

## Passive immunoprophylaxis of edema disease in weaned piglets

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**ABSTRACT:** Hyperimmune sera against Shiga toxin Stx2e with antibody titres 8 000–32 000, prepared by immunization of slaughter pigs, were administered subcutaneously to piglets ( $n = 73$ ) 3 days after weaning. The titres of Stx2e neutralizing antibodies in sera of piglets ranged from 16 to 512 following application of the immune serum. In most of the piglets, passively acquired antibodies disappeared during 4 to 8 weeks after application. The recorded serum titres were in all of the piglets 8 or higher fourteen days following administration. In another experiment, an immune porcine serum with antibody titre against Stx2e of 130 000 was applied subcutaneously into a knee fold to 30 piglets. The serum was applied at the dose 3 ml, 1.5 ml, 0.75 ml, 0.4 ml, 0.2 ml, 0.1 ml, and 0.05 ml based on the group of piglets. Antibody-free serum was applied to pigs of the control group ( $n = 5$ ). The following day, supernatant of STEC culture with Stx2e titre of 150 000 was applied to piglets of both experimental and control groups into the opposite knee fold that the above serum. Piglets that received 0.2 to 3 ml of serum remained unaffected throughout the experiment following application of supernatant containing Stx2e. Piglets that received 0.05 ml of serum and those of the control group showed severe clinical manifestations of edema disease following Stx2e application and were sacrificed ante finem. Marked signs of the disease could already be observed 18 hrs following Stx2e application. In a group of 3 piglets that received 0.1 ml of serum, mild paralysis was observed in 1 piglet 18 hrs following Stx2e application.

**Keywords:** coliinfections; *Escherichia coli*; Shiga toxin; Stx2e; verotoxin; VT2e

Edema disease of pigs is a serious affection causing great losses of weaned piglets. *Escherichia coli* strains producing verotoxin and denoted also as verotoxigenic *E. coli* (VTEC) have been implicated as the cause of edema disease in swine. These strains colonize intestine of weaned piglets and produce a toxin in the gut that is lethal for Vero cells and therefore termed verotoxin (Konowalchuk et al., 1977). Verotoxins of *E. coli* are also called Shiga-like toxins (SLT) because of their close relationship to Shiga toxin produced by *Shigella dysenteriae* (O'Brien and LaVeck, 1983). At present, designation which is based on relationship to Shigella toxins is recommended and the toxins are called Shiga toxins (Stx1, Stx2, Stx2c, Stx2e) (Calderwood et al., 1996), and *E. coli* strains producing toxins are designated Shiga toxigenic *E. coli* (STEC). Shiga toxin produced

by *E. coli* isolates from pigs with edema disease is a variant of Stx2 (Marques et al., 1987) and is designated Stx2e. This term corresponds with the previous designation VT2e or SLTIIIv. Most of the edema disease *E. coli* strains are assigned to the serogroup O139. In some of the strains, fimbrial antigen F18ab has been demonstrated (Rippinger et al., 1995; Wittig et al., 1995).

In pigs, edema disease is being developed as a toxemia. It is chiefly a disease of weaned pigs with signs occurring within one week after weaning. In case of feeding medicated feed mixtures to piglets immediately after weaning, the condition may develop in older animals. Data are known from the literature about sporadic cases of edema disease in older animals (Sydler et al., 1996). In herds, in which edema disease occurs, either all piglets are

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affected in a litter or only individual piglets in another litter. Some litters are not affected with the disease at all.

Clinical signs of edema disease include swelling of the eyelids, staggering gait followed by paralysis, and peculiar squeal or snoring sound. Severe infections may result in sudden deaths without apparent clinical signs. *Apost mortem* examination determines pathological changes in various parts of the body. Most frequently edema is seen in the eyelids, in the frontal area, over the belly, the wall of stomach and mesocolon. Histological examinations reveal edema in the brain. In piglets, Stx2e induces characteristic lesions as is fibrinoid necrosis of arterioles, brain hemorrhage. Higher doses of Stx2e result in surface epithelial necroses of colon and lesions of kidney (Gannon et al., 1989).

Experimental induction of edema disease is very difficult. Susceptibility of piglets to edema disease depends on several factors, especially on genetic resistance, diet, and immunity (Smith and Linggood, 1971; Bertschinger et al., 1978; Bertschinger and Gyles, 1994). Susceptibility to infection with strains possessing the colonization factors F18ab is conditioned by the presence of a receptor on enterocytes. Sensitivity to adhesion is governed by a dominant (B) allele designated ECF18R, and resistance by a recessive (b) allele on the chromosome 6 (Vogeli et al., 1996).

The absence of circulating neutralizing antibodies against Stx in serum is considered to be the decisive factor in edema disease pathogenesis (Gannon et al., 1988). Smith et al. (1983) carried out a large study with *E. coli* Shiga toxins and concluded that Shiga toxins of *E. coli* strains isolated from pigs are very poor immunogens. To induce production of antitoxic antibodies, a long-term immunization was needed. Crude supernatants of porcine STEC strain cultures contain a small amount of Stx2e (Smith et al., 1983; Alexa et al., 1998) and it is probably the cause of its putative weak immunogenicity.

MacLeod and Gyles (1991) immunized pigs with a purified toxoid Stx2e, inactivated with glutaraldehyde. They demonstrated induction of neutralizing antibodies in pigs and a subsequent protection against Stx2e lethal dose. Johansen et al. (1997) used in field trials a vaccine prepared from an inactivated purified toxin with a lipid adjuvant to vaccinate piglets in herds with persisting edema disease. In the vaccinated groups of piglets, a considerable decrease of mortality was recorded, compared to control groups. The increase in antibodies neutralizing

Stx2e was observed in blood sera of piglets. Based on our experimental results (Alexa et al., 2002), a toxoid vaccine for active immunoprophylaxis of piglets against edema disease has been commercially produced in the Czech Republic.

MacLeod and Gyles (1991) prepared a serum with high titre of neutralizing antibodies to VT2e by immunization of pigs. Application of the sera confirmed its protective effect against lethal dose of VT2e. Based on our preliminary results (Alexa et al., 1998) and data from the literature (Johansen et al., 2000), application of antitoxic antiStx2e serum appears to be a possible protection against edema disease. The objective of our study was to determine the protective effect of parenterally applied antitoxic serum against Stx2e, the minimum dose and the period of persisting of antibodies in porcine sera.

## MATERIAL AND METHODS

**Antitoxic serum.** The serum was prepared by repeated immunization of slaughter pigs with vaccine prepared from a toxoid of Stx2e and a lipid adjuvant (Alexa et al., 2002). After slaughtering of pigs, blood was aseptically collected and the serum was recovered and consequently preserved with 0.5% phenol.

**Titration of Shiga toxin neutralizing antibodies.** This examination was carried out on VERO cell line. Blood sera inactivated by heating to 56°C for 30 min were serially diluted starting with 1 : 2 in MEM supplemented with antibiotics. Equal quantities of *E. coli* supernatant containing Shiga toxin Stx2e at the concentration 10 CD<sub>50</sub>, diluted in MEM, were added to all dilutions. Cytotoxic dose CD<sub>50</sub> is a dose of toxin, inducing cytopathic effect in 50% of Vero cell lines. The mixtures were allowed to stay overnight at room temperature. The following day, 60 µl of the mixtures from different dilutions were applied to the wells of Nunclon plates and 90 µl volume of Vero cell suspension at a concentration 3 × 10<sup>5</sup>/ml was added. The results were evaluated microscopically following incubation at 37°C in 5% CO<sub>2</sub> for 72 hours. The last dilution of serum, which inhibited degeneration of Vero cells by 50%, gave the titre of neutralizing antibodies.

**The dynamics of antibodies in the sera of pigs.** The dynamics of passively acquired antibodies to Shiga toxin St2e in serum was determined in conventional weaned pigs (*n* = 73) without clini-

cal signs. Pigs 10–18 kg body weight, 3 days after weaning, were applied subcutaneously antitoxic sera at the dose of 3 ml per animal prepared by immunization of slaughter pigs. The titres of Stx2e neutralizing antibodies in the applied sera ranged between 8 000 and 32 000. Blood was collected from pigs the following day after serum application and then on day 7, 14, 28, 42 and 56 after the application to determine the titres of Stx2e neutralizing antibodies.

**Determination of the toxic dose.** Supernatant of Shiga-toxigenic *E. coli* strain with a titre of Stx2e 150 000 CD<sub>50</sub> was applied intramuscularly at a dose 0.5 ml and 1 ml to conventional suckling piglets ( $n = 12$ ), 5–6 kg body weight, prior to weaning. Health status was monitored for 7 days. The sucklings were chosen to eliminate a possible effect of Shiga toxin developed after a natural infection of weaned pigs by STEC. During health status monitoring, rectal swabs of piglets were examined for the presence of STEC in the gut.

**Determination of the protective dose of antibodies to Stx2e.** Thirty conventional piglets (7–9 kg body weight) immediately after weaning were included into the experiment. Two experiments of the same design were carried out. Experimental and control groups were formed by randomly selected pigs. Porcine immune serum with antibody titre to Stx2e of 130 000 was applied subcutaneously into the knee fold to piglets in the experimental groups (3–5 animals each). The dose of serum in the individual groups was 3 ml, 1.5 ml, 0.75 ml, 0.4 ml, 0.2 ml, 0.1 ml, and 0.05 ml. The serum that was determined to be applied at the dose of 0.2 ml to 0.05 ml was diluted with physiological saline 1 : 10 so that the applied dose was not lower than 0.5 ml. Piglets of the control groups were applied 3 ml of porcine serum containing no antibodies to Stx. The following day, 3 ml of STEC culture supernatant with Stx2e titre of 150 000 CD<sub>50</sub> was applied to pigs of both experimental and control groups into the opposite knee fold than the above serum. Rectal swabs were collected continuously so that STEC could be detected in the gut of experimental piglets. Health status of pigs was monitored for 14 days following serum application. Piglets with severe health disorders were euthanized ante finem using thiopental. Dead or euthanized pigs were subject to pathological examinations.

**Examinations of rectal swabs.** Rectal swabs were cultured on blood agar containing 5% ovine blood, and on McConkey agar. Five preferentially hemo-

lytic *E. coli* strains were isolated from each of the swabs. In these strains, typing of O-antigen was carried out by a slow agglutination with specific antisera, and in the representatives of the same O-serogroups, examination to determine production of Stx2e using PCR was performed as described previously Alexa et al. (2000).

## RESULTS

### The dynamics of antibodies following serum application

Twenty-four hours after serum administration, a considerable increase in titre of serum neutralizing antibodies was observed. The titres of Stx2e neutralizing antibodies ranged in piglets from 16 to 512, depending on the dose and body weight. In most piglets, the passively acquired antibodies disappeared within 4 to 8 weeks following administration. Fourteen days following administration, serum titres were in none of the piglets lower than 8.

After administration of serum with higher antibody titre, serum titres in piglets were higher than in piglets to which serum with lower titre had been administered. However, the level of antibody titre in the serum of piglets was not directly dependent on antibody titre in the applied serum. Variability of titres existed in all groups, which was likely due to individual differences in antibody resorption following administration. Although, variability of the initial serum titres was relatively high among the pigs, a similar course of titre decrease was observed in all the pigs (Figure 1).

### Toxic dose of STEC culture supernatant

Only sporadic mild changes of health status were observed in piglets to which STEC culture supernatant at the dose of 0.5 ml or lower was applied. Of 6 piglets injected with 1 ml supernatant, 3 developed the signs of edema disease characterized by swelling of eyelids and neurological disturbances. The other 3 piglets showed no clinical signs of edema disease. Based on these results, supernatant at the dose of 3 ml was applied to other 3 piglets. All 3 piglets developed severe signs of the disease within 24 hours. Staggering gait was followed by loss of control of hind limbs and paralysis. Two piglets died within 48 hours following supernatant admin-

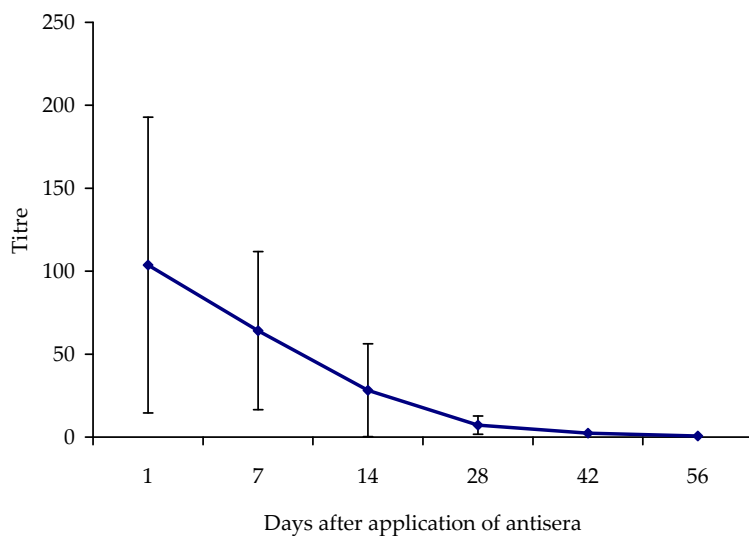


Figure 1. The dynamics of antibody titres neutralizing Sx2e in sera of piglets following application of antitoxic sera

istration, one animal was sacrificed *ante finem*. *Post mortem* examinations revealed subcutaneous edema over the body and in mesocolon.

### Determination of protective dose of serum

The results of our experiments are summarized in Table 1. Piglets which received 0.2 to 3 ml of serum remained healthy throughout the experiment after application of Stx2e. Neither health disorders nor pain at the injection site and around were observed immediately after subcutaneous administration of Shiga toxin to the piglets.

Table 1. Protective effect of anti Stx2e immune serum applied subcutaneously to piglets against *E. coli* culture supernatant with Stx2e. The titre of Stx2e neutralizing antibodies in the administered serum was 150 000. The titre of the administered Stx2e was 130 000

Dose of serum (ml)	Dose of Stx2e (ml)	Clinical ED/total (No. of piglets)	Dead/total (No. of piglets)
3.0	3	0/5	0/5
1.5	3	0/5	0/5
0.75	3	0/3	0/3
0.4	3	0/3	0/3
0.2	3	0/3	0/3
0.1	3	1/3	1/3
0.05	3	3/3	3/3
Control	3	5/5	5/5

Mild disturbance of movement was observed in 1 piglet 18 h following application of 0.1 ml serum. The symptom progressed and after 24 hours loss of control of the limbs was observed. The animal was sacrificed and the subsequent *post mortem* examination revealed no apparent signs of edema disease. The only thing observed were jelly-like infiltrates at Shiga toxin application site. The remaining two piglets appeared clinically normal over the experimental period.

Piglets of a group that received 0.05 ml serum and those of the control groups developed signs of severe edema disease after Stx2e administration. Considerable signs could be already observed 18 hours after Stx2e application. The animals moved with difficulties, muscle tremor was seen and one piglet died within the following 24 hours. Recumbency without a coordinated movement was seen in other animals. The affected piglets were euthanized. Necropsy of piglets that died following Shiga toxin application revealed changes at the application sites, characterized by tissue edema, jelly-like infiltrates and local congestions.

Marked subcutaneous edema and edema of mesocolon could be seen in all piglets at necropsy. No STEC were detected in the rectal swabs of experimental and control animals.

### DISCUSSION

The increase in Stx2e neutralizing antibodies was observed following the application of immune serum. Based on our experience, anti Stx2e antibod-

ies in the serum of piglets are acquired by serum application. Our previous examinations did not reveal Stx2e neutralizing antibodies in non-vaccinated piglets and in those to which antibodies were not applied. The same situation was observed even though Stx2e neutralizing antibodies were detected in dams' sera (Alexa et al., 1998). For that reason, anti Stx2e antibodies were not examined in piglets prior to serum administration.

Although we assumed that antibody titres in piglets of the same body weight will be the same at the same dose and titre of serum, variability among individuals occurred. In spite of variability, the mean titre in the group that received serum dose of higher titre was higher compared to the group with serum dose of lower titre. There can be several causes why the titre in piglets following serum application did not strictly correlate with the dose. The differences can be caused by different rate of resorption from the application site. A partial absorption of neutralizing antibodies could occur by STx2e resorbed into blood after natural STEC infection. In some cases, natural infection of piglets with STEC strains could not be excluded as the experiment was carried out under the conditions of conventional breeding. Propagation of verotoxigenic *E. coli* strains in the intestine of piglets and subsequent drop of antibody titre could not be prevented during the experiment. In spite of the above facts, fourteen days following administration serum titres were in all piglets higher than 8. The curve characterizing the course of antibody decline in sera was nearly identical in all piglets.

In experiments determining the protective dose of serum, a three-fold dose inducing the signs of mild edema disease in 50% of piglets was taken as a base. This was a lethal dose to all piglets in a consequent application. Some of the piglets were euthanized ante finem. MacLeod et al. (1991) induced mild edema disease by injecting 1.5 ng Stx2e, and more severe signs of edema disease by a two-fold dose of the toxin when 3 pigs of 8 had to be euthanized. We assumed the dose of toxin used in our experiment to be sufficient for the assessment of protective effect of the serum. Under the natural course of edema disease, animals with mild signs can be observed in a herd, as well as those severely affected that do not survive. However, in our experiments all piglets that received 3 ml of supernatant of STEC culture with Stx2e titre 150 000 died. MacLeod and Gyles (1991) used in their experiments for demonstration of the protective effect of anti Stx2e neutralizing antibodies 20 ml of porcine serum preparation with

antibody titre of 400 000. Thus, the piglets were protected against 6 ng of purified Stx2e per 1 kg of body weight. In some of the piglets, mild signs of edema disease could be seen. In our experiments, lower doses of Stx2e were used. However, even the lower doses were lethal for the control pigs and those that had received the lowest dose 0.05 ml of serum. Regarding lower dose of Shiga toxin, a respectively lower dose of antiserum could be used as the protection.

Active immunization of piglets requires starting vaccination shortly after farrowing to obtain sufficient time for inducing active immunity. To protect pigs effectively against edema disease, the onset of immunity has to be immediately after weaning. This is difficult to obtain, especially in weaning earlier than at the age of 25 days. Our results confirm the possibility to protect pigs against edema disease by parenteral application of serum with Stx2e neutralizing antibodies. The results obtained so far in field experiments are consistent with the previous observations. Anti Stx2e antibodies in blood of piglets neutralize the resorbed toxin, however, they have no effect on colonization of intestine with STEC strains. It can be assumed that due to gut colonization, local immunity against the surface STEC structures will be developed, especially against the colonization factors F18. After the antitoxic passive immunity disappears, the piglets are protected against gut colonization with Stx2e by natural local immunity. This fact is in accordance with previous results (Alexa et al., 1998; Johansen et al., 2000).

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