Chicken Anti-Idiotypic Antibodies As an Alternative to the Antimicrobial Treatment for the Control of Escherichia coli K 88 in Domestic Animals

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ABSTRACT

Antimicrobials have been widely used to control diarrheal infections in domestic animals. However, the misuse of these antimicrobials can lead to human health hazards. The purpose of this research was to evaluate the efficacy of anti-idiotypic antibodies that mimic Escherichia coli K88 antigens in combating diarrheal infections in piglets. Polyclonal anti-idiotypic antibodies (Ab2) were produced in laying hens against several monoclonal (mAb1) and polyclonal (pAb1) antibodies that recognized Escherichia coli K88 fimbrial antigen. Preliminary evaluation showed that anti-pAb antibodies (Ab2) were superior to anti-mab antibodies. Therefore, the efficacy of two Ab2 preparations [Ab2 produced against rabbit anti-K88 IgG2a and its F(ab')2] were evaluated following an intragastric administration to neonatal piglets challenged with E. coli K88. Partial protection (62.5% survival) was observed in the Ab2 treated piglets, while only 12.5 % of control piglets (no-Ab2) survived. Our results indicate that Ab2 are excellent surrogate antigens for immunization and could also be useful in the control of E.coli infection in piglets.

Keywords: Anti-idiotypic antibodies, E. coli K88, Internal image.

INTRODUCTION

In this paper, monoclonal antibodies (mAb1) were produced in mice against K88 fimbriae, while polyclonal antibodies (pAb1) were produced in rabbits against K88 fimbriae. The idiotype antibodies are the anti-fimbrial mAb1 or pAb1. Polyclonal anti-idiotypic antibodies are referred to as Ab2 and were produced in chickens against mAb1 or pAb1. Ab3 are polyclonal anti-anti-idiotypic antibodies and were produced in mice against Ab2.

Diarrhea caused by enterotoxigenic Escherichia coli K88 (ETEC) is one of the most prevalent diseases in neonatal piglets worldwide (Yokoyama et al., 1992). The ability of ETEC to colonize the small intestine is a virulence factor; i.e a prerequisite for the production of diarrhea in both humans and animals (Erickson et al., 1992; Nystrom, 1995). This colonization is facilitated by the binding of bacterial fimbriae to specific receptors on the villus epithelium (Hacker, 1992; Klemm, 1985; Smith, 1992).

Antimicrobials have been widely used for the past few decades and are considered the therapy of choice for the control of the ETEC diseases. However, their use on a routine basis for prophylactic and therapeutic purposes has contributed widely to the appearance of multiple drug resistance in bacteria such as Salmonella and E. coli (Hays, 1986). Enhanced immunity by oral
administration of antibodies with predefined specificity derived from serum (Elliot, 1978; Spier et al., 1989), colostrum (Shimizu et al., 1988) or monoclonal antibodies (Sherman et al., 1983; Smith and Lida, 1990) has been used to combat certain intestinal diseases. However, it is prohibitively expensive to obtain large amounts of these antibodies or simply it is impractical (Yokoyama et al., 1992). The egg yolk has shown to be a good source of antibodies. It is possible to obtain antigen-specific chicken IgY by immunizing laying hens with the desired antigen (Bartz et al., 1980; Otake et al., 1991; Yokoyama et al., 1992; Yolken et al., 1988). This convenient source of antibodies could replace the need for serum, colostrum or monoclonal antibodies. Chicken IgY has been successfully used against murine rotavirus infection in mice (Bartz et al., 1980; Yolken et al., 1988), dental carries caused by Streptococcus mutants in rats (Otake et al., 1991), neonatal diarrhea caused by E. coli K88, K99 or 987P in piglets (Ikemori et al., 1992; Maraquardt et al., 1999; Yokoyama, 1992) and E. coli F18 infections in weaned piglets (Zuniga et al., 1997). Therapeutic antibodies that are currently available are mainly produced by immunizing the host with attenuated or inactivated bacteria or viruses, which often requires elaborate and expensive purification. In addition, these pathogens possess certain risks to the working personnel, and the complex nature of the bacterial outer membrane proteins and lipopolysaccharides might cause severe reactions at the site of injection in the animal.

Numerous studies have provided evidence that anti-idiotypic antibodies (Ab2) that bear an internal image to a particular antigen can be produced by immunizing a host with the antibody (Ab1) that recognizes the antigen (Jerne, 1974; Rico et al., 1989). The portion of Ab2 that bears an internal image to the antigen can induce an antigen specific immune response in individuals that have never encountered the antigen (Ertl and Bona, 1988; Rico et al., 1989). Anti-idiotypic antibodies have been used to induce protective immunity to schistosoma, trypansomosis, Nesseria gonorrhea, E. coli K13, herpes virus, influenza virus, Streptococcus mutants, Listeria monocytogens and Brucella abortus (Anders et al., 1989; Beauclair and Khansari, 1990, Brossay, 1993; Crzych et al., 1985; Kaufmann et al., 1985; Kennedy et al., 1986; McNamara et al., 1984; Sacks et al., 1982). Despite the large number of reports on the use of anti-idiotypic antibodies as vaccine, there are only two reports that have addressed the use of anti-idiotypic antibodies as therapeutic agents or for passive immunization. (Reck et al., 1988) and (Jackson et al., 1990) demonstrated that anti-idiotypic antibodies bearing an internal image to Staphylococcus enterotoxin B and to an antigen on Streptococcus mutants provided partial protection against emetic response and diarrhea in monkeys and dental carries in rats, respectively. This study was designed to determine if egg-yolk anti-idiotypic antibodies that bear an internal image to the K88 fimbriae can interfere with the colonization of E. coli K88 in the pig intestine by blocking its receptors and therefore prevent infection by this organism.

MATERIALS AND METHODS

Animals. Female Balb/c mice, 6-8 weeks of age were obtained from the Central Breeding Facility, University of Manitoba, Winnipeg. Twenty-week old Leghorn chickens were obtained from the University of Manitoba poultry barn, Winnipeg, and White New Zealand rabbits were obtained from the Blue Farm Rabbitry, Winnipeg, Manitoba. Three days old Cotswold piglets were obtained from a local farm, Winnipeg, Manitoba. All animals were treated according to the guidelines established by the Canadian Council on Animal Care, CCAC (1980).

Bacteria and Culture Conditions. E. coli K88 was
obtained from the Pennsylvania State University E. coli Reference Center (University Park, PA). It was cultured on trypticase soy agar slants. E. coli K88 was grown in 8 L tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) for 36 hours at 37°C. Cells were then centrifuged, washed and used immediately for extraction of fimbriae. Alternatively, harvested bacteria were suspended in physiological saline and their concentrations were adjusted at OD at 640 nm to give 10^9 CFU/ml which was used to challenge immunized mice. For the piglet experiment, a Loopfull of E. coli K88 stock was inoculated into 250 ml TSB and incubated for 18 hours at 37°C. This broth (200 µl) was spread onto blood agar plates and incubated for 20-24 hours at 37°C. Bacteria were removed by scraping, suspended in saline and used for the challenge studies. Absorbance of the suspension was adjusted at 640 nm to give 10^10 CFU/ml. Each piglet was challenged with 5 ml of the bacterial suspension.

**Extraction and Purification of K88 Fimbriae**

Purification of K88 fimbriae was done according to the method of Erickson et al. (1992) with modifications. Briefly, bacteria were cultured as described above in TSB for 36 hours at 37°C. They were then harvested by centrifugation at 3000 × g for 15 minutes, washed once with 0.01 M Phosphate Buffered Saline (PBS) (pH 7.2), resuspended in the same buffer and heated in a water bath for 30 minutes at 60°C to release the fimbriae. While still hot, the bacteria were blended for 10 minutes at high speed in IKA-Ultra-Turrax T-25 homogenizer (IKA Laborotechnik, Staufen, Germany). Bacterial cells were removed by centrifugation (14,000 × g, 15 minutes), the supernatant was filtered through a 0.45 µm syringe filter (Nalgene, Rochester, NY) and fimbriae were precipitated by gradually adding 2.5 % citric acid until pH reached 4.0. The fimbriae were incubated for 2 hours at 4°C, centrifuged at 14,000 × g for 30 minutes and the precipitation procedure was repeated three more times. Purity of fimbrial extract was determined by SDS-PAGE electrophoresis followed by silver staining.

**Anti-K88 Monoclonal Antibody (mAb1) Production and Purification.** Antibody producing cell lines were obtained from fusion of spleen cells of mice immunized with pure K88 fimbriae and myeloma cells (P3x63-Ag8.653) using polyethylene glycol MW 4000 (Merck, Darmstadt, Germany) as described previously by Jaradat and Zawistowski (1996). Antibody producing hybridomas were screened by ELISA using microtiter plates coated with K88 antigen. Several anti-K88 mAbs were produced and isotyped using a mouse isotyping kit (BioRad, Hercules, CA). Antibodies were produced by inducing ascites in mice and purified using either protein-A Sepharose for IgG2b following a procedure described by Harlow and Lane (1988) or Fast Protein Liquid Chromatography (FPLC) (Pharmacia, Uppsala, Sweden) gel filtration for IgM following the manufacturer’s instructions.

**Production and Purification of Anti-K88 Polyclonal Antibodies (pAb1) and Their F(ab′)2 Fragments**

Serum from white New Zealand rabbits immunized with K88 fimbriae was fractionated into IgG1, IgG2a and IgG2b using protein A-Sepharose column according to the method of Ey et al. (1978). F(ab′)2 fragments was prepared from the purified rabbit IgG2a. Briefly, about 20 mg of pure IgG2a were suspended in 0.1M citrate buffer (pH 4.2) and pepsin was added at a ratio of 1:33 enzyme/substrate ratio (Coligan et al., 1992). The mixture was incubated for 8 hours at 37°C and the reaction was stopped by adjusting the pH to 8.0 with 2 M Tris-base. The F(ab′)2 fraction was then purified using FPLC Superose 12 HR 10/30 gel filtration column and each fraction was tested for activity against K88 fimbriae by ELISA.

**SDS-PAGE and Immunoblotting**

The specificity of the produced monoclonal antibodies
was assessed using SDS-PAGE and Immunoblotting. Purified fibrial protein (10 µg /lane) was subjected to SDS-PAGE, transferred to nitrocellulose and probed with the Ab1 preparations. Membranes were then incubated with anti-mouse antibody conjugated to alkaline phosphatase and were then developed to visualize the bands recognized by the antibodies.

**Production of Anti-idiotypic Antibodies (Ab2) in Chicken.** Twenty week-old white Leghorn laying hens (2 hens per treatment) were immunized with three mAb1, two rabbit pAb1 and F(ab')2 of rabbit IgG2a that were purified. Initially, the birds were injected intramuscularly (IM) with 250 µg of the immunogen emulsified in Complete Freund’s Adjuvant (CFA) at 1:1 ratio of immunogen to CFA. In the second and subsequent injections, each of 250 µg of immunogen were emulsified in IFA and administered biweekly. After the fifth injection, serum was tested for titer and eggs were collected daily. Antibodies from chicken sera were purified by FPLC using a Superose 12HR 10/30 gel filtration column. Alternatively, egg-yolk was separated from egg-white and freeze-dried using a Var Tis freeze dryer model Genesis 25 LE (Gardiner, NY). The titer of the Ab2 was measured by indirect ELISA.

**Competitive Elisa for Detection and Quantification of Anti-Idiotype Antibodies (Ab2) in Chicken Serum.** The detection and quantification of anti-idiotypic antibodies that bear an internal image to fimbriae were performed by an indirect competitive ELISA. Plates were coated with 1 µg/well of Ab1 [IgG, IgG2a or the latter antibody F(ab')2; fragments from rabbits, or IgG2b, IgM1 or IgM2 from mice], diluted in 0.05 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. After blocking the plates with 5% skim milk, a mixture of serially diluted K88 fimbriae (inhibitor) and a fixed amount (0.4 µg/well) of purified chicken serum were added to each well and incubated for 1.5 hour at 37°C. Alkaline phosphatase-conjugated rabbit anti-chicken and ρ-nitrophenyl phosphate (ρNPP) were added as the secondary antibody and substrate, respectively. The color was developed by incubating the plates for 30 minutes at 37°C and OD was read at 405 nm using the microtiter plate reader. The percent inhibition was calculated according to the following formula:

\[
\% \text{ Inhibition} = \frac{\text{OD without inhibitor} - \text{OD with inhibitor}}{\text{OD without inhibitor}} \times 100\%
\]

**Analysis of Serum Antibodies (Ab3) by ELISA.** The production of anti-anti-idiotypic antibodies (Ab3) in mice was detected by indirect non-competitive ELISA according to Jaradat and Zawistowski (1996).

**Mice Immunization for the Challenge Study.** FPLC purified preparations of different anti-idiotypic antibodies (Ab2) were used as surrogate antigens for mice immunization. All vaccinated and non-vaccinated mice were then challenged with an IP injection of \(5 \times 10^8\) CFU of *E. coli* K88 in 200 µl of PBS one week after the last antigen injection. The mice were then observed for signs of infection every 2 hours. Those mice that appeared to suffer from a severe infection were killed by CO2.

**Pig Challenge with *E. coli* K88.** The procedure was similar to that described before (Marquardt et al., 1999). Twenty four 3-day-old Cotswold piglets were obtained from a local farm and transferred to the University of Manitoba’s Animal Research Unit. The piglets were fed milk replacer (skim milk, 109 g; whey powder, 77 g; methyl cellulose, 4 g; calcium carbonate and phosphate, 1 g each; water soluble vitamin mix, 4 g; and oil mix, 35 ml per one liter). Piglets were randomly divided into three groups; containing 8 piglets each, and were challenged with \(5 \times 10^{10}\) CFU of *E. coli* K88 in 5 ml of saline at zero time. Five hours before *E. coli* challenge, two groups were given Ab2 produced against either
IgG2a or its F(ab’)_2 fragments, while the third group was left without Ab treatment as a positive control. The experiment was conducted for 72 hours with antibodies (2g of egg yolk suspended in water and containing an antibody titer of >10^6) being administered by gastric intubation to each piglet in the treated groups every 6-8 hours (total 9 times). Both E. coli and anti-idiotypic antibodies (Ab2) were delivered directly to the stomach using a syringe and a polyethylene tube. The clinical responses of the piglets were monitored throughout the experiment for the occurrence of diarrhea, weight loss and mortality.

RESULTS

Three monoclonal antibodies (2 IgMs and one IgGb2) were produced against E. coli K88 fimbriae using the hybridoma technology, propagated in mice to produce ascites and purified using affinity chromatography. Anti-idiotypic antibodies were produced in chicken against the MAbs, and rabbit whole IgG, IgG2a and its F(ab’)_2 fraction. Monoclonal antibodies (mAb1) were found to react with only one major band corresponding to 28 kDa which is the molecular weight of the K88 fimbriae (Jacobs and DeGraaf, 1985) and one minor band (Figure 1, lanes 1,2 and 3). The polyclonal antibodies also mainly recognized the 28 kDa fimbrial protein. Polyclonal antibodies also reacted to a minor degree with other protein bands that were not detected by silver staining (Figure 1, lane 4).

Figure 1: Immunoblot analysis of K88 fimbriae probed with monoclonal antibodies (lanes 1-3) and rabbit polyclonal (lane 4) that was obtained from mice and rabbits immunized with K88 fimbriae.

Characterization of Anti-idiotypic Antibodies

The anti-idiotypic antibodies were in part characterized by indirect competitive ELISA. The competitive ELISA was used to demonstrate the presence of anti-idiotypic antibodies that bear an internal image for K88 in chicken serum. In this assay, the competition was between a fixed amount of Ab2 and a variable amount (serially diluted) of fimbriae (inhibitor)
for binding of the corresponding Ab1 (Figure 2, A and B). Anti-idiotypic antibodies produced against pAb1 had at least a two-fold higher percent inhibition (ca. 28-35 %) than for those produced against mAb1 (ca. 6-15 %). There was no difference in percent inhibition within each of the two groups.

![Graph A](image1.png)

**Figure 2, A and B: Inhibition of the binding of pure K88 fimbriae to Ab1 by anti-idiotypic antibodies (Ab2) as tested by competitive ELISA.** In the assay, equal amounts (100µl of 10µg/ml/well) of pure Ab1 were coated onto the plates and allowed to compete with fixed amounts of pure Ab2 from chicken serum (0.4 µg/well in 50 µl PBS) and variable amounts of K88 fimbriae. Upper frame (A) represents Ab2 produced against rabbit pAb1 [IgG2a (■), F(ab’)_2 (Δ) or IgG (♦)]. Antibodies (Ab2) in the lower frame (B) were produced against mouse mAb1 [IgG2b (Δ), IgM1 (♦) or IgM2 (■)]. The best fit lines were obtained using Microsoft Excel. Values are the average of a triplicate set of analysis.

**Humoral Response in Mice Immunized with Anti-idiotypic Antibodies.** Balb/c mice were immunized with different anti-idiotypic antibodies (Ab2) and with pure K88 fimbriae to determine if the anti-idiotypic antibodies bear...
an internal image of the original immunogen (K88 fimbriae). The antibody response of mice was evaluated by direct non-competitive ELISA. Figure 3A shows the progress in the production of anti-idiotypic antibodies (Ab3) in mice against chicken (Ab2) over a 6-week period. It appeared that anti-idiotypic antibodies produced against all Ab2 antigens gave similar overall results. This indicates that the different anti-idiotypic antibodies from chicken serum (Ab2) produced a similar nonspecific Ab3 response in mouse serum. The ability of the Ab3 in the same serum to react specifically with K88 fimbriae was also tested (Figure 3B). The results indicated that the greatest reaction between Ab3 and fimbriae was obtained after 6 weeks when the antigen (chicken Ab2) was produced against rabbit pAb1 [IgG2a, F(ab')2 and IgG]. The lowest value for Ab3 was obtained when Ab2 was produced against mAb1. The data also show that the degree of reaction of fimbriae with mouse anti-K88 fimbriae (pAb1) was greater than it was with the anti-anti-idiotypic antibodies (Ab3). The titers for K88 fimbriae in the various Ab3 serum from mice were also determined (Figure 4A). The absorbance values for serum from mice after 6 weeks of immunization which is a measure of titer, followed a pattern similar to that obtained in Figure 3B. In these studies, all anti-idiotypic preparations gave a similar titer (about 4000) considering an absorbance value of > 0.1 as positive. However, at a dilution of 1:30, sera from mice that were immunized with anti-idiotypic antibodies (Ab2) against F(ab')2 and IgG2a showed an OD of 2.5, followed by sera from mice immunized with anti-IgG Ab2 (OD= 2.0). In contrast, sera from mice immunized with chicken (Ab2) from the different anti-mAb1 exhibited lower OD values (1.0). A titer curve for mouse pAb1 against K88 fimbriae (Figure 4B) indicated that the anti-K88 antibody titer for pAb1 was about 20,000-fold greater than the highest titer present in the different Ab3. In these comparisons, the titer was the dilution obtained at half maximum absorption.

![Figure 3, A and B: Detection of anti-anti-idiotypic antibody (Ab3) response in serum of Balb/c mice over a 6-week period by indirect non-competitive ELISA. Balb/c mice were immunized with Ab2 that were produced in chicken against anti-K88 rabbit polyclonal IgG2a (○), F(ab')2 (■) and IgG (▲), or mouse anti-K88 monoclonal](image)
IgG2b (☐), IgM1 (◆), IgM2 (★). A) detection of Ab3 response to Ab2 in serum. The plates were coated with a fixed amount of Ab2 (100 µl of 10 µg/ml diluted Ab2/well) and 100 µl of Ab3 diluted 1:2000 were added to each well. This assay provides a measure of the non-specific plus K88 specific antibody in serum. B) Detection of Ab3 (diluted 1:30) in response to K88 fimbriae. In this assay, fimbriae were coated on the plate (100 µl of 10µg/ml/ well). This assay provides a measure of the K88 specific antibodies in serum. Figure 3B also shows the antibody response for mice immunized with K88 fimbriae (●). Serum was collected prior to each antigen boost. Other procedures were described in Materials and Methods. Each point represents the average of a triplicate set of analysis.

Figure 4, A and B: Titration curves of serum (Ab3) of mice (6 mice per group) after 6 weeks of immunization as tested by indirect non-competitive ELISA against K88 fimbriae. A) Mice were immunized with chicken Ab2 that was produced against rabbit anti-K88 polyclonal IgG2a (◆), F(ab′)2 (☐), IgG (▲) or mouse anti-K88 monoclonal IgG2b (Δ), IgM1 (●), IgM2 (★). The titer plate wells in this assay were coated with 100 µl of 10 µg/ml pure K88 fimbriae followed by 100 µl of serially diluted Ab3 (initially diluted 1:30) B) Titration curves of serum (1:250 dilution) from mice immunized with K88 fimbriae and tested against the same antigen. Serum was collected prior to each boost.
**Immunoblot Analysis of Mice Serum (Ab3) Immunized with Various Anti-idiotypic Antibodies (Ab2)**

Purified K88 fimbriae (10 µl/lane) were subjected to SDS-PAGE electrophoresis and transferred onto nitrocellulose membranes to further demonstrate the specificity of Ab3 to K88 fimbriae. The membranes were then probed with mice serum containing Ab3 against the different anti-idiotypic antibodies (Ab2). Serum from all mice had anti-K88 anti-anti-idiotypic antibodies that recognized the K88 antigen, while non-immunized serum failed to recognize the K88 fimbriae (Figure 5). Further, all the Ab3 preparations recognized the K88 fimbriae band to a similar degree as indicated by the apparent intensity of the band.

**Mouse Protection Experiment.** Protection of vaccinated mice against IP administered *E. coli* K88 was evaluated seven days after the last injection with the Ab2. The protective effects of each Ab2 are shown in Table I. The survival rate for all mice that were immunized with Ab2 antibodies was 100% in both experiments 1 and 2. In contrast, the survival rate for non-immunized mice was only 25% for experiment 1 and 18% for experiment 2. All immunized and non-immunized mice, except for those immunized with pure K88 antigen, showed signs of infection within 4 hours as indicated by a marked decrease in their activity. However, after 24 hours these signs disappeared from all immunized mice, while the survivors of non-immunized mice continued to show signs of infection for at least one more day.

Figure 5: Immunoblott analysis of K88 fimbrial antigen when probed with serum (Ab3) from mice immunized with Ab2 that was obtained from chicken serum produced against anti-rabbit (pAb1) IgG2a, F(ab')2, or IgG (Lane 1, 2, 3), respectively. Lanes 4, 5 and 6 were chicken Ab2 that were produced against anti-mouse (mAb1) IgG2b, IgM1 or IgM2. Lane 7 was the pre-immune serum.
In experiment 1 and 2, mice were injected IP with 250 µl of 2 x 10⁸ CFU per mouse of \textit{E. coli} K88. One group of mice was immunized with K88 fimbriae as a positive control, while another group was left without any treatment as negative control. The other groups were immunized with different antibodies to produce anti-anti-idiotypic antibodies (Ab3).

The rabbit and chicken antibodies are polyclonal, while the mice were monoclonal. Ab1 were produced against K88 fimbriae and Ab2 were produced against Ab1. Ab2 contained anti-K88 anti-idiotypic antibodies.

The disease status was considered to be positive (+) in mice where their activity markedly decreased and they stopped eating and drinking. Otherwise they were considered to be normal.

This antibody was not tested in experiment 1.

Not determined.

**Passive Protection of Piglets.** Young piglets were infected with \textit{E. coli} K88 by gastric intubation and some were orally treated with two of the chicken anti-idiotypic antibodies [anti- rabbit IgG2a and anti- rabbit F(ab')₂] that had the highest activity against K88 fimbriae as determined by competitive ELISA. The results of this experiment demonstrated that most of the piglets challenged with \textit{E. coli} developed diarrhea within 24 hours (day 1). This was also apparent from the severe weight loss observed after 24 hours of the challenge (Table II) which was mainly caused by the loss of body fluids.

The \textit{E. coli} challenged and non-Ab2 treated group (group 1) showed the highest weight loss after 24 hours with the average being 541 g which was equal to 25 % of the body weight before treatment. The weight loss in the two treated groups was much less being 269 g (10 %) for those treated with anti-F(ab')₂ antibodies and 347 g (17 %) for those treated with anti-IgG2a antibodies. Although the averages in weight loss showed variation among different groups, statistical analysis showed no significant differences \((P < 0.05)\) among groups 1, 2 and 3. The mortality rate was the highest, 87.5 % among the...
group that was challenged with \textit{E. coli} but not treated with Ab2, while it was 37.5\% in groups 2 and 3 (the \textit{E. coli} and Ab2 treated groups).

**Table II:** Incidence of diarrhea, fecal scores, loss in body weight and mortality in piglets that were challenged with \textit{E. coli} K88 and treated with anti-idiotypic antibodies (Ab2) or left without treatment.

<table>
<thead>
<tr>
<th>Groups(^a)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
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<tr>
<td><strong>Treatments</strong></td>
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<td>\textit{E. coli}</td>
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<td>+</td>
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<tr>
<td>Ab2(^b)</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Type of Ab2</td>
<td>Anti-Fab2</td>
<td>Anti-IgG2a</td>
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<tr>
<td><strong>Incidence of Diarrhea (%)(^c)</strong></td>
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<tr>
<td>Day 1</td>
<td>87.5</td>
<td>75</td>
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<tr>
<td><strong>Weight loss (G) per piglet after 24 h</strong></td>
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<td></td>
<td>541(^d)</td>
<td>269(^d)</td>
<td>374(^d)</td>
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<tr>
<td><strong>Mortality (%)</strong></td>
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<td>37.5</td>
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\(^a\) All groups were given 5 ml of 10^{10} CFU/ml \textit{E. coli} K88 suspended in saline on day 0 by gastric tubing. Data for day 0 and 1 are only given as only one piglet survived in group one after this period.

\(^b\) Groups 2 and 3 were given chicken Ab2 6 h prior to the bacterial challenge and every 8 h after the challenge until the end of the experiment while group 1 was left without treatment as a negative control.

\(^c\) Percent of piglets developed diarrhea as indicated by the fecal consistency.

\(^d\) Means within a row followed by the same alphabet superscript are statistically not significant at \(p < 0.05\).

**DISCUSSION**

The control of animal diseases is of prime importance in the production of safe foods. To control these diseases, farmers tend to use extensively antimicrobials that leave residues in the slaughtered animals in most of times. Finding an alternative to the use of antimicrobials will greatly benefit this industry. Therapeutic antibodies have a promise in solving this problem or at least minimizing it. Anti-idiotypic antibodies are one strategy to combat animal diseases.

Antibodies contain idiotypes which are regions located in the antigen binding site. These areas are capable of acting as antigens to produce antibodies that are called anti-idiotypic antibodies (Jerne, 1974). The proportion of anti-idiotypic antibodies carrying an internal image to the original antigen can be used as surrogate vaccines. Such vaccines have shown to induce protective immunity in animals that had never been exposed to the nominal antigen (Beauclair and Khansari, 1990; Brossay et al., 1993; Collins et al., 1991; Paque et
al., 1990; Percival et al., 1990; Tanaka et al., 1994). *E. coli* K88 strain is responsible for the majority of diarrhea outbreaks in piglets. The major subunit of the K88 fimbrial antigen is believed to be responsible for the attachment and colonization of *E. coli* to the pig intestine (Smyth et al., 1994). We have produced mAbs and pAbs for this major subunit as shown by the hemagglutination test and immunoblotting of detergent treated K88 fimbriae (data not shown). These antibodies were used to produce anti-idiotypic antibodies carrying an internal image of this major subunit as the anti-idiotypic antibodies were able to inhibit the binding of pure K88 fimbriae to Ab1 in a concentration dependent manner when tested by a competitive ELISA. These antibodies were distinct from anti-idiotypic preparations that are devoid of this fraction. In addition, the competitive assay developed in this study was able to quantitatively determine the proportion of the anti-idiotypic antibodies that carry an internal image (% inhibition) to the original antigen. Interestingly, the anti-idiotypic antibodies produced against anti-K88 pAb1 had at least twice the percent inhibition (ca. 28-35 %) of that of the anti-idiotypic antibodies produced against anti-K88 mAb1 (ca. 6-15 %). The reason for this difference could be related to the specificity of Ab1 where the monoclonal antibodies, being unspecific, would have recognized only one epitope (10-12 amino acids) on the K88 fimbrial major subunit, while the polyclonal antibodies being multispecific, would have contained a battery of antibodies that recognize all epitopes in the fimbrial subunit. Therefore, when both were injected into chicken, polyclonal antibodies would have induced the production of anti-idiotypic antibodies that contain a higher proportion of antibodies bearing an internal image for a wide range of the K88 antigen determinants in comparison to a limited specificity range of the monoclonal antibodies.

In this study, we also demonstrated that administration of anti-K88 anti-idiotypic antibodies could induce immunity in mice against subsequent infection with *E. coli* K88. The immunity was manifested by complete protection against an *E. coli* IP challenge of immunized mice (100 % survival in both experiments 1 and 2, Table I), while only 25% and 18 % of un-immunized mice survived the challenge in these experiments. Our results seem to contradict results obtained by Paque et al. (1990). They produced anti-idiotypic antibodies against antibodies that recognize an *E. coli* strain possessing type 1 fimbriae. In their study, the anti-idiotypic antibodies were unable to induce protective immunity in mice against an IP challenge with *E. coli*. The reason for the difference may be that in the current study, antibodies (Ab1) were produced against the K88 major subunit which contains the adhesion that recognized the receptor. Therefore, Ab2 carry an internal image to the K88 antigen, consequently it conferred protection in challenged mice. However, type 1 fimbiae consist of eight subunits (A to H) with only Fim H subunit being responsible for the adhesion to receptors. Therefore, anti-idiotypic antibodies produced against antibodies that recognize the major subunit (A), which seems to be immunodominant, were either devoid of or contained only minute amounts of anti-idiotypic antibodies that bore an internal image of Fim H subunit and, consequently, failed to protect mice challenged with the *E. coli*.

It is noteworthy that there was no difference in protection among different anti-idiotypic preparations. This indicates that the mice immune system needed only a small amount of anti-idiotypic antibodies bearing an internal image of K88 to induce the production of protective antibodies against *E. coli* K88 infection. Further, there were no differences in the intensity of the bands recognized by any of the anti-idiotypic antibody
preparations from mouse serum (Ab3) used in the immunization studies against K88 fimbriae. However, when the serum was taken from mice at two week intervals and tested for the presence of anti-K88 antibodies (Ab3) by direct ELISA, it was clear that serum from mice immunized with anti-idiotypic antibodies produced against anti-K88 pAb1 exhibited much higher Ab3 titers against fimbriae compared to mice that were immunized with anti-idiotypic antibodies against anti-K88 mAb1. These results were consistent with results obtained by using the competitive ELISA.

A second study was undertaken to evaluate the efficacy of two anti-idiotypic preparations in protecting piglets against diarrhea caused by \textit{E. coli} K88. Two anti-idiotypic antibody preparations that exhibited the highest percent inhibition in the competitive ELISA and highest Ab3 titer in the mice protection experiment were selected. It is known that \textit{E. coli} K88 attaches to receptors in the intestinal mucus and epithelium layer as a prerequisite step prior to colonization and toxin release. The anti-idiotypic antibodies that bear an internal image to K88 when administered prior to bacterial challenge should block these receptors in an analogous manner to the corresponding fimbriae. Thus, the anti-idiotypic antibodies that bear internal images to the K88 would compete with bacteria for binding to K88 receptors, and would prevent bacterial colonization so that bacteria will be washed out through normal cleansing mechanisms. In this experiment, egg-yolk containing anti-idiotypic antibodies was administered through gastric intubation to piglets in order to block the K88 receptors. Partial protection (62.5 % survival rate) was observed in piglets treated with anti-idiotypic antibodies and \textit{E. coli} K88, while the survival rate was only 12.5 % among the groups that were challenged with \textit{E. coli} and did not receive the anti-idiotypic antibodies. This latter group exhibited a considerable weight loss (541 g) due to severe diarrhea, while the Ab treated groups did not lose as much weight (269 and 347 for groups 2 and 3, respectively). These data imply that the anti-idiotypic antibodies were able to partially block \textit{E. coli} K88 receptors resulting in partial protection against infection with the organism.

There are two reasons that could explain the inability of the anti-idiotypic antibodies to provide complete protection in challenged piglets. These are: i) the proportion of the antibodies that bear an internal image of K88 fimbriae were not enough to block all the receptors, bearing in mind that a high ($5 \times 10^{10}$ CFU) \textit{E. coli} K88 dose was administered to each piglet by gastric tubing and ii) some of the anti-idiotypic antibodies might have digested or inactivated while passing through the stomach.

Our results appeared to be consistent with results obtained by Jackson et al. (1990). They reported incomplete protection of rats against dental carries when anti-\textit{Streptococcus mutans} anti-idiotypic antibodies, were administered orally to rats. The anti-idiotypic antibodies used in this study were polyclonal which means that only a certain proportion of the pool was effective. However, Reck et al. (1988) reported a complete protection of monkeys against diarrhea and emetic response induced by \textit{Staphylococcus enterotoxin B}. They used a monoclonal anti-idiotypic antibody that mimicked the toxin.

The results of the current study indicate that anti-idiotypic antibodies against \textit{E. coli} K88 fimbriae when administered to mice were able to induce active immunity in mice that completely protected them against an IP challenge with \textit{E. coli} K88. The same anti-idiotypic antibodies when used to provide a passive immunity against intragastric challenge of piglets by \textit{E. coli} K88 also provide protection, but the protection was only partial. The protective effect with anti-idiotypic
antibodies was greater when polyclonal antibodies compared to monoclonal antibodies were used as the source of idioype antibodies. Also, the protective effect of the polyclonal antibodies in mice obtained with the anti-idiotypic antibodies was considerably less than that obtained when they were immunized directly with fimbrial antigen. These results demonstrate that anti-idiotypic antibodies can be useful in the control of *E. coli* infection in pigs and mice, but may not be nearly as effective as idioype antibodies produced directly against the antigen.

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Anti-idiotypic (BRORTIN TAHAKY) ANTIGEN K88 EXISTING IN E.coli (IN THE ENTRANCE OF THE PHYSICAL EXISTENCE) STUDY

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