Prevention of edema disease in pigs by passive immunization

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Abstract

The effect of treatment with verotoxin 2e (VT2e) specific antiserum was evaluated in 3 Danish pig herds with edema disease (ED). The antiserum was prepared by immunizing horses with a VT2e toxoid. The study was performed as a randomized blind field trial with parallel treatment and control groups. There were approximately 50 piglets in each group in each of the 3 herds and 741 piglets were included in the study (244 from herd A, 249 from herd B, and 247 from herd C). Treatment groups received 2, 4, or 6 mL anti-VT2e serum intramuscularly the day before weaning. Control groups were treated with 6 mL normal horse serum or 6 mL RPMI 1640 medium as placebo. All pigs that died in the trial period (1 d before weaning to 44 d after weaning) were examined pathologically and microbiologically. Mortality due to ED, mortality due to other causes, and adverse effects due to treatment were recorded. As there was no mortality due to ED, herd B was excluded from statistical calculations on mortality. The content of horse antibodies specific to VT2e in serum from pigs was analyzed in an indirect ELISA. A higher dose of anti-VT2e serum was reflected in higher optical density values in the indirect ELISA. Transient adverse reactions, seen as vomiting, ataxia, and cyanosis, occurred shortly after the injection of horse serum in 1.5% of the pigs, and one pig died. There were no statistically significant differences in mortality due to other causes among the 3 treatment groups in herds A and C. Only pigs from which F18+, VT2e+, ST−, LT− hemolytic *E. coli* (O139 or O-rough) was isolated were diagnosed as dead due to ED. Deaths due to ED in the control groups were 8.1% and 12.0% in herds A and C, respectively, compared with 0% and 0.7% in the corresponding serum groups. The difference between treatment and control groups was statistically significant ($P < 0.0001$). It was not possible to establish an effect of dose (2, 4, or 6 mL) of anti-VT2e serum, because only one pig died of ED in the treatment groups. It was concluded that passive immunization by intramuscular injection of a VT2e-specific antiserum can be used for protecting piglets against ED.

Résumé

L'effet d'un traitement à l'aide d'un antisérum spécifique contre la vérotoxine 2e (VT2e) fut évalué dans trois troupeaux danois au prise avec la maladie de l'œdème (ED). L'antisérum fut préparé par immunisation de chevaux avec un toxoïde de VT2e. L'étude fut réalisée à l'aveugle selon un modèle expérimental randomisé comptant en sus un groupe traité et un groupe témoin. Il y avait environ 50 porcelets dans chacun des groupes dans chaque élevage, totalisant 741 porcelets (241 sur la ferme A, 249 sur la ferme B et 247 sur la ferme C). Les animaux dans les groupes traités ont reçu 2, 4 ou 6 mL de sérum anti-VT2e par voie intra-musculaire le jour précédant le sevrage. Les groupes témoins furent traités avec 6 mL de sérum de cheval normal ou 6 mL de milieu RPMI 1640. Tous les porcs qui moururent durant la période de l'essai (un jour avant le sevrage jusqu'à 44 jours après le sevrage) furent soumis à un examen pathologique et microbiologique. Les mortalités causées par ED ou par une autre cause, ainsi que les effets adverses dus aux traitements furent pris en note. Étant donné le fait qu'aucune mortalité associée à ED n'y fut observée, le troupeau B fut exclus des calculs statistiques de mortalité. La quantité d'anticorps équins spécifiques contre VT2e présents dans le sérum des porcs fut mesurée au moyen d'une méthode ELISA indirecte. Des réactions adverses transitoires, telles que vomissement, ataxie et cyanose, se sont produites peu de temps après l'injection du sérum de cheval chez 1,5 % des porcs et 1 animal est mort. Aucune différence significative ne fut notée dans les mortalités associées à d'autres causes parmi les 3 groupes de traitements dans les troupeaux A et C. Un diagnostic de mortalité causée par ED fut émis seulement pour les animaux à partir desquels des souches F18+, VT2e+, ST−, LT− de E. coli *hémolytiques (O139 ou O-rugueux) furent isolées. Dans le groupe témoin, les mortalités dues à ED étaient de 8,1 % (troupeau A) et 12,0 % (troupeau C) alors qu'elles étaient de 0 % et 0,7 % dans les groupes traités correspondants, la différence entre les groupes témoins et les groupes traités étant statistiquement significative (P < 0,0001). Aucun effet associé à la dose reçue (2, 4 ou 6 mL) de sérum anti-VT2e ne put être établi. L'immunisation passive par injection intra-musculaire d'antisérum spécifique dirigé contre VT2e pourrait s'avérer efficace pour protéger les porcelets contre ED.*

(Traduit par docteur Serge Messier)

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Received February 17, 1999.

Introduction

Edema disease (ED) in pigs caused by *Escherichia coli* was first reported in 1938 by Shanks (1), but in Denmark this disease was first diagnosed microbiologically and pathologically at the beginning of 1994. Several outbreaks of ED were diagnosed in Danish pig herds through 1994 and 1995, whereafter the number of new outbreaks decreased (2). Edema disease in pigs can be caused by infection with a number of different serogroups of hemolytic *E. coli*, e.g. O138, O139, and O141 (3), but in Denmark the majority of outbreaks of ED have been caused by hemolytic *E. coli* O139, which produce F18-fimbriae and verotoxin 2e (VT2e) (2). If the verotoxigenic *E. coli* have the ability to produce enterotoxins [heat stable (ST) or heat labile (LT)], diarrhea is usually the dominating clinical picture in the herd (2,4).

The pathogenesis of ED and the characteristic clinical and pathological manifestations of the disease have been described (3,5). The significance of F18 and VT2e for development of typical clinical signs and pathological lesions of ED has been demonstrated in previous studies (4,6,7). Protection against the disease has been obtained by vaccination of pigs with purified VT2e inactivated by glutaraldehyde. Protection was demonstrated using an experimental ED model (8) and in field trials in 2 production herds with post weaning ED (9). Vaccination with a genetically modified VT2e-toxoid has also been shown to be effective in protecting pigs against ED using an experimental infection model (10). Vaccination probably requires 2 injections with an interval of 14 d in order to provide protective immunity from 1 wk after the second injection (9). In contrast, a specific antiserum may provide immediate passively acquired protective immunity in pigs. Alexa et al (11) have demonstrated good protection against ED under field conditions by treating weaned pigs with an antiserum, which was produced by injecting the supernatant from a culture of a verotoxigenic *E. coli* strain in slaughter pigs. The objective of the present study was to evaluate the effect of prophylactic treatment of pigs with 3 different doses of VT2e-specific antiserum produced in horses.

Materials and methods

Preparation of anti-VT2e serum

Preparation of horse anti-VT2e serum was performed by Section for Biological Products, Department of Pathology and Epidemiology, Danish Veterinary Laboratory. Antiserum was prepared by immunizing horses with purified, glutaraldehyde-inactivated VT2e (8,12) (100 µg/mL) with 15% Emulsigen (MVP Laboratories, Ralston, Nebraska, USA) as adjuvant. Horses were injected by the intramuscular (IM) route with doses of 2 mL of the vaccine. The first 3 injections were given with 21 d between injections; thereafter injections were given at intervals of 4 wk. Starting 1 wk after the first injection, blood samples were collected every week. The content of specific antibodies to VT2e was analyzed in an indirect enzyme-linked immunosorbent assay (ELISA), described below. The ELISA titer measured for horse serum was defined as the geometrical mean of the reciprocal value of the highest dilution that resulted in an optical density (OD) value of ≥ 0.500. After the 4th injection, each horse was bled 10 and 13 d after each injection, giving portions of 1.0 to 2.2 L of serum. Individual serum samples were analyzed by ELISA and serum was pooled so that the batch used in this study had an ELISA titer of ≥ 32 000.

Experimental design

The treatment was performed as a randomized blind field trial with parallel treatment and control groups in 3 Danish herds with 300 sows (herd A), 150 sows (herd B), and 300 sows (herd C), respectively. The experimental design is summarized in Table I. Herds A and C were free from toxin-producing *Pasteurella multocida*, *Serpulina hyodysenteriae*, and *Sarcoptes scabiei* var. *suis*. Herd A was also free from *Actinobacillus pleuropneumoniae* serotypes 2 and 6 (other serotypes are very rare in Denmark). Herd B had no declared health status. Before the start of this trial the herds had used the antiserum as prophylaxis against ED in weaned pigs.

In the present study, the piglets were treated with 2, 4, or 6 mL of antiserum. Pigs in control groups were given either 6 mL normal serum from non-immunized horses or 6 mL RPMI 1640 medium (Gibco BRL, Life Technologies, USA) (placebo) colored the same as the serum. To blind the experiment, all injection doses were standardized to 6 mL by adding normal horse serum. All treatments were given as a single treatment the day before weaning. The injected material was delivered as 2 IM injections of 3 mL each, one on each side of the neck.

At the time of injection, the pigs were ear-tagged. The treatments were performed in 2 consecutive batches of pigs in each herd. After treatment the pigs were put back into the pens. The following day, the pigs were weaned and pigs from different treatment

Table I. Summary of the study design to evaluate effect of an anti-VT2e serum in 3 Danish pig herds on post weaning mortality due to edema disease

	Herd A	Herd B	Herd C
Herd size (no. of sows)	300	150	300
No. of sows in trial	28	26	25
Weaning age (d)	27 (24–39)[a]	26 (15–47)	29 (23–43)
Time of treatment		One day before weaning	
Time of 1st blood sample		Before treatment	
Time of 2nd blood sample		1 wk post injection	
Time of 3rd blood sample		4 wk post injection	
Time of 4th blood sample		6 wk post injection	

[a] Data are expressed as mean (range)

Table II. Effects of different doses of anti-VT2e serum and normal horse serum on post weaning mortality due to edema disease and due to other causes

	Treatment	Herd A Dead/Total	Herd C Dead/Total
Mortality due to edema disease	2 mL antiserum group	0/49[a]	1/50
	4 mL antiserum group	0/49	0/49
	6 mL antiserum group	0/48	0/48
	Normal serum group	4/50	3/50
	Placebo group	4/48	9/50
	Total	8/244	13/247
Mortality due to other causes	2 mL antiserum group	2/49	2/50
	4 mL antiserum group	4/49	3/49
	6 mL antiserum group	5/48	2/48
	Normal serum group	4/50	5/50
	Placebo group	4/48	3/50
	Total	19/244	15/247

[a] Number of pigs

groups were mixed. Prophylactic measures normally taken against ED (administration of antibiotics and zinc oxide in the feed) were discontinued during the experiment. Furthermore, the piglets were fed standard diet ad libitum after weaning. The effect of antiserum treatment was measured by mortality due to ED in the nursery period.

Enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA was performed using VT2e-toxoid as antigen as previously described (9), except that an ELISA plate-washer (Microwash II, Skatron Instruments, Lier, Norway) was used for washing the plates, and horse antibodies were detected using peroxidase-conjugated sheep anti-horse immunoglobulins (PP260, The Binding Site, Birmingham, United Kingdom). A pool of normal serum from 10 non-immunized horses was used as a negative control.

Serology

The content of horse antibodies specific to VT2e in serum samples from pigs was analyzed in the indirect ELISA. Samples were applied in 2-fold dilution series, in duplicate, starting with a 1:2 dilution in phosphate-buffered saline (2.3 mM KH_2PO_4, 7.7 mM Na_2HPO_4, 140 mM NaCl, pH 7.2) with 0.05% Tween 20 (v/v) and 1% bovine serum albumin (w/v). Serum samples were obtained from approximately 25% of the animals in each group immediately before injection, 1 wk post injection, 4 wk post injection and from approximately 10% of the animals in each group at 6 wk post injection. Results of the ELISA analysis were presented as the OD values obtained at serum dilution 1:64.

Pathological and microbiological examinations

All piglets that died in the trial period (1 d before weaning to 44 d after weaning) were examined at the Danish Veterinary Laboratory. Pathological and microbiological investigations were performed. Hemolytic *E. coli* were typed by slide agglutination tests (serogroups O8, O45, O64, O101, O138, O139, O141ab, O141ac, O147, O149, O157) and tested by PCR for the genes of the fimbriae F4, F5, F6, F18 and F41 and the genes of the toxins LT, STa, STb and VT2e (13). Only pigs from which F18+, VT2e+, ST−, LT− hemolytic *E. coli* was isolated were categorized as "dead due to ED." The O serogroup was not used as a criterion for presence of edema disease. Isolation of verotoxigenic *E. coli* also carrying genes for enterotoxins was not taken as evidence of ED.

Statistical analysis

Mortality due to ED, other causes, and overall mortality were analyzed by a logistic regression model. Three explanatory variables were included: treatment (dose), batch, and herd. The effect of serum doses (2, 4, or 6 mL) was examined. If no effect of dose could be established, the data from the 3 serum groups were aggregated. Significant interactions between explanatory variables, using a significance level of 5%, were included in the final models.

Optical density values obtained from blood samples were analyzed non-parametrically in order to avoid normality assumptions (Kruskal-Wallis test). The computations were performed using SAS software (14).

Results

Mortality

In herd B there was no mortality due to ED; therefore, herd B was excluded from the statistical analysis of mortality. Eight pigs from herd A and 13 pigs from herd C were categorized as "dead due to ED," translating to 38% of the overall mortality in the 2 herds (Table II). Nineteen pigs in herd A and 15 pigs in herd C died from other causes according to the applied method for categorizing causes of death. In the VT2e antiserum treated groups only 1 pig died of ED. Seven pigs from the normal horse serum group and 13 pigs from the placebo group died of ED.

There was no statistical difference in mortality between the normal horse serum and placebo groups or between the 3 antiserum treatments groups. In the final statistical model, the normal horse serum and placebo groups were aggregated in 1 control group,

Table III. Effect of treatment with VT2e antiserum on mortality due to edema disease (ED) and due to other causes

Herd	Batch	Mortality due to ED		Mortality due to other causes	
		Serum	Control	Serum	Control
A	1	0% (0/93)	7.8% (5/64)	6.5% (6/93)	7.8% (5/64)
	2	0% (0/53)	8.8% (3/34)	9.4% (5/53)	8.8% (3/34)
	All	0% (0/146)	8.1% (8/98)	7.3% (11/146)	8.1% (8/98)
C	1	1.4% (1/70)	18.8% (9/48)	1.4% (1/70)	10.4% (5/48)
	2	0% (0/77)	5.8% (3/52)	7.8% (6/77)	5.8% (3/52)
	All	0.7% (1/147)	12.0% (12/100)	4.8% (7/147)	8.0% (8/100)
A+C combined	All	0.3% (1/293)a	10.1% (20/198)	6.1% (18/293)	8.1% (16/198)

[a] Difference in ED mortality in serum and control groups is statistically significant ($P < 0.0001$)

Table IV. Results from statistical analysis: odds ratio estimates, 95% confidence intervals and *P*-values

Effect of VT2e anti serum		Odds ratio	95% Confidence interval	*P*-value
Overall mortality	Control	1.00		
	Treatment	0.31	0.16–0.58	< 0.0001
Mortality due to ED	Control	1.00		
	Treatment	0.03	0.001–0.19	< 0.0001
Mortality due to other causes	Control	1.00		
	Treatment	0.74	0.35–1.61	0.51

and the 3 antiserum groups were aggregated in 1 treatment group. In the combined control group from herd A and herd C mortality due to ED was 10.1%, and due to other causes 8.1% (Table III). In the combined treatment group from herd A and herd C, mortality due to ED was 0.3%, and due to other causes 6.1%. Due to the low mortality in the serum groups it made no sense to estimate an effect of serum dose. The odds ratios (OR) and confidence intervals (CI) for effect of antiserum treatment on mortality due to ED, other causes, and overall mortality are shown in Table IV. The results indicate that pigs treated with anti-VT2e serum had 69% lower overall risk of dying than pigs in the control groups (OR = 0.31). The effect of herd and batch was significant only due to the higher level of ED-caused mortality in the first batch of animals in Herd C (see Table III). This effect has been adjusted for in Table IV, but it has only a marginal influence on the results.

The results indicate that pigs treated with anti-VT2e serum had 97% lower risk of dying of ED than pigs in the control groups (OR = 0.03, see Table IV) ($P < 0.0001$). The 2 mL treatment group in which one pig died of ED also showed a significant reduction in ED-caused mortality when compared to the control animals, with an estimate of OR = 0.09 ($P = 0.02$).

There was no statistically significant difference in mortality due to other causes between control and treatment groups. The results are shown in Table IV.

Pathological and microbiological examinations

Characteristic pathologic changes of ED, such as edema in subcutis, stomach, or mesocolon, were found in only a few pigs in this investigation. In herd A, hemolytic *E. coli* F18+, VT2e+, ST−, LT− was typed as O139 in only 1 pig and as O-rough in 7 pigs. In herd C, hemolytic *E. coli* F18+, VT2e+, ST−, LT− was typed as O139 in 6 pigs and as O-rough in 7 pigs. Since all of these isolates were verotoxigenic and negative for enterotoxins, the pigs were categorized as "dead due to ED."

In herd A, there were two F18+, VT2e+ hemolytic O-rough *E. coli* that were STb-positive. These pigs were categorized as "dead due to other causes." Neither of the two F18+, VT2e+, STb+ pigs showed pathological findings consistent with ED.

In herd A, as well as in herd C, catarrhal enteritis was found in 6 pigs from which hemolytic *E. coli* O149 was isolated from the intestines. Further, catarrhal enteritis was found in 5 pigs without any isolation of pathogenic *E. coli*, and in 1 pig with catarrhal enteritis, hemolytic *E. coli* O45 was isolated. In 1 pig, hemolytic *E. coli* O139, F18+, VT2e+ was isolated from the small intestine and colon and hemolytic *E. coli* O149 was isolated from the colon. The pathology of this pig was inconclusive and it was categorized as "dead due to ED."

Seven pigs were diagnosed with polyserositis/septicemia, 4 pigs with pneumonia, and in 5 pigs there were autolysis and no specific pathologic or bacteriologic findings. Concerning "death due to other causes," there was no indication of significant differences between herds or treatment groups.

Serological evaluation

Statistical analysis showed that after treatment with 2 mL antiserum there was a statistically significant difference in OD values between herds. Six weeks after treatment with 4 mL antiserum there was a statistically significant difference in OD values between herds. Four and 6 wk after treatment with 6 mL antiserum there was a statistically significant difference in OD values between herds. The results are shown in Table V.

In all groups and herds, the OD values before treatment were very low and on similar levels. There were no statistically significant differences in OD values between groups before treatment. The serological reaction in serum samples taken before treatment was interpreted as the background reaction in the ELISA, since no horse antibodies were present in these samples. In the placebo group and normal serum group, the OD values after treatment were, as expected, similar to the OD values before treatment. The pigs

Table V. Effects of different doses of anti-VT2e serum and normal horse serum on the VT2e specific horse antibodies in pig serum measured in the indirect ELISA

		Treatment				
	Herd	2 mL antiserum	4 mL antiserum	6 mL antiserum	Normal horse serum	Placebo
Before injection	A	0.04[a]	0.04	0.03	0.04	0.03
	B	0.03	0.08	0.03	0.04	0.04
	C	0.04	0.04	0.03	0.03	0.03
1 wk post injection	A	0.95	1.44	2.09	0.03	0.03
	B	0.96	1.48	1.99	0.04	0.04
	C	0.79	1.31	1.61	0.04	0.04
4 wk post injection	A	0.31	0.58	0.79	0.03	0.03
	B	0.36	0.64	0.77	0.04	0.04
	C	0.21	0.44	0.62	0.05	0.04
6 wk post injection	A	0.10	0.22	0.35	0.03	0.04
	B	0.30	0.45	0.39	0.05	0.10
	C	0.08	0.16	0.21	0.03	0.04

[a] OD (median) at serum dilution 1:64

treated with antiserum showed the highest OD values 1 wk after treatment. Higher dose of antiserum was reflected in higher OD values after treatment. The differences between OD values for the different anti-VT2e serum treatments were statistically significant 1 and 4 wk after injection in all herds ($P < 0.01$). In conclusion, the effects of treatment with anti-VT2e serum on OD value in this study were: 1) higher dose of antiserum was reflected in higher OD values after treatment, and 2) OD values for same dose of antiserum at the same point in time differed significantly between herds.

Adverse effects

One pig injected with normal horse serum reacted to the injection, and showed adverse reactions shortly after the injection. Seven pigs from the antiserum groups also showed transient adverse reactions shortly after the injection, and 1 pig died within 1 h of the injection. The clinical signs were vomiting, ataxia, and circulatory disturbance with cyanosis. No adverse reactions were observed in herd B. The percentage of pigs that received horse serum and showed adverse reactions was 1.5%.

Discussion

Although the treatment was performed on a limited number of pigs and the mortality due to ED during the trial was only 8.1% in the control groups, the effect of the anti-VT2e serum was substantial and it was possible to demonstrate a statistically significant reduction in mortality due to ED. It was not possible to establish the effect of dose of anti-VT2e serum because only 1 pig died of ED in the treatment groups. The results of this study indicate that treatment with anti-VT2e serum produced in horses can prevent ED in pigs. The results are consistent with findings in field trials performed with antiserum produced in pigs where the overall mortality was 67–92% lower in the serum treated than in the control groups (11). In our study, the overall mortality was 69% lower in the serum treated than in the control groups.

Post mortem diagnosis of ED is not always clear-cut, because characteristic lesions of edema are often absent. Therefore, in this study, diagnosis of ED was based primarily on the isolation of hemolytic *E. coli* F18+, VT2e+, LT−, ST−, and concomitant absence of significant lesions indicating other causes of death. Verotoxigenic *E. coli* also producing enterotoxins most often causes diarrhea (2,4). Thus, the 2 pigs from which O-rough hemolytic *E. coli* F18+, VT2e+, STb+ was isolated as the only pathogen were categorized as "dead due to other causes."

The isolation of both O-rough and O139, F18+, VT2e+, LT−, ST− hemolytic *E. coli* from the same herd has been observed before in Danish pig herds with ED (15). In that study, ribotyping and pulsed field gel electrophoresis showed identical patterns for O139 and O-rough isolates. In this context it was reasonable to consider both O139 and O-rough isolates as causative agents of the ED if they carried the genes for F18 and VT2e and no other virulence factors.

The adverse effects seen in some piglets may cause some reservations concerning the applicability of serum treatment. The adverse reactions observed in the present study were characterized as peracute anaphylactic reactions, presumably caused by lack of compatibility of the horse serum in the pigs. The combination of stress and circulatory disturbance after injection of the serum from horses might be fatal for a few pigs. In the above-mentioned study (11), using 5 mL antiserum from pigs injected subcutaneously 4 d after weaning no adverse effects were reported (Alexa, personal communication). However, production of antiserum in pigs or sows would presumably be more costly and difficult to standardize compared to production of antiserum in horses. A cost-benefit analysis of the use of serum treatment can be done using the formula: X*Y/100 = Z, where X is the price of a pig, Y is the reduction in mortality due to ED in percent, and Z is the price of a dose of serum. For example, if the price of 1 dose of serum is 2 US$ and the value of a pig is 25 US$ the reduction in mortality should be at least 8% for the treatment to be economically worthwhile.

In conclusion, the effects of treatment with anti-VT2e serum in this study were 1) provision of protective passive immunity against infection with F18+, VT2e+ *E. coli*, seen as significant reduction in mortality due to ED, 2) adverse reactions in some piglets, regardless of dose. However, it seems possible to overcome severe adverse

reactions by avoiding stressful situations for the pigs immediately after serum treatment. The results show that passive immunization of pigs by IM injection of a VT2e-specific antiserum can be used for protecting piglets against ED.

Acknowledgments

We would like to acknowledge the skillful technical assistance of Heidi Pia Andersen and Jutta Hammermuller. We also acknowledge Rikke Hoff-Jørgensen for proposing and initiating the concept of using anti-VT2e serum for treatment of ED in pigs.

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