Use of clinical signs in efficacy testing of erysipelas vaccines

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Summary

The efficacy of vaccines for veterinary use is usually ensured by well-controlled laboratory experiments in which vaccinated and untreated animals of the target species are challenged. The challenge should reflect as much as possible the natural conditions of the infection. Erysipelas challenge tests may cause extreme suffering of unprotected animals. High fever, apathy and large skin lesions are characteristics of acute erysipelas infection. A standardized challenge model and clear guidelines for humane endpoints should be given to allow treatment of these animals as soon as possible. We present an overview of clinical signs of erysipelas in pigs after experimental intradermal infection. The challenge model considers both the need for standardized challenge conditions and animal welfare issues.

Swine erysipelas is a worldwide bacterial disease of great economic importance. Vaccines are very effective in preventing the disease. The efficacy of vaccines for veterinary use has to be demonstrated by well-controlled vaccination (challenge) experiments in the target animal. The challenge should mimic as much as possible the natural conditions of the infection (Lensing *et al*. 1995). However, the challenge with *Erysipelothrix rhusiopathiae* strains may cause extreme suffering in the case of unprotected animals. On the other hand, *E. rhusiopathiae* is very susceptible to antibiotic or antiserum treatment and so unnecessary suffering can be avoided if clear guidance on humane endpoints is given.

So far, methods for challenge tests in pigs (Fortner & Dintner 1944, Möhlmann *et al*. 1961, Wellmann 1966, Anonymous 1982) (Table 1) use different modes of infection and challenge strains which may cause different clinical signs and different time courses for the disease. Therefore, a standard test for defining humane endpoints is required.

The *European Pharmacopoeia* monograph No. 64 for inactivated swine erysipelas vaccines does not contain regulations for such a test (European Pharmacopoeia Commision 1997). However, the monograph is under revision and will include a production section which requires an immunogenicity/efficacy test in pigs (Anonymous 1997). We propose a standard challenge model for assessing experimental infection, including clear guidelines for humane endpoints.

Materials and methods

Animals

We used 78 pigs for three test periods. They were 10-week-old females or castrated males, with a body weight of 20–25 kg. They were ordinary fattening pigs from a commercial pig farm and were free of antibodies against erysipelas.

Husbandry

Eight animals were randomly allocated to each group and housed together on straw under standardized conditions. They were fed with standard pellets twice a day.

Table 1 Advantages and disadvantages of different infection models

	Challenge methods			
Requirements	Intradermal	Scarification	Intramuscular	Intraconjunctival
Natural route of infection	Yes	Yes	No	Yes
Simultaneous use of different challenge strains (several serovars) possible	Yes	Yes	No	No
Application of a defined number of bacteria	Yes	No	Yes	Difficult
Spreading of bacteria from inoculation side	No	Yes	No	Possible
Assessment of local skin reaction possible	Yes	Yes	No	Difficult (other bacteria may cause conjunctival infection)

Table 2 Pig challenge trials

Trial No.	No. of vaccines tested	Immunization	Challenge strains (serovar)
A	2	Single vaccination	Gießen R 2 (1) NF 4 (2)
B	3	Single vaccination	A 360 (1) NF 4 (2)
C	2	Repeated vaccination after 3 weeks	A 360 (1) NF 4 (2)

Immunization

We used 7 erysipelas vaccines (3 mono and 4 combined vaccines) from different manufacturers. The pigs were immunized according to the route recommended by the manufacturer. The immunization schedule is shown in Table 2.

Challenge strains

We used a bacterial suspension of *E. rhusiopathiae* serovar 1 (strain A 360 or strain Gießen R 2) and *E. rhusiopathiae* serovar 2 (strain NF 4) . The challenge concentration of serovar 1 was about 10^7 cfu/ml and for serovar 2 was 10^6 cfu/ml. The inoculation volume used for each strain was 0.1 ml.

Challenge procedure

The challenge was performed 2 weeks after double vaccination or 3 weeks after single vaccination, respectively. Prior to vaccination, the skin was cleaned and disinfected and the inoculation site was marked with a water-resistant pen. 0.1 ml of each strain (serovar 1 and 2) was inoculated intradermally on one side with a disposable syringe (needle size: 0.33×12 mm) at a right angle and 1 cm deep into the skin, and at a distance of 5 cm from the mark (to allow for better observation of skin reactions). The animals were closely monitored for 10 days after the challenge.

Clinical signs monitored

Skin reaction, body temperature and changes in behaviour were used as clinical signs. If the rise in body temperature exceeded 2°C compared with day 0, the animals were treated with penicillin. Detailed criteria for the treatment are given in Table 3.

Table 3 Clinical signs of erysipelas after experimental intradermal infection

Course of disease	Skin reaction	Temperature	Behaviour	Remarks
With specific dermal erysipelas signs	Diamond-shaped skin lesions only at the inoculation site	High increase (rise > 2°C)	Apathy, anorexia	Therapy is necessary (penicillin or antisera application for at least 4 days)
	Diamond-shaped skin lesions at the inoculation site *and* generalized erysipelas	High increase (rise > 2°C)	Apathy, anorexia	Results in fever decrease within 12 h
	Diamond-shaped skin lesions at the inoculation site, *possibly* generalized erysipelas	No or only moderate increase (< 2°C)	No major change, still eating	No therapy necessary
Without specific dermal erysipelas signs	No skin reaction	High increase (rise > 2°C)	Apathy, anorexia	Therapy is necessary (penicillin or antisera application for at least 4 days) Results in decrease of body temperature within 12 h

Results

Skin lesions

All unvaccinated animals showed skin lesions typical of erysipelas at the inoculation side of serovar 1 within the first 24 h after infection. Mostly diamond-shaped skin lesions with a pink to dark red colour, sometimes with a dark necrotic centre, appeared, and in some cases generalized skin reactions were seen. The clinical signs of the vaccinated pigs were not as consistent as those of the control animals. A few vaccinated animals had skin reactions without pyrexia. This occurred only in trials A and B, where the pigs had only one immunization.

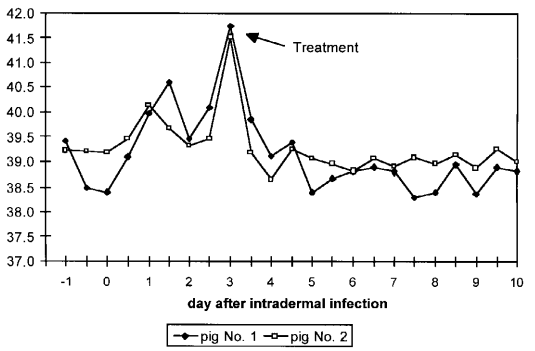

Figure 1 Body temperature of two unvaccinated pigs during the observation time

Fever

The body temperature did not correlate with the skin lesions in all cases. If fever occurred, the increase in body temperature took place within the first 3 or 4 days. The increase in body temperature was accompanied by sluggish behaviour, apathy and inappetence. Fig 1 shows the typical course of body temperature in the case of two control animals. Typical skin reactions of erysipelas were always evident at the inoculation site before body temperature increased. However, not all animals with skin lesions developed high fever. After treatment with long-acting penicillin the temperature dropped rapidly. All the pigs recovered completely within 2 days.

Overall, 82% of the vaccinated pigs from the three test periods showed no signs of disease. All vaccinated pigs with a re-vaccination after 3 weeks showed no signs of illness (Table 4).

Table 4 Results of erysipelas challenge tests in pigs (see also Table 2)

Trial→	A		B		C	
	Vaccinates	Controls	Vaccinates	Controls	Vaccinates	Controls
Skin reaction:						
Only serovar 1	1/16	1/8	4/24	5/8	–	3/6
Serovar 1 + 2	1/16	4/8	1/24	2/8	–	2/6
Generalized	–	3/8	1/24	1/8	–	1/6
None	14/16	–	18/24	–	16/16	–
Body temperature (compared with day 0)						
$< 2°C$	16/16	3/8	18/24	5/8	16/16	2/6
$> 2°C$	–	5/8	4/24	3/8	–	4/6

Discussion

Vaccination-challenge tests in the target species are required to demonstrate the immunogenicity and efficacy of veterinary vaccines. At present, no reference method is mentioned in the *European Pharmacopoeia*. However, the monograph on inactivated swine erysipelas vaccine is currently under revision and the new version will include procedures for such a test in pigs (Anonymous 1997). Several infection modes such as intramuscular application (Anonymous 1982), intraconjunctival (Möhlmann *et al.* 1961) or skin scarification (Fortner & Dintner 1944) are used, each of which has various disadvantages (see Table 1).

We propose an intradermal challenge method because it fulfils the requirements for a standard procedure. Furthermore, it offers the possibility of assessing clinical signs and of defining clear humane endpoints, at which treatment can be given at an early stage of the disease. The suffering of the pigs due to the systemic spread of the microorganism is thus minimized. We propose that this test model be included in the new version of the *European Pharmacopoeia* monograph on erysipelas vaccines. A detailed standard operating procedure and bacterial reference strains are available on request.

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