Endotoxin in porcine vaccines: clinical signs and safety aspects

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Summary

Endotoxin (lipopolysaccharide) is a constituent of the cell walls of Gram-negative bacteria and of vaccines prepared from such bacteria. High levels of endotoxin may give rise to a range of pathophysiological reactions, and adverse reactions due to the endotoxin content tend to be seen in animals following vaccination. For several porcine vaccines the maximum content of endotoxin is limited to 1×10^6 IU/dose by the *European Pharmacopoeia* but very little data are available to justify this limit. In this study, pigs of various ages were vaccinated with licensed vaccines and their endotoxin content was determined using the Limulus Amoebocyte Lysate (LAL) test according to the *European Pharmacopoeia*. The animals were intensively monitored for 48 h after vaccination, their body temperatures measured and blood samples taken for analysis including plasma endotoxin concentration. There was a clear relationship between the vaccine endotoxin content and changes in blood cell counts and the clinical picture, with elevated plasma endotoxin levels being correlated with an initial leucopenia followed by leucocytosis and clinical signs. In general, normal physiological values were regained 24 h after the injection of the vaccine, but young animals weighing between 10 and 40 kg were found to be very sensitive to a high endotoxin content. However, large differences between individual reactions were observed, and this was due not only to differences in the vaccine's endotoxin content but also in the type of bacteria used and the composition of the vaccine.

Vaccination is the most important preventive measure for avoiding infection in livestock. Usually healthy animals are vaccinated with inactivated or attenuated microorganisms. Depending on the kind of vaccine used, toxic products from the microorganism itself or from the production process may contaminate it. There should be a strict risk–benefit analysis between the possible damage due to the disease and the risks of a prophylactic vaccination. The safety of vaccines for veterinary use has to be thoroughly demonstrated as part of the licensing procedure. Safety tests are performed as part of the clinical studies to ensure a lack of adverse reactions in the target species after the application of a single dose, an overdose and the repeated administration of one dose of a vaccine: no significant local and systemic reactions should occur. Even reproductive performance and immunological functions have to be examined. Later, each batch of a licensed vaccine is tested in the target species, so a large number of animals of the target species is required for the safety tests.

Vaccines derived from Gram-negative bacteria may contain considerable amounts of endotoxin. Endotoxins are lipopolysaccharides (LPS) (Hitchcock *et al.* 1986) and are constituents of the bacterial cell wall. Free endotoxin can only be found after lysis of the bacteria. Endotoxin resists most common means of inactivation (heating, chemical detergents) and is found in varying amounts in all vaccines derived from Gram-negative bacteria. Systemic exposure to high levels of endotoxin in humans or other mammals results in numerous adverse sequelae (Cort & Kindahl 1980, Culbertson & Osburn 1980). Clinical signs such as fever, tachypnoea, vomiting, as well as changes in haemodynamics are seen after the injection of vac-

cines containing an elevated amount of LPS (Hussaini & Ready 1981). Fattening pigs are also very sensitive to stress, which can be induced e.g. by mass vaccination. In these animals, therefore, it is particularly difficult to distinguish between the effects of the vaccination procedure and those of harmful ingredients in the vaccine.

For several pig vaccines a maximum endotoxin limit of 1×10^6 IU/dose is accepted by the *European Pharmacopoeia* unless a higher amount has been shown to be safe, but pharmaco-vigilance reports and our own safety tests have indicated that vaccines derived from Gram-negative bacteria may cause untoward reactions due to their endotoxin content. The aim of this study was to set criteria for the assessment of adverse reactions in pigs caused by high levels of endotoxin in the vaccine, and the presenting clinical signs and blood parameters were investigated.

Materials and methods

Animals

Female and castrated male pigs (hybrid breed between Pietran, Landrace & Yorkshire) aged between 2 and 4 months were reared in groups of 5–6 animals in pens with straw bedding under conventional conditions. The animals were supplied with commercial compound feed twice a day, and water was provided *ad libitum* through nipple drinkers.

Vaccines

Eleven inactivated (8 mono- and 3 multi-component) products as well as 2 live attenuated vaccines, all licensed for the German market, were examined (Table 1).

Determination of endotoxin concentration in vaccines

The endotoxin concentration in vaccines was determined by the LAL test by the gel clot method according to the *European Pharmacopoeia*.

Immunization and blood samples

Each group of animals was treated with a different vaccine. At the beginning of each experiment (0 h) two pigs in each group were vaccinated with a single dose, two others received twice the recommended dose (inactivated products) or 10 × the recommended dose (live vaccines). Physiological saline was administered to the control animals. Blood samples were taken prior to vaccination and 1, 2, 4, 6 and 24 h after injection. The blood was centrifuged and the plasma stored at −20°C until the determination of its endotoxin concentration. Blood cell counts were determined by automatic blood cell analysis (Coulter-JT, Coulter USA)

Clinical signs

The animals were monitored closely for 24 h after vaccination for activity, food intake, behaviour as well as the functioning of their respiratory and cardiovascular systems. Rectal temperature was measured just before each blood sampling.

Determination of endotoxin concentration in plasma

A chromogenic kinetic method of the LAL was used to determine the concentration of endotoxin in the plasma (Spectramax 250,

Table 1 Vaccines used in the experiments and their endotoxin content

Vaccine	No. of vaccines being tested	Range of endotoxin concentration IU × 10^6/dose	No. of vaccinated animals (no. of controls)
Escherichia coli	4	0.24–2.4	23 (3)
Actinobacillosis	4	0.12–4.8	14 (5)
Multi-component	3	0.48–4.8	10 (4)
Live	2	0.30–3.0	7 (3)

Molecular Devices Corporation, USA; Pyrochrome LAL, Pyroquant Diagnostik GmbH, D-Weiterstadt).

Results

Clinical signs, described as endotoxin effects, were seen during the first 24 h after inoculation of the vaccines and were most pronounced in the first 2 h. However, due to the great individual differences between the animals, the spectrum (and degree) of clinical signs varied considerably. The results listed in Table 2 specify the percentage of pigs that showed typical signs of endotoxaemia. We noted a marked reduction in general activity, with some pigs in a somnolent dog-sitting position and others unable to rise for a certain period. Shivering was often seen in younger animals with a body weight of 10–40 kg. There was a loss of appetite after the administration of vaccines with an endotoxin content of more than 1×10^6 IU/dose, and some animals refused food for the whole of the observation period. The respiration rate was accelerated and elaborated, sometimes gasping. Pale mucous membranes and body surfaces were signs of circulatory disorders.

The increase in rectal temperature was nearly 1.5°C during the first hours after vaccine application (see Fig 1A).

After the application of high doses of endotoxin the number of circulating leucocytes initially decreased but then increased above the starting values; however, with

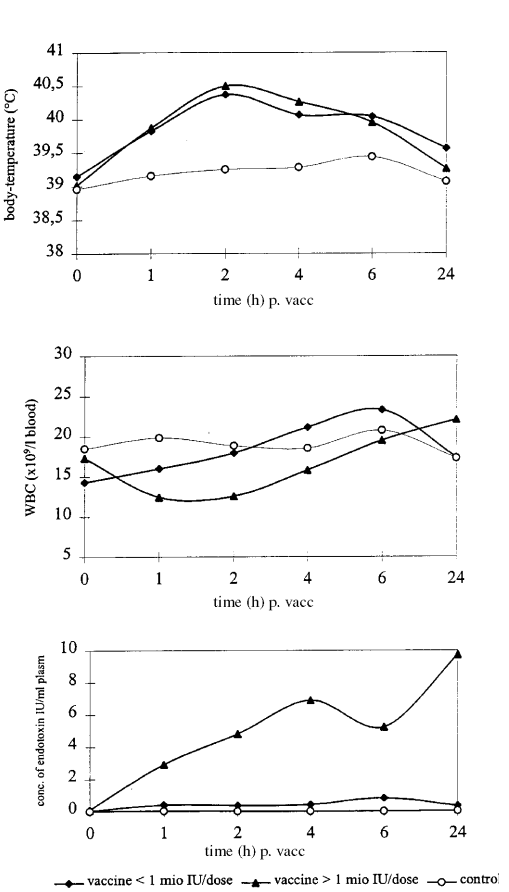

Fig 1 Body temperature (A), white blood cell count (B) and concentration of plasma endotoxin (C) after injection of vaccines containing less ($n = 44$) or more ($n = 25$) than 1×10^6 IU/dose endotoxin

Table 2 Clinical signs after injection of vaccines containing endotoxin less ($n = 44$) or more ($n = 25$) than 1×10^6 IU/dose

Clinical signs	Criteria	Vaccine endotoxin conc. IU/dose	% showing signs after 15 min	1 h	2 h	4 h	6 h	24 h
Behaviour	Restless, trembling	$<1\times10^6$	6.8	22.7	11.3	11.4	18.2	0
		$>1\times10^6$	36	60	52	28	24	12
Activity	Reluctant to move, recumbent	$<1\times10^6$	2.3	15.9	22.7	18.2	2.3	2.3
		$>1\times10^6$	12	44	52	48	12	8
Food intake	Refused	$<1\times10^6$	4.6	18	22.7	22.7	25	2.3
		$>1\times10^6$	16	60	60	60	48	16
Respiratory system	Accelerated respiratory rate	$<1\times10^6$	2.3	11.4	15.9	11.4	2.3	0
		$>1\times10^6$	32	40	36	8	12	0
Cardiovascular system	Pale mucous membranes, blue ears	$<1\times10^6$	6.8	13.6	13.6	6.8	13.6	4.5
		$>1\times10^6$	16	40	24	16	16	8

lower doses only a leucocytosis was observed (Fig 1B).

The analysis of endotoxin in the plasma of vaccinated animals demonstrated high concentrations in those animals which had been given endotoxin above the 1×10^6 IU/dose. Animals which had been given vaccines containing lower endotoxin levels showed endotoxin plasma concentrations just above the limit of detectability (see Fig 1C).

Control animals showed no abnormalities at all.

Discussion

Our results showed that animals absorbed endotoxin from the vaccine in a dose-dependent manner. The effects were more severe after intramuscular injection than after subcutaneous application (data not shown). Elevated endotoxin concentrations in plasma led to an increase in body temperature and immune parameters like cytokine levels (data not shown) and white blood cell counts were also affected.

Due to today's breeding and housing conditions, pigs are very stress-susceptible. However, the control pigs in our experiments never showed abnormal reactions due to the sham-vaccination or bleeding, and so the effects noted in vaccinated animals are almost certainly due to the endotoxin content of the vaccines. Therefore, the endotoxin limits in veterinary pig vaccines should be re-evaluated, as the results of our study clearly show the need to establish specific criteria for safety testing of endotoxin-containing vaccines to avoid unnecessary suffering in vaccinated pigs. It should be clearly stated in the various *Pharmacopoeias* how test animals should be monitored, e.g. using clinical signs and score sheets, and the measurement of leucocytes seems to be a good indicator for distinguishing between the immunostimulating effect of low doses of endotoxin and the immunosuppressive effects of high levels. Attempts to optimize test procedures by improved clinical examination parameters should result in a reduction of animal usage and be in line with the thinking of Russell and Burch (1959) and Winckler and Breves (1997). A refinement in the test procedure might also lead to a reduction in the number of animals used for batch testing.

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