0.1 IU/ml is protective against enterotoxaemia. The experiments showed that dosing sheep with phenothiazine in therapeutic doses can induce enterotoxaemia in animals harbouring *Clostridium perfringens* type D in their intestines, but an antibody level of 0.1 IU/ml prevents the development of the disease (Jansen 1960b). Webster et al. (1985) found that an antitoxin titre lower than 0.1 IU/ml caused susceptibility. Our results revealed that the sera of 12 of the 13 sheep contained protective levels of antibodies 8 weeks after one vaccination, only one animal remaining unprotected. Jansen (1960a) reported that a serum ε-antitoxin level of 0.15 IU/ml induced total protection against enterotoxaemia under experimental conditions, when the disease was generated by administration of dextrin and bacterium culture to the duodenum. In our experiment 12 sheep examined displayed protection at the higher requirement, too.

Table 1 shows the time schedule of blood sampling and the immune response indices of the sheep revaccinated 2, 4 and 8 weeks after the first immunization in test two. The highest antibody levels were found in group A, where the animals were revaccinated 8 weeks later, and in group B where the animals were revaccinated 2 weeks later. There was no correlation between the extent of the immune response and the length of the period between the first and second immunisations. As concerns continuous protection against *Clostridium perfringens*

D, an 8-week-long interval is suggested between the vaccinations among the three tested possibilities, because the first vaccination protects the animals efficiently for 8 weeks, and the antibody peak resulting from the second vaccination appears later than in the cases of the shorter intervals; with this schedule, therefore, the animals possess a protective antibody level for the longest period of time.

Optimální interval mezi vakcinací a revakcinací ovcí proti *Clostridium perfringens* typu D

Cílem studie bylo stanovit optimální interval mezi první vakcinací a revakcinací ovcí proti *Clostridium perfringens* typu D pomocí monovalentní vakcíny s aluminium hydroxidem. Za 8 týdnů pro vakcinaci 13 ovcí ve věku 6 měsíců byl u 12 zvířat titr protilátek vyšší a u 1 zvířete nižší než 0,1 IU/ml (za přirozených podmínek zajišťuje tento titr dostatečnou ochranu). Mezi velikostí imunní odpovědi a délkou intervalu mezi imunizací a revakcinací nebyla nalezena korelace u 24 ovcí vakcinovaných 2x v rozmezí 2, 4 nebo 8 týdnů. Přesto autoři navrhují revakcinaci po 8 týdnech od první vakcinace. V takovém případě je většina zvířat kontinuálně chráněna až do termínu revakcinace, a druhý vrchol koncentrace protilátek se objevuje později. Toto vakcinační schéma poskytuje nejdelší imunitu.

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