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# Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets

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**Abstract**

The protective effects of egg-yolk antibodies obtained from hens immunized with fimbrial antigens from a local strain (*Escherichia coli* K88+ MB, Manitoba, Canada) of K88+ piliated enterotoxigenic *E. coli* (ETEC) were evaluated in 3- and 21-day-old piglets in which ETEC diarrhea was induced and also in early-weaned piglets in a commercial farm. The results demonstrated that the *E. coli* K88+ MB-induced diarrhea in 3-day-old piglets was cured 24 h after treating with egg-yolk antibodies while those treated with egg-yolk powder from conventional hens continued to have diarrhea and 62.5% of them died of severe diarrhea. For 21-day-old weaned piglets, those fed egg-yolk antibodies had transient diarrhea, positive body weight gains and 100% survival during the period of the experiment, whereas control piglets that were treated with placebo had severe diarrhea and dehydration and some died within 48 h after infection. In the field trial, the incidence and severity of diarrhea of 14–18-day-old weaned piglets fed egg-yolk antibodies were much lower than in those fed a commercial diet containing an antibiotic. These results indicate that the neonatal and early-weaned piglets that received the egg-yolk antibodies were protected against ETEC infection. 

*Keywords:* Egg-yolk antibody (IgY); *Escherichia coli* K88+; Diarrhea; Piglet

## 1. Introduction

It has been well recognized that diarrheal disease caused by enterotoxigenic *Escherichia coli* (ETEC) is by far the most common enteric colibacillosis encountered in neonatal and early-weaned pigs [1–3]. It is also known that colonization of the small intestine of the pig by ETEC adhering to the epithelium accounts for most gastrointestinal disorders in both neonatal and post-weaning piglets [1,2]. Among the

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different ETEC, those expressing the K88+ fimbrial antigen are the most prevalent form of *E. coli* infection found world-wide wherever pigs are raised in large numbers [4]. It has been estimated that K88+ ETEC are responsible for 50% of the 10 million piglet deaths each year [5].

Antibodies raised against ETEC fimbrial antigens have been administered orally to piglets and have offered potential prophylactic and therapeutic value in controlling the disease [6]. The egg yolk has been shown to be a convenient source of polyclonal antibodies. Chicken egg-yolk IgY can be used as an inexpensive and effective source of antibodies for the passive immunization of animals suffering from intestinal diseases. Our in vitro study clearly demonstrated that purified antibodies from the egg yolk of the chicken against the fimbriae of *E. coli* K88+ were able to block the binding of *E. coli* K88+ to the mucosal receptor [7]. The objective of the present study was to evaluate the efficacy of chicken egg-yolk antibodies against experimentally induced ETEC diarrhea in neonatal and early-weaned piglets and also against diarrhea in a field trial.

## 2. Materials and methods

### 2.1. Bacteria and culture condition

A local strain of hemolytic (H) ETEC K88+ bacterium (K88+ MB) was obtained from the Animal Health Center, Veterinary Services Branch, Manitoba Agriculture, Winnipeg, Man., Canada. The isolate, which was obtained from the feces of a piglet suffering from diarrhea, was shown to give a positive agglutination test with anti-K88 ETEC antibody but not with anti-K99 ETEC antibody. Primary cultures of ETEC strains were grown overnight in tryptic soya broth (TSB, Difco) at 37°C using a 1% inoculum from stocks stored at −20°C in 30% glycerol. *E. coli* K88+ MB used for infection were prepared as follows: *E. coli* K88+ MB were grown overnight in TSB (Difco) at 37°C using a 1% inoculum from stocks. Cells were harvested by centrifugation at $2000\times g$ for 15 min in phosphate-buffered saline (PBS, pH 7.2), washed twice and resuspended in PBS. The suspension concentrated to $10^{10}$ or $10^{12}$ CFU $ml^{-1}$ was used for infection.

### 2.2. Production of egg-yolk antibodies (IgY) against ETEC K88+ antigen

The K88+ MB fimbrial antigen was purified using a modification of the method as previously described [7]. White Leghorn laying hens (Shaver SX 288) that were approximately 20 weeks of age were immunized and used for egg production in accordance with standard animal care regulations [7,8]. The egg-yolk antibodies were prepared as previously reported [7]. Briefly, the eggs were broken, the egg whites discarded and the yolk rolled on filter paper (Whatman No. 1) to remove residues of egg white. The yolks were then transferred into a funnel fitted with filter paper that was opened at the bottom of the funnel, the yolk membranes were ruptured and yolks were collected in a measuring cylinder leaving the yolk membrane attached to the paper. The yolks were diluted (1:9, v/v) with acidified double distilled water to obtain a final pH of 5.0, frozen at −20°C overnight, defrosted and centrifuged at $10\,000\times g$ for 30 min at 15°C. The supernatant was filtered two times with filter paper. The egg-yolk antibody powder was obtained by freeze-drying the water-soluble protein fraction of egg yolks. The yolks were also freeze-dried without purification. The titer of the egg-yolk antibody was tested by direct ELISA [7].

### 2.3. Experimental design of passive protection of piglets

#### 2.3.1. Experiment I

Twenty-six 3-day-old Cotswold piglets were obtained and transferred to the University of Manitoba's Animal Research Unit. They were fed with milk replacer (skim milk powder, 109 g; whey powder, 77 g; methyl cellulose, 4 g; minerals, 2 g; oil mix, 35 g; and vitamin mix, 20 ml, for one liter) immediately after arrival for 3 days. The piglets were then randomly divided into three groups. Group 1 containing 10 piglets was used as negative control (not treated with ETEC or antibodies). The other two groups each containing eight piglets were treated with placebo (egg yolk with no antibodies, as a positive control, group II) and egg-yolk antibodies (treatment, group III). The piglets in groups II and III were challenged with 5 ml of *E. coli* K88+ MB at a dose of $10^{10}$ CFU $ml^{-1}$ per piglet at time 0 h. The

bacterial suspension was delivered orally to each piglet with a syringe attached to a polyethylene tube held in the piglet's oral cavity. The piglets in group III were treated three times (−3, 0 and 3 h after the ETEC challenge) on the first day and once a day for the following two consecutive days each time with 1.5 g of egg-yolk antibodies at a titer of 140 000 (100 000–168 000). The antibody or placebo was mixed with 5 ml of PBS buffer and the slurry was administered orally from a syringe into the stomach via a polyethylene tube. The clinical response of each piglet was monitored throughout the experiment in terms of occurrence of diarrhea, fecal consistency score, weight loss and mortality.

#### 2.3.2. Experiment II

Twenty 21-day-old healthy Cotswold piglets were randomly divided into two groups, i.e., the control and the treatment, of 10 piglets each. All piglets were challenged twice each with 5 ml of *E. coli* K88+ MB at a dose of $10^{12}$ CFU $ml^{-1}$ per piglet. The suspension or *E. coli* K88+ MB was administered at 0 and 5 h of the experiment. After challenge, the piglets in treatment group were treated with 0.5 g of egg-yolk antibodies at a titer of 140 000 (100 000–168 000) three times a day at times −1, 4 and 9 h each for two consecutive days after the first *E. coli* challenge, whereas the control received placebo treatment. The bacterial suspension or egg-yolk antibody was delivered as described in experiment I. The clinical response of each piglet was monitored throughout the experiment in terms of occurrence of diarrhea, fecal consistency score, weight loss and mortality.

#### 2.3.3. Experiment III (field trial)

This experiment was carried out in the Puratone Research Barn in Ste. Agathe (Winnipeg, Man., Canada). Three hundred and six 14–18-days-old piglets (weaning) were selected and allotted to 18 experimental groups (pens) each having 17 piglets. All diets were formulated to meet NRC requirement [9] and had a similar protein and energy content. The experimental treatments were randomly distributed among pens in the barn. Piglets in group I were fed the commercial diet containing an antibiotic (Mecadox 55); those in group II were fed a diet with 2% spray-dried egg yolk (SDEY) with no antibodies against *E. coli* and without antibiotic; and those in group III (treatment) were fed a diet with egg-yolk antibodies including 0.2% anti-K88+ and 0.1% anti-K99+ antibodies and 1.7% SDEY with no antibodies. The clinical response of each piglet was monitored throughout the experiment in terms of occurrence of diarrhea, fecal consistency score, weight loss, mortality and enumeration of *E. coli* K88 and K99 from anal swabs. Values for occurrence of diarrhea and fecal consistency score were the average of two scores as determined by two independent evaluations.

The piglets were fed the three experimental diets for a period of 8 days. Body weight, general performance and swabbing (test for *E. coli* K88 and K99) were done on days 0, 3 and 8 of the experiment. Four animals for swabbing from each pen were identified by number at the start of the experiment (day 0). The presence of the *E. coli* K88 or K99 strains was analyzed by an immunoassay. The assay is highly specific with the sensitivity of approximately 10 000 CFU $ml^{-1}$.

## 3. Results

### 3.1. Production of egg-yolk antibodies

Two types of antibodies were produced, one type from hens immunized with the attenuated whole organism and one type using the fimbriae of the ETEC K88+ MB. The antibody titer was much greater when the fimbriae were used as an antigen (140 000) compared with that of antibodies with the whole cells (12 000). Also purification of the anti-K88 fimbria antibodies dramatically increased the titer. The titer of egg-yolk antibodies obtained from freeze-drying was 140 000, varying from 100 000 to 168 000, and these antibodies which were 15-fold greater than that of the original egg yolk were used for the following passive protection experiments. The semi-purified egg-yolk antibodies were used for the immunoassays.

### 3.2. Experiment I

The results demonstrated that within 24 h of the

Table 1
Clinical response of 3- or 21-day-old pigs after challenge with ETEC K88+ MB and treatment with egg-yolk antibodies (experiments I and II)

| Treatment | No. of pigs | No. of pigs with diarrhea (FC score[a]) after | | | Weight gain (g) | No. of pigs dead (%) |
|---|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | | |
| *3-day-old piglets*[b] | | | | | | |
| Group I | 10 | 1/10 (0.1) | 1/10 (1.0) | 1/10 (1.1) | 60.3 | 1/10 (10.0) |
| Group II | 8 | 7/8 (2.3) | 4/5 (2.4) | 4/5 (2.4) | 19.0 | 5/8 (62.5) |
| Treatment | 8 | 1/8 (0.4) | 2/7 (0.6) | 2/7 (0.7) | 46.7 | 1/8 (12.5) |
| *21-day-old piglets*[c] | | | | | | |
| Control | 10 | 3/10 (1.0) | 8/10 (2.0) | 4/7 (3.0) | −36.2 | 3 (30.0) |
| Treatment | 10 | 5/10 (1.6) | 0/10 (0.0) | 0/10 (0.5) | +90.6 | 0 (0) |

[a]FC score is the mean fecal consistency score: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. Pigs with a fecal score of ≤1 were considered not to have diarrhea.
[b]For 3-day-old piglets, those in group II and the treatment group were challenged orally with K88+ MB two times (at 0 and 5 h) at a dose of $10^{10}$ CFU $ml^{-1}$ of viable organisms per piglet. Piglets in group I were not challenged with K88+ MB. Piglets in the treatment group were treated with egg-yolk antibodies, three times a day (−3, 0 and 3 h after the first ETEC challenge) for the first day and once a day for the following two consecutive days.
[c]All the 21-day-old piglets were challenged orally with K88+ MB two times (at 0 and 5 h) at a dose of $10^{12}$ CFU $ml^{-1}$ of viable organisms per piglet. Piglets in the treatment group were treated with 0.5 g of egg-yolk antibodies, three times a day (−1, 4 and 9 h after the first ETEC challenge) for the first day and once a day for two consecutive days after the first ETEC challenge.

challenge with *E. coli* K88+ MB there was 182 and 106 g loss in weight in *E. coli* K88+ MB challenged piglets while the unchallenged group gained 191 g weight (data not shown). The weight loss was mainly attributed to severe diarrhea induced by *E. coli* K88+ MB. Piglets treated with egg-yolk antibodies recovered and gained weight after 48 h of administration of egg-yolk antibodies while untreated pigs continued to lose weight. After 3 days, pigs in the treatment group and those from the unchallenged group had similar body weight gains that were significant higher ($P < 0.05$) than those challenged with *E. coli* without antibody treatment.

The severity of diarrhea was monitored using a 0–3 scoring system. The piglets in group I at most only had an occasional slight incidence of diarrhea. In contrast, both of the *E. coli* K88+ MB infected groups developed severe to moderate diarrhea within 10 h of *E. coli* K88+MB challenge (data not shown). The *E. coli* K88+ MB induced diarrhea was cured 24 h after treating with egg-yolk antibodies, whereas those treated with egg-yolk powder without K88 antibodies (group II) still continued to have diarrhea resulting in 62.5% death (Table 1). In contrast, only one piglet died in each of group I and the treatment group.

### 3.3. Experiment II

Control piglets that were treated with the placebo developed severe diarrhea within 12 h and were dehydrated and lost weight within 48 h (data not shown). Thirty percent of the pigs died of severe diarrhea. In contrast, the pigs treated with egg-yolk antibodies from the immunized hens exhibited no signs of diarrhea 24 or 48 h after treatment, had a positive weight gain and none of the pigs died (Table 1). The results indicate that the piglets that were treated with antibodies from the hens immunized against ETEC were protected against the deleterious effects of this organism.

### 3.4. Experiment III

The incidence and severity of diarrhea was least for pigs fed egg-yolk antibodies, and highest in those fed a diet with an antibiotic (Table 2). Three piglets died in group I while no piglets died in the other two groups. No *E. coli* K88+ or K99+ were detected between 0 and 8 days when egg-yolk antibodies were fed. Pigs in the other two groups were infected with either one or the other strain of *E. coli* (4.8–8.3%) especially by day 8 of the study. There was no

Table 2
Body weight gain, incidence of scours, mortality and presence of *E. coli* in fecal swabs of pigs fed diets with/without antibodies (experiment III)

| Treatment[a] | No. of pigs | Body weight gain (kg day$^{-1}$)[b] | No. of pigs with diarrhea[c] (%) (FC)[d] | Mortality (No.) | *E. coli* K88+ in fecal swab (%) on day | | | *E. coli* K99+ in fecal swab (%) on day | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0 | 3 | 8 | 0 | 3 | 8 |
| Group I | 102 | 0.18 ± 0.01 | 5.8 (1.3) | 3 | 0 | 0 | 8.3 | 0 | 4.2 | 4.2 |
| Group II | 102 | 0.16 ± 0.01 | 3.9 (1.5) | 0 | 0 | 0 | 0 | 4.2 | 4.2 | 4.2 |
| Group III | 102 | 0.16 ± 0.01 | 1.9 (1.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Group I pigs were fed a commercial diet; group II were fed a modified diet containing 2% spray-dried egg yolk (SDEY) with no antibodies while group III were fed the same diet as group II except that they received 0.1, 0.2 and 1.7% SDEY containing K88, K99 and no antibodies, respectively.
[b]Values of body weight gain are means ± S.D.
[c]Percent diarrhea was determined on pigs in each group during the first week.
[d]FC score is the mean fecal consistency score: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. Pigs with a fecal score of ≤1 were considered not to have diarrhea.

significant difference in average daily gain among all the groups.

## 4. Discussion

Previous studies have demonstrated that the egg yolk of the chicken is an excellent source of an abundant supply of relatively inexpensive antibodies [10]. In the present experiment, the egg-yolk antibodies were preserved by lyophilization. It is clear that these methods yielded antibodies suitable for use in agglutination, ELISA and in vitro adhesion-inhibition tests ([7], Kim, unpublished).

The results in the present experiment demonstrated that egg-yolk antibodies could protect both neonatal and 21-day-old weaned piglets from diarrhea induced by a challenge with *E. coli* K88+ MB. The results are consistent with the findings of other researchers [1,11–14]. Wiedemann et al. [11] found that chicken egg-yolk antibodies were as successful as a common antibiotic therapy in curing piglets with diarrhea. Yokoyama et al. [1] showed that antibodies prepared from the yolk of eggs from hens immunized with fimbrial antigens of *E. coli* were protective in newborn piglets against a challenge with homologous ETEC strains. Zuniga et al. [13] reported that pigs eating egg powder with antibody against the same fimbrial variant were fully protected when the pigs were challenged with *E. coli* F18+. Imberechts et al. [14] also reported that *E. coli* F18ab antibodies significantly reduced the excretion of the *E. coli* strain used for infection. Treated pigs that were infected with the F18+ *E. coli* strain were clinically better protected.

The present results showed that the occurrence of diarrhea and mortality was significantly lower in pigs fed the diet incorporated with chicken egg-yolk antibodies in a commercial farm. There are scant reports on the efficacy of egg-yolk antibodies applied in commercial farms except that Wiedemann et al. [11] reported that 92% of diarrhea affected piglets could be cured over a period of 5 days by the sole application of lyophilized yolk from immunized hens.

The mechanism by which egg-yolk antibodies protect pigs against ETEC induced diarrhea has been proposed as that the antibodies against the fimbriae of ETEC react with the receptor binding component of ETEC (fimbriae) thereby preventing them from attaching and adhering to the mucosa of the intestine of piglets [1,6]. This hypothesis is supported by in vitro adhesion-inhibition tests [1,7]. In our previous study, it was clearly demonstrated that anti-K88+ MB fimbrial antibodies from chicken egg yolk when added to *E. coli* K88+ MB prevented their binding to receptors in the mucus isolated from the intestine of piglets. The effectiveness is related to two factors, i.e., dose of antibodies and the concentration of ETEC. The supply of antibodies in the intestine must therefore be sufficiently high to prevent binding of *E. coli* to the intestinal receptors. The antibody

must also be continuously or nearly continuously available in the intestine. The present study showed that 1.5 g per day per piglet was sufficient to prevent the diarrhea induced by infection with $10^{10}$ ETEC and the addition of 0.2% of egg-yolk antibodies in the diet was preventive against diarrhea in the commercial farm.

It can be concluded that chicken egg-yolk antibodies were able to prevent the diarrhea experimentally induced by ETEC in neonatal (3-day-old) and 21-day-old weaned piglets, and also lower the occurrence of diarrhea of early-weaned piglets in a field trial.

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