

Humane Endpoints in the Efficacy Testing of Swine Erysipelas Vaccines

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Summary

For licensing the efficacy of vaccines for veterinary use has to be demonstrated by well-controlled laboratory experiments in which vaccinated and untreated animals of the target species are challenged. Erysipelas challenge tests cause extreme suffering of the unprotected animals with high fever, apathy, large skin lesions, and even death.

This paper describes a standardised procedure for the vaccination challenge test and gives due consideration to the welfare of the animals. By monitoring and using clinical signs observed during the test it is possible to minimise animal pain and distress, thus preventing unnecessary animal suffering.

Zusammenfassung: Humane Endpunkte in der Wirksamkeitsprüfung des Rotlauf-Impfstoffes

Für die Zulassung von Tierimpfstoffen muss die Wirksamkeit durch streng kontrollierte Laborexperimente belegt werden. Hierbei werden geimpfte und ungeimpfte Tiere der Zieltierart infiziert. Rotlaufbelastungsversuche verursachen hochgradige Leiden bei ungeschützten Tieren, die mit hohem Fieber, Apathie und grossen Hautläsionen einhergehen und auch tödlich verlaufen können. Diese Arbeit beschreibt eine standardisierte Methode zur Durchführung eines Belastungsversuchs und berücksichtigt hierbei vor allem Tierschutzaspekte, in dem es durch eine intensive Überwachung und die Dokumentation auftretender klinischer Zeichen möglich wird, die Schmerzen der Tiere zu verringern und so unnötige Leiden zu vermeiden.

Keywords: *erysipelas vaccines, humane endpoints, vaccination challenge test, refinement*

1 Introduction

Erysipelas is a very old bacterial disease with a world-wide distribution. The disease has always been of great importance in pig farming but also for other farm animals such as sheep and poultry. It is a zoonosis that can cause wound infections in butchers, farmers and veterinarians but fortunately, the bacterium is highly sensitive to many antibiotics.

Erysipelothrix (E.) rhusiopathiae is persistent and wide-spread in the environment and can be found in the soil and slurry of piggeries. The following forms of the disease are known:

- Acute septicaemic form: accompanied by very high fever
- Urticarial form: showing pathognomonic urticarial plaques
- Chronic forms: associated with endocarditis and polyarthritis.

More than 20 different serovars of *E. rhusiopathiae* have been identified on

the basis of somatic antigens (Norrung, 1979), but Serovars 1 and 2 account for > 80% of all isolates from pigs (Wood and Harrington, 1979).

Soon after the first description of the germ by Robert Koch in the 1880's, the history of erysipelas vaccination started when Louis Pasteur proposed a vaccination scheme with attenuated cultures (Cussler, 1998). Nowadays, mainly inactivated vaccines which were invented in the 1940's are used. To guarantee their quality and efficacy, German scientists developed a challenge test in pigs in which they scarified the skin and applied virulent erysipelas strains (Fig. 1). By 1950, this kind of testing was introduced into German guidelines for state approved erysipelas vaccines (Cussler, 1998). In those days erysipelas was a notifiable disease in pigs.

Vaccination is still the most effective means of fighting the disease. Today, often combined vaccines are used. Even though the disease can easily be treated with antibiotics, infection is still widespread in the pig population and has a negative impact on productivity. Especially outdoor rearing bears a high risk for pigs to contract this disease. Therefore the use of erysipelas vaccines

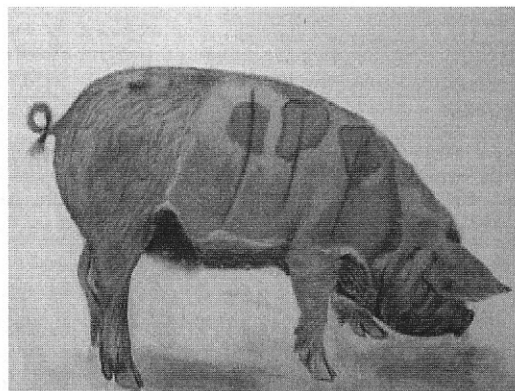


Fig. 1: Erysipelas skin lesions after cutaneous infection by scarification

contributes to improve the state of health and to reduce the use of antibiotics in farm animals. Consequently, vaccines are routinely used and are required to undergo extensive efficacy testing, in order to meet the licensing requirements.

Today, it is a legal requirement in many countries that animals have to be treated with respect and they should only be used for research when this is absolutely necessary (i.e. when there are no non-animal alternatives). When animals have to be used, as is the case for erysipelas vaccine testing at the moment, two further criteria have to be met. Firstly, only the minimum number of animals should be used to ensure statistical validity and, secondly, the suffering of animals should be reduced to a minimum, whilst still meeting the scientific objectives. This approach is defined as the Three Rs: Replacement, Reduction and Refinement. It was developed by two British scientists, Russell and Burch, in 1959 and is now part of the European regulations (e.g. Council of Europe, 1986).

The severity of the erysipelas challenge test raises animal welfare concerns and makes the test an ideal case for applying the Three Rs. While there are promising efforts to replace the challenge test in pigs by the use of serological methods (Gyra et al., 1998), there is insufficient evidence to rely on them to date. In particular, due to the high variability of individual animals and the large differences in the composition of the various vaccines it is very difficult to determine a protective antibody level in pigs. Keeping in mind the long period necessary for validation and acceptance of these kinds of test methods, it is realistic to assume that regulatory bodies will still require challenge tests for several years.

For registration of a new vaccine, its efficacy has to be shown for the target species in well-controlled reproducible experiments under laboratory conditions. Target animals are vaccinated and subsequently challenged with the micro-organism. The challenge should mimic the natural conditions of infection as much as possible, in particular with reference to the quantity and route of bacterial challenge. Such efficacy tests are diffi-

cult to perform because many different parameters have to be taken into consideration (Lensing et al., 1995). A standard operating procedure should include:

1. a route of infection that can be easily reproduced;
2. reference strains of micro-organisms of defined virulence;
3. specification of clinical signs;
4. the number and percentage of animals with clinical signs; and
5. the pass, fail and retest criteria.

The efficacy of vaccines containing more than one serovar of a micro-organism has to be demonstrated against each of the serovars of the pathogen that are of relevance in the field in separate experiments.

Inactivated erysipelas vaccines are usually produced using strains of serovar 2, which provides cross-protection against most other serovars (Takahashi et al., 1984), and efficacy has to be demonstrated in pigs (Johannes et al., 1998). A standard method and standard reference organisms are necessary to avoid practical problems (Sawtell, 1998) and to provide a reliable test (Lensing et al., 1995).

Regulations concerning the quality of inactivated erysipelas vaccines are laid down in the European Pharmacopoeia (Ph. Eur.) monograph 0064 (Council of Europe, 2000) and in the Code of Federal Regulations (USDA, 1982).

In Europe, immunogenicity and efficacy of erysipelas vaccines in pigs is demonstrated by intradermal challenge with strains of virulent *E. rhusiopathiae*. In the United States, potency testing of erysipelas bacterins in swine is performed using only one challenge strain and an intramuscular route for infection (USDA, 1982).

We describe a refined test that causes less animal suffering and involves the use of humane endpoints. Clinical signs, such as an increase in body temperature or skin lesions, are used as reliable surrogates to terminate a test at the earliest possible stage to avoid causing unnecessary suffering and death. The method allows us to combine

the application of two (or more) serovars of *E. rhusiopathiae* in one experiment, thus reducing the number of animals required. Furthermore, the test conditions and the challenge cultures are standardised and have been evaluated in several laboratories (Cussler et al., 2001). Consequently, no additional tests should be necessary to establish or evaluate this test.

2 Animals and Methods

The test according to Ph. Eur. requires using 15 pigs of the same origin and more than twelve weeks old. They must be free from erysipelas antibodies to ensure sensitivity to the infection. Ten pigs are vaccinated with the product under test and five are used as unvaccinated controls. Three weeks after vaccination the pigs are challenged with virulent strains of Serovars 1 and 2 of *E. rhusiopathiae* (Council of Europe, 2000) which are now available as European Biological Reference Preparations (BRP). The freeze-dried cultures are dissolved in the ampoules with saline and thoroughly mixed, but to ensure that the bacterial concentration is high enough, colony-forming units should be confirmed by plate counting.

Blood samples should be taken from all animals before vaccination to ensure freedom from antibodies against erysipelas. The persistence of maternal antibodies and the ubiquitous existence of the bacterium make it necessary to check the antibody status of the test animals using a sensitive method such as

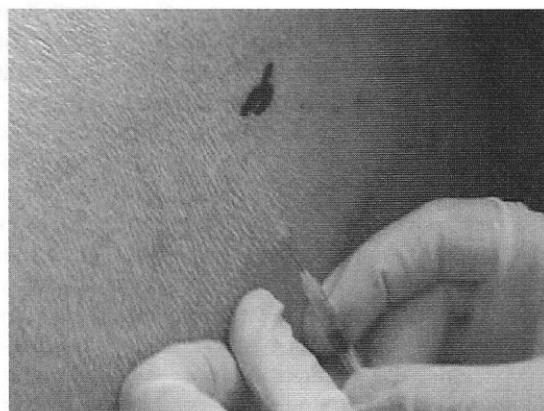


Fig. 2: Intradermal injection of *E. rhusiopathiae* culture



Fig. 3: Pathognomonic urticarial plaque at the injection site ("diamond skin disease")

ELISA before testing (Cussler et al., 1998).

The challenge is performed by an intra-dermal injection of 0.1 ml of a bacterial suspension (placed 5 cm below a water resistant pen skin mark for site location purposes, see Fig. 2). To ensure the deposition of a defined challenge dose at the inoculation site, the injection should be made with a thin needle to avoid reflux (e.g. 22G), parallel to the skin and 2 to 3 mm into the epidermis.

In older animals, it is possible to insert a 5 to 10 mm long needle vertically into the skin and to inject the challenge inoculum into a deeper region of the skin. However, experience to date indicates that the former technique results in a more consistent appearance of skin lesions (Cussler et al., 2000). To ensure that the challenge was appropriate and in accordance with the Ph. Eur., at least four out of the five control animals must show typical signs of infection with erysipelas, and at least 9 out of 10 vaccinated animals must be protected and must not show any signs of disease (Council of Europe, 2000).

3 Results

The requirements concerning challenge conditions for potency testing of veterinary vaccines as proposed by Lensing et al. (1995) are very difficult to achieve. The availability of reference strains is most important for a standardised test system. The BRP Serovars 1 and 2 used have been tested in several laboratories

to ensure that the disease is reproducibly induced (Cussler et al., 2001).

These challenge tests are an important animal welfare issue as they cause infectious disease and extreme distress at least in the unprotected animals. Refinement of these methods is therefore of great importance. The use of humane endpoints e.g. fever reactions and clinical signs such as skin reactions in the case of erysipelas disease are easy to monitor in pigs and should therefore be suitable parameters. The method also offers the possibility to test protection against several serovars simultaneously in the same pigs, thereby reducing the number of animals required.

3.1 Skin reactions

The use of intradermal inoculation has the great advantage of developing typical pathognomic signs at the inoculation site. Therefore, the development of infection can easily be monitored and controlled. The appearance of a small urticarial lesion at the inoculation site is the typical sign of the onset of infection soon after challenge inoculation, and often a necrotic centre develops in the lesion. However, the deposition of fluid in the epidermis may also result in a small reaction, such as induration on the first day after injection, but in contrast to an erysipelas skin infection, these reactions will not enlarge or spread and can be clearly distinguished over the next few days. During the following days, the lesions develop in unprotected animals to show a typical brick- or diamond-shaped appearance ("diamond skin disease", see Fig. 3) but other irregular shapes may also be found which can be extensive (Sawtell, 1998).

Lesions may spread from the inoculation site and appear on different parts of the body and, in severe cases, the whole animal can be covered. The appearance of skin lesions distant from the

inoculation site indicates a generalised infection (Fig. 4). In most cases, skin lesions appear at the inoculation sites but variability in their appearance has been reported especially for Serovar 2. Differences such as the SPF status of the pigs or the injection technique may have caused this variation but the numbers of animals affected so far have been too few to clarify the issue (Cussler et al., 2001).

3.2 Fever reaction

Fever is a typical reaction of the body to combat bacterial infection such as erysipelas. Monitoring body temperature in the animals should start a few days before vaccination to determine their baseline temperature and so they become used to the procedure. Monitoring should continue for at least 8 days after challenge and be measured twice daily. After intradermal challenge, a clear fever reaction usually accompanies the appearance of skin lesions. A short peak of fever may already appear on the first day after inoculation and this will result in a typical biphasic body temperature curve. The fever can reach very high levels and may kill the pigs, if they do not receive treatment. Development of high fever for 2 consecutive days after intramuscular infection is used to classify animals as non-protected in the erysipelas challenge test currently required in the United States (USDA, 2000). On the other hand, protected animals show no significant increase in temperature.

In acute erysipelas, a high body temperature may occur without any skin reactions. This can happen when the infection dose is very high or when the



Fig. 4: Generalised erysipelas infection

animals are extremely susceptible. In these cases, blood cultures should be taken before the experiment is terminated by antibiotic treatment or euthanasia to determine the erysipelas bacteria and serovar as the cause.

The results of studies performed independently in several laboratories have confirmed the suitability of the reference challenge strains (Cussler et al., 2001). All unvaccinated pigs challenged with these strains developed typical signs of acute erysipelas (high fever > 41.0°C and/or local skin reaction).

3.3 Use of humane endpoints

Erysipelas is a severe disease in pigs and the control animals in these tests may succumb to the disease. The clinical signs of erysipelas can be easily monitored using score sheets that document the clinical signs of skin lesions and fever (Johannes et al., 1999). The experiment should be terminated in the following circumstances:

- Reaction at the skin injection site together with high body temperature (> 2°C above baseline, or > 41.0°C).
- Very high fever reaction (> 41.0°C), anorexia and recumbency without skin reaction. In these cases, animals should be treated, but blood samples for culture should be taken first to demonstrate bacteraemia.

Depending on the kind of laboratory and the regulatory restrictions it may be necessary to euthanise the animals. However, the bacterium is still very sensitive to antibiotic and antiserum treatment and therapeutic intervention is possible. Treatment, e.g. Penicillin G 10000-30000 IU daily or long-acting penicillin for a minimum of five days should begin as soon as typical clinical signs have developed to ensure that unnecessary suffering is avoided. Effectiveness of treatment will be seen as the animals showing normal behaviour and feeding the next day, but it may take several days for the skin lesions to heal and disappear. Using this scheme and treatment, no case of death under test has so far occurred among the pigs. Antibiotic treatment should also be given to all unprotected animals at the end of the test observation period to avoid chronic erysipelas lesions such as endocarditis or arthritis.

4 Conclusion

We describe a vaccination-challenge test to demonstrate the efficacy of swine erysipelas vaccines. It utilises humane endpoints to avoid unnecessary animal suffering. The use of reference strains in a well-defined challenge procedure ensures good reproducibility and allows a better comparison between results obtained by different laboratories than existing protocols. This fact, together with the possibility to test more than one serovar in the same animal, considerably reduces the number of animals necessary for vaccine efficacy testing.

The increase in body temperature and the appearance of other typical clinical signs are reliable and permit appropriate humane endpoints to be used in these pig challenge tests. If a significant increase in body temperature (e.g. more than 2°C above baseline, or the temperature exceeds 41.0°C) and/or skin lesions occur, either in combination or separately, then the experiment should be terminated, either by administering an antibiotic treatment for at least five days or by humane killing of the animals. This reduces animal suffering considerably. The clinical signs of erysipelas are easy to observe and staff can be trained to recognise early stages of the disease.

This test design is in agreement with the revision proposal for the Ph. Eur. monograph on "Vaccines for Veterinary Use" which now includes a new paragraph on animal tests to encourage the use of the Three R alternatives approach (Council of Europe, 2001). Hopefully, in future many institutions and companies will use humane endpoints instead of lethality. Our moral and legal obligations to practise humane science and avoid unnecessary animal suffering has to be respected. It is anticipated that this refinement method is only an interim step on the way towards replacing the erysipelas challenge test by a serological method.

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