Passive immunization of piglets against enterotoxigenic colibacillosis by vaccinating dams with K88ac pili bearing bacterins

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Day-old piglets were passively immunized by vaccinating the pregnant sows with K88ac enterotoxigenic Escherichia coli (ETEC) vaccine. High level of ETEC specific antibodies was excreted in colostrum (3733.33 ± 1152.13) and maintained a detectable level (100.00 ± 0.00) up to 21 day post partum (DPP). The IgG was the predominant immunoglobulin followed by IgA and IgM. Piglets born of vaccinated dam (group A) and unvaccinated dam (group B) were challenged in 7 day of age. Clinical and faecal scores were significantly (P<0.01) low in group A than that of group B. Piglets of group A developed mild diarrhoea (33.33%), while all the control piglets developed profuse diarrhoea and 3 of these died before 14 day of challenge infection.

Enteric colibacillosis is one of the major causes of death in neonatal piglets. Passive transfer of lacteal immunity from the vaccinated dams can protect young from enterotoxigenic Escherichia coli (ETEC) infection1. Different ETEC derived immunogens have been used to vaccinate pregnant dams2,3. However, most promising results were obtained using pili antigens4. The bacterins preserve both capsular and pili antigens render strong passive immunity to calves and protect against challenge infection5. In this study we have evaluated passive immunoprotective response in pigs against K88ac ETEC by vaccinating the dams. Also specific immunoglobulin isotypes in milk were estimated.

Preparation of vaccine—Enterotoxigenic E coli strain (0149 : K91 : K88ac : LT+ STa+ STb+) was grown in minca broth at 37°C for 24 hr. Bacteria were inactivated by formalin (0.4% v/v) at 4°C for 24 hr. Inactivated bacterial suspension was precipitated by addition of 3.0 ml of a 10% (w/v) solution of aluminum potassium sulphate and 1.5 ml of a 7.4% (w/v) solution of potassium hydroxide per 100 ml. Bacterial concentration was adjusted to 2 X 10^9 CFU per ml prior to inactivation by pour plate technique6. However, the volume of the bacterial suspension was kept constant even after inactivation.

Immunization of dams—Three pregnant sows (crossbred Hampshire×Dum variety) of second lactation were selected from the breeding stock of the Network Project on Pigs, Khanapara, Guwahati. These animals were naturally exposed to E coli as their serum samples were found positive for ETEC. The pregnant sows primed with E coli were vaccinated with K88ac ETEC vaccine intra-muscular 6 week before farrowing. One ml of bacterin was emulsified in equal volume of Montanide ISA 25 (Seppic 75, quai-d'Orsay, Paris) adjuvant. Booster vaccination was given at 2 weeks before farrowing. One ml of bacterin injected at the base of the mammary tissue of each fore and hind pair teats.

Antibodies in colostrum and milk—Colostrum and milk samples were collected from the vaccinated sows for first 7 days and at 2nd, 3rd, and 4th week of post farrowing periods. Milk samples were centrifuged at 700 g for 30 min at 4°C to remove the fat. The rennin (Sigma Chemical Co., USA) was added @ 1 unit per 10 ml and incubated at 37°C for 2 hr. The milk wheys were obtained by centrifugation at 8000 g for 15 min at 4°C and pH was adjusted to 7.4 and stored at -20°C.

Indirect ELISA—K88ac specific whole antibodies and isotype specific antibodies were assayed by indirect ELISA. The polystyrene plates (Nunc, Denmark) were coated with a 1:100 diluted ETEC extract in 0.05 M carbonate bicarbonate buffer (pH 9.6) over night at 4°C. All reagents, except the substrate, were diluted in 0.01 M T-PBS containing 5% FCS. The whey was added in a volume 100 µl but other reagents at a predetermined optimal concentration in a volume of 50 µl. After each incubation step plates were washed five times with 0.05% w/v tween 20 in 0.01 M PBS.
For detection of K88ac specific whole antibodies, coated plates were incubated with 2-fold dilutions of whey (starting at 1:100) at 37°C for 1 hr. Subsequently rabbit anti-swine IgG (1:2000, Sigma Chemical Co.) and goat anti-rabbit HRPO conjugate (1:3000, Sigma Chemical Co.) were added and incubated at 37°C for 1 hr. However, to detect specific isotype antibodies, coated plates were incubated with MoAb to swine IgA (1:500), IgM (1:500) and rabbit anti-swine IgG (1:3000) antibodies. Finally, anti-mouse or anti-rabbit IgG conjugated with HRPO (1:2000, Dakopatts) was added and incubated at 37°C for 1 hr. The OPD (Sigma Chemical Co.) was used as substrate and after 15 min absorbance was measured in dynatech ELISA reader at 490 nm. The reciprocal of the highest dilution of a test sample yielding an OD value (OD of test sample- OD of negative whey control) of ≥ 0.1 was taken as titre. As a negative control whey of unvaccinated sow was used.

Protection test — On seven day of age piglets born to ETEC vaccinated sow (group A, 6 nos) and unvaccinated sow (group B, 4 nos) were challenged orally with 1×10⁹ CFU of live K88ac ETEC. The bacterial suspension prepared in 10 ml sterile PBS (pH 7.4) was administered into the back of the mouth. The gastric acid was neutralized 15 min before with 1.4% NaHCO₃. The piglets were examined at challenge and two times daily for 21 post challenge (PC). At each examination clinical and faecal scores were recorded as described by Lanza⁶. The clinical scores were ranged in value from 0 to 3 as follows: 0=normal rectal temperature (100-102°F), progressive weight gain; 2=no weight gain; 3=subnormal temperature (<98°F), loss of weight and dead. The faecal scores were fixed between 0 to 3 and criteria were: 0=firm faeces, no K88ac ETEC; 1=sof faeces, 1-2 K88ac ETEC; 2=semi-liquid faeces, 4-5 K88ac ETEC; 3=faeces watery and projectile, 5 K88ac ETEC. Rectal temperature and faecal consistency were recorded twice daily up to 7 day PC. The weight gain was calculated weekly up to 4 week of age. The excretion of ETEC in the challenged piglets was studied in the rectal swab up to 7 PC. Ten randomly selected colonies were screened for K88ac ETEC by indirect ELISA using pili specific antiserum.

Protection of ETEC challenged piglets — In the control group (group B) on 2nd day of challenge all piglets exposed to ETEC developed diarrhoea which became profuse and watery by 4 PCD. The challenged ETEC bacteria were excreted in high numbers from 4 PCD onwards. Animal became weak and lost their body weight. Three out of four piglets died before 14 PCD. Piglets born to sow immunized with K88ac ETEC developed mild diarrhoea (2/6) within 72 hr and recoverd before 6 PCD. However, 4/6 piglets excreted challenge bacteria up to 4 PCD. There was a marginal loss of body weight in piglets born to vaccinated dam (group A). The results of passive protection studies and statistical analysis are in Table 1. Mean clinical and faecal scores in piglets born to vaccinated sow (0.21 ± 0.07; 0.43 ± 0.10) and unvaccinated sow (2.22 ± 0.17; 2.03 ± 0.23) differed significantly (P<0.01).

High fatalities due to coli in piglets occur mostly in first week of life⁷. It is, therefore, essential to vaccinate the pregnant dams to transfer ETEC specific lacteal antibodies passively to the newborns. A promising results obtained by vaccinating with whole bacterial cells possessing common pili antigen⁸. Previous study⁹ indicated that a high degree of protection was evoked against coli in piglets which suckled by dams immunized with a partially purified K88 preparation. The protection afforded was mostly attributable to anti-K88 antibodies. Acrès⁷ demonstrated that in addition to the pili antibodies, K30 and 09 antibodies have contributed in preventing diarrhea and death in calves.

The data presented here indicate that the lacteal immunity against K88ac antigens prevented severe diarrhoea and death in piglets experimentally challenged at 7 day of age with homologous ETEC strain. All piglets (100%) nursing unvaccinated sow

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of animals</th>
<th>Piglets with diarrhoea/ total (%)</th>
<th>Mean* clinical score (± SE)</th>
<th>Mean* faecal score (± SE)</th>
<th>Duration of diarrhoea (day)</th>
<th>No. of piglets dead/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>2/6 (33.33)</td>
<td>0.21 (± 0.07)</td>
<td>0.43 (± 0.10)</td>
<td>2</td>
<td>0/6 (0.00)</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>4/4 (100.00)</td>
<td>2.22 (± 0.17)</td>
<td>2.03 (± 0.23)</td>
<td>3-5</td>
<td>3/4 (75.00)</td>
</tr>
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* P < 0.01
A=piglets received colostrum from vaccinated sow.
B=piglets of non-vaccinated sow.

Table 1 — Protection of piglets fed with colostrum and milk from vaccinated sow after ETEC challenge
developed severe diarrhoea within 24 hr and 3/4 succumbed before 14 PCD. The colostrum of the unvaccinated sow did not contain antibody to K88ac ETEC. This results confirmed the virulence and age susceptibility of the challenge ETEC strain. The piglets (6/6) suckled by the vaccinated dam with > 1000colostral antibody titre survived the challenge infection. Although 2/6 (33.33%) piglets developed mild diarrhoea for a brief period. Shedding of ETEC strain was low and delayed by 48 hr comparing to the control piglets. Sero-positive sow vaccinated IM and IMm boost up the level of immunoglobulins in the lacteal secretion and thus prevented the colonization of K88ac ETEC. ETEC specific IgG immunoglobulin was predominant in the colostral period but the mean titre was reduced 3-fold at the transition of colostrum to milk. IgA and IgM antibody titres were also decreased steadily after the colostral period. In other studies, seropositive sow immunized with TGEV IM and IMm evoked high milk IgA and IgG antibodies, but in seronegative sow high level of IgG antibodies were detected in milk and serum.

Antibody response — Results of K88ac ETEC specific whole antibodies and specific immunoglobulin isotypes at different stages of lactation are in Table 2. In unvaccinated control sow, antibodies could not be detected in the lacteal secretion. But in vaccinated sow high concentration of antibodies was present on the colostrum of first 4 days (3733.33 to 1066.66) as well as in milk up to 14 DPP (150.00). IgA (666.66) and IgM (538.33) isotypes appeared in the first colostrum. But IgA maintained a detectable level up to 7 DPP (133.33).

The results of the study showed that the protection of piglets against ETEC challenge infection was mainly due to IgG and IgA antibodies. However, SIgA play an important role in the protection of the gut against pathogens [6,10]. Maternal vaccination to elicit IgA antibody in milk thus is contingent to mammary gland in response to the antigen [10,12]. It is, therefore, necessary to understand the gut-mammary mechanism to boostup the level of neutralizing antibodies in milk.

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References
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