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The control of necrotic enteritis in sucking piglets by means of a *Clostridium perfringens* toxoid vaccine

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Abstract

Necrotic enteritis in sucking piglets constitutes a serious problem in piglet rearing units because of the high morbidity and mortality associated with the disease. The primary causal agent is *Clostridium perfringens* type C. The β-toxin plays a decisive role in the pathogenesis of this disease. A toxoid vaccine for use in sows has been developed and studied in field trials. The European Pharmacopoeia Monograph on vaccines for use in animals lays down a method of the efficacy testing based on the immunization of rabbits, the collection of pooled sera and the subsequent assay of anti-toxin antibodies in mice using an appropriate test toxin. The vaccine is regarded as effective if it induces a minimum of 10 IU of β-anti-toxin per ml of rabbit serum. We have established a range of 17.14–98.23 IU β-anti-toxin per ml rabbit serum induced by a sample of *C. perfringens* toxoid vaccine. The vaccine has been used under field conditions in different rearing units at the same time, mostly in the form of emergency vaccinations following the outbreak of disease. The outcome of vaccination was evaluated by recording the total numbers of piglets born alive and the piglet losses. Use of the vaccine, coupled with other measures, resulted in an approximately 30% reduction in the number of losses.

Keywords: *Clostridium perfringens*; β-Toxin; Swine; Necrotic enteritis vaccine

1. Introduction

Necrotic enteritis (NE) of sucking piglets may have a peracute, acute or subacute to chronic course and is mainly seen in the first 2 weeks post partum. In addition to the peracute disease course, resulting in death, most cases are characterized by a red-brownish diarrhoea, sometimes containing blood. This is accompanied by a hemorrhagic to hemorrhagic necrotizing inflammation of the middle and posterior sections of the small intestine. In view of the high morbidity and mortality rates, NE is a cause of serious financial losses in pig rearing.

Clostridium perfringens type C [3] is the primary etiologic agent of NE in sucking piglets. Type B has also been observed [2].

Experimental infection with *C. perfringens* type A was found to induce a serous catarrhal enteritis. No toxemic general disease, associated with the high mortality and characteristic for NE, was observed [7–10].

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The birth of weak piglets following infection of the population with PRRSV has recently been proposed as a predisposing factor for the appearance of disease [2]. In addition, infections with enterotoxic *Escherichia coli*, rotaviruses, coronaviruses and coccidia also exert a considerable influence on the clinical course of NE and its severity.

Oral infection of piglets, in most cases through teats smeared with faeces, leads to replication of *C. perfringens* type C in the intestines, resulting in the production of protein toxins (exotoxins), including β-toxin. The β-toxin is not degraded because of the low synthesis of digestive enzymes by piglets and the high anti-trypsin content of the sows' milk. It consequently has a decisive influence on the pathogenesis of NE.

The β-toxin initially causes considerable damage to the intestinal enterocytes and can lead to the complete disappearance of the brush border. At the same time, it causes disturbances in the terminal vascular system in the region of the villous vessels. The presence of β-toxins can be demonstrated in the mesenterial lymph nodes by this time, indicating that absorption takes place at an early stage [6]. The action of the toxins on the enterocytes culminates in their necrosis. With the epithelium exposed, the clostridia are now in a position to attach themselves to the villous stroma, i.e. to the basal membrane. Here, they rapidly bring about massive necrosis of the mucosa and submucosa, together with destruction of the villous vessels, causing leakage of erythrocytes into the lumen of the bowels. The level of β-toxin in the blood rises massively, resulting in enterotoxemia.

Vaccines are currently available for the prophylaxis of NE and to fight disease in piglet populations. They contain the toxoid of the β-toxin as their active ingredient. The European Pharmacopoeia Monograph '*C. perfringens* vaccines for use in animals' specifies requirements for the efficacy of the vaccine [1].

2. Materials and methods

2.1. Vaccine

These studies were performed with *C. perfringens* type C toxoid vaccine (Per-C-porc®), licensed for use in swine.

2.2. Efficacy testing under laboratory conditions

A total of three batches of the *C. perfringens* type C toxoid vaccine were tested in accordance with the Monograph in the European Pharmacopoeia. The underlying principle of the assay is to administer the vaccine twice in rabbits and then to determine the anti-toxin titer of the rabbit sera using a suitable test toxin in the mouse. The vaccine must induce at least 10 IU of β-anti-toxin per ml rabbit serum.

For each batch of toxoid vaccine, 10 female SPF rabbits, (CRL New Zealand Whites, 2–2.5 kg, Charles River, Germany) were given two doses at an interval of 21 days by the subcutaneous route.

Blood was collected from the rabbits, 14 days after the last vaccination by cardiac puncture. The sera of 10 animals per vaccine batch were pooled and frozen at −20°C.

The test toxin, LFA 2-1/94, used for testing the pooled rabbit sera in the mouse, was derived from a young liquid culture of *C. perfringens* type C (MS 3011/001/01/81). The culture was purified by centrifugation, ultrafiltration and precipitation of the ultrafiltrate with ammonium sulfate [5]. The sterile-filtered raw toxin fraction was then lyophilized.

The international standard for *C. perfringens* β-anti-toxin (Central Veterinary Laboratories, Weybridge) was adopted for calculation of the test toxin dose, by determination of the LD_{50} and the L+ dose for the mouse. The value was 0.0966 mg per mouse.

After establishing the test dose per mouse, the efficacy of the pooled rabbit sera was determined in accordance with the European Pharmacopoeia Monograph in one preliminary study and two main studies. The mice used for the study were female NMRI outbred mice, 18–20 g (Moellegaard Breeding Centre, Germany). Probit analysis (SPSS 5.0.2 for Windows) was used to determine the content of β-anti-toxin per ml rabbit serum.

2.3. Efficacy testing under field conditions

The vaccine Per-C-porc®, Batch number 50 05 92, was used in a piglet breeding unit which had suffered

Table 1
Results of efficacy testing of vaccine batches in accordance with the EP Monograph on *C. perfringens* vaccines

Batch number	Efficacy in IU β-anti-toxin ml^{-1} rabbit serum	
	Mean value	Confidence interval
50 05 92	17.14	13.87–24.95
51 04 93	45.17	41.65–53.59
52 06 93	98.23	85.84–128.87

massive losses through *C. perfringens* type C. Pigs of the breed 'Deutsches Edelschwein' were bred and farrowing was carried out in a 3-week cycle. Once the presence of the disease was established, the piglets were given a penicillin preparation by mouth in the first 3 days post partum (groups 188/189). From group 190 onwards, all dams were additionally vaccinated at 5 and 2 weeks ante partum with a single dose of Per-C-porc®. The efficacy of the vaccine was assessed through the total number of piglets born alive and the piglet losses before and after the prophylactic measures.

3. Results and discussion

3.1. Efficacy testing under laboratory conditions

The results for the efficacy of the vaccine batches are presented in Table 1. It can be seen that all batches satisfied the requirements of the EP Monograph on *C. perfringens* vaccines in that they induced at least 10 IU of β-anti-toxin per ml rabbit serum. The fluctuations in titer are related both to the toxin concentration of the fermentation product and the unavoidable biological fluctuations of the methods.

3.2. Efficacy testing under field conditions

The piglet losses before and after the use of the *C. perfringens* type C vaccine Per-C-porc® in a piglet rearing unit are presented in Table 2. The clinical picture of NE was observed from sow group 187 onwards. Once the disease was confirmed by pathological/anatomical and microbiological findings, the piglets of groups 187/188 were given 40 000 IU of benzylpenicillin potassium in the first 3 days post partum [4,11]. Administration of the penicillin shifted the peak of piglet losses from the first days post partum to the second week after birth. There was no reduction in the actual number of losses. The sows of groups 190, 191 and 192 were additionally vaccinated twice with the Per-C-porc®. The subsequent farrowing was characterized by a continuous reduction in losses and by the end of the study period had fallen to the baseline values. The clinical picture of NE was still seen, however, in isolated cases after vaccine use. These were probably piglets which had not received colostrum immediately after birth.

The studies clearly show that vaccination of sows with the toxoid vaccine and concomitant administration of a penicillin preparation in the piglets leads to a drastic reduction in piglet losses. At the same time, one batch which lays only marginally above the required 10 IU β-toxin per ml rabbit serum was nevertheless found to have a high level of efficacy under field conditions.

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Table 2
Losses before and after use of Per-C-porc®

Sow group	Piglets born alive	Losses (absolute)	Losses (in %)
186	1277	80	6.3
187	1370	100	7.3
188	1110	228	20.5
189	1315	468	35.6
190/191	3166	491	15.5
192	1443	78	5.4

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