VETERINARSKI ARHIV 71 (6), 381-387, 2001

Elimination of enterotoxigenic *Escherichia coli* in experimentally infected piglets by passive immunization with bovine colostral antibodies

Nagendra Nath Barman[1]*, Dilip Kumar Sarma[1], and Bhabendra Nath Neog[2]

[1]*Department of Microbiology, College of Veterinary Science, Guwahati, India*

[2]*Network Project, Indian Council of Agricultural Research, College of Veterinary Science, Guwahati, India*

BARMAN, N. N., D. K. SARMA, B. N. NEOG: Elimination of enterotoxigenic *Escherichia coli* in experimentally infected piglets by passive immunization with bovine colostral antibodies. Vet. arhiv 71, 381-387, 2001.

ABSTRACT

When piglets fail to receive colostrum from the sow, as an alternative means passive immunization can be carried out with antibodies derived from non-maternal sources. In this study, pregnant cows were vaccinated intramuscularly and intramammarily with killed ETEC vaccine and developed a high level of colostral antibodies. These antibodies were specifically of IgG and IgA. Day-old piglets fed with colostrum containing a high titre of IgG survived the challenge infection. However, 75% piglets fed with colostrum from unvaccinated cows, and 100% piglets receiving no colostrum, died from challenge infection.

Key words: *E. coli*, passive immunization, piglets

* Contact address:

Associate Professor Nagendra Nath Barman, Department of Microbiology, College of Veterinary Science, Assam Agricultural University Khanapara, Guwahati – 781022, Assam, India, Phone: +091 361 331 501/332 768, Fax: +091 361 337 700, E-mail: aaucvs@ren. nic. in

ISSN 0372-5480
Printed in Croatia

Introduction

Maternally derived passive immunity may break down due to a failure on the part of the dam to provide protective colostral antibodies or where piglets are unable to obtain access to the colostrum (FAIRBROTHER, 1992). In such a situation, colostral antibodies from a heterologous species would be an alternative source for providing passive immunity to the neonates. There is substantial evidence supporting the use of passive antibodies from heterologous species to prevent enteric diseases in animals (CORDLE et al., 1991). Enterotoxigenic *Escherichia coli* (ETEC) is the most common enteric colibacillosis occurring in piglets (HUMSKI et al., 1998; NAGY and FEKETE, 1999). Thus, it is essential that the disease be controlled. In this study, the ability of bovine antibodies to prevent diarrhoea caused by ETEC in piglets has been evaluated.

Materials and methods

Enterotoxigenic *Escherichia coli* was isolated from diarrhoeic piglets. The isolated strain belonged to serotype O149: K91: K88ac. Heat labile enterotoxin was determined by rabbit ligated ileal loop bioassay (SPIRA et al., 1981). The villus adhesion of ETEC in the experimental piglets was studied retrospectively using histological and immunofluorescence techniques.

Bovine antibodies to pig ETEC were prepared by vaccinating three pregnant Jersey x Holstein-Friesian dairy cows using a formalin-killed whole bacterin containing K88ac pili. The bacterin (prepared from approximately 3×10^{10} bacteria per dose) was precipitated with alum and adjuvanted with Montanide ISA 25 (Seppic 75, Quai-d'Orsay, Paris) adjuvant. Three pregnant Jersey x Holstein-Friesian dairy cows were immunized intramuscularly six weeks prior to calving. Cows were given a booster immunization two weeks prior to calving by intramammary infusion with bacterin without adjuvant. Upon calving, one-time milking from each of the three immunized and five un-immunized cows was collected daily for five days. Each day, collections from each group were pooled, defatted by centrifugation and processed to a whey fraction by precipitating the casein using rennin (Sigma Chemical Co.). The pH of whey samples was adjusted to 7. 4 with 8% $NaHCO_3$ and stored at -20 °C.

Mammary secretions from immunized cows were collected for the first seven days and at 2, 3 and 4 weeks of the post-calving period. ETEC specific antibody titres in whey were determined by indirect ELISA. Whole antibody titre was determined using rabbit anti-bovine peroxidase conjugate (1:3000, Dakopatts, Denmark). ETEC specific immunoglobulin isotypes were estimated using antibovine monoclonal antibody of IgA (1:4000), Ig M (1:2000) and IgG (1:2000), followed by rabbit anti-mouse peroxidase conjugate (1:3000, Dakopatts, Denmark). All the reagents except the enzyme were diluted in PBS-Tween containing 0.5 M NaCl and 10% horse serum, while the enzyme conjugate was diluted in 4% horse serum. An extinction value of the highest dilution ≥0.1 was considered antibody titre.

A litter of colostrum-deprived piglets born of a gilt (Hampshire x Dum variety) was separated at birth and divided into groups A, B and C, comprising four piglets in each group. Piglets of group A received colostrum of the vaccinated cows, group B from unvaccinated cows. Group C piglets were not provided with cow colostrum. Within 2–3 hours of birth each newborn piglet of groups A and B was provided with 100 ml pf pooled colostrum daily in four divided doses for five consecutive days. The piglets were kept in a clean-air station and provided sterilized creep ration throughout the experimental period. Piglets were challenged with 1×10^9 CFU of ETEC per ml at 7 days of age through oral route. Gastric acid was neutralized by 1.4% $NaHCO_3$ 15 minutes prior to infection. Challenged piglets were examined for 21 days post-infection. Clinical scores ranged from 0–3 and were based on loss of body weight. Faecal scores were fixed between 0 and 3 on the basis of faecal consistency and excretion of ETEC. Data were presented as mean ± SE and were analysed by analysis of variance. Significance was accepted at $P<0.01$ level.

Results and discussion

There is growing interest in the use of orally administered non-maternal or heterologous species-derived passive antibodies to prevent enteric diseases. Passive antibodies have a number of attractive features, such as a high potential value for safety, specificity, immediate effectiveness and easily controlled doses in immune-compromised animals. Results of the

present study describe the utility of bovine antibodies to ETEC in preventing diarrhoeal diseases caused by the same pathogen.

In the present study, a high level of anti-ETEC antibodies was detected in the colostrum of the immunized cows during the first three days postpartum (Fig. 1). The mean reciprocal values of whole antibody on 1st, 2nd and 3rd days postpartum were 2346.0 ± 1142.21, 1493.35 ± 460.85 and 533.33 ± 87.09, respectively. Antibody titre declined gradually and reached near to the baseline on the 28th day postpartum. Colostral antibodies specific to ETEC were primarily of IgG and IgA. A high IgG mean antibody titre prevailed consistently up to 7 days postpartum (1120.0 ± 598.0) and remained in the colostrum up to 4 days of lactation. Prevalence of IgG immunoglobulin also clearly indicated that systemic priming and intramammary booster enhanced the selective transport of IgG immunoglobulin to the colostrum. In ruminants, IgG_1 was identified as the

Table 1. Percentage protection of piglets fed with colostrum from vaccinated cows after ETEC challenge

Animal Group	N°. of animals	Piglets with diarrhoea	*Mean ± SE		Duration of diarrhoea (day)	No. of piglets dead
			Clinical score	Faecal score		
A	4	2 (50.00)	$0.17^{a} \pm 0.06$	$0.68^{a} \pm 0.12$	2 – 3	0 (0.00)
B	4	3 (75.00)	$1.95^{ab} \pm 0.18$	$1.79^{ab} \pm 0.19$	4 – 6	3 (75.00)
C	4	4 (100.00)	$2.40^{b} \pm 0.22$	$2.07^{b} \pm 0.27$	4 – 5	4 (100.00)

* $P<0.01$

( ) Figures in parentheses indicate percentages

The means in a column bearing different superscripts are significantly different at $P<0.05$

A = piglets fed colostrum from vaccinated cow; B = piglets received colostrum from unvaccinated cow; C = piglets received no colostrum

most predominant immunoglobulin isotype present in the milk (SAIF, 1985). IgG_1 was the predominant isotype of sero-positive cows vaccinated parenterally with rotavirus and coronavirus (SAIF, 1985). This study showed that the local antigenic stimuli induced the mammary gland to recruit more antibodies from the circulation.

The protective effect of anti-ETEC bovine colostral antibodies is summarized in Table 1. Suckling piglets fed with colostrum from vaccinated cows (Group 1) survived the challenge infection. However, 50. 0% of animals developed mild diarrhoea for a brief period without losing significant body weight. Piglets receiving colostrum from unvaccinated animals (Group B) and animals of the control group (Group C) developed watery diarrhoea and rapidly lost body weight. Three out of 4 piglets in Group B, and all piglets in Group C, died within 14 days of the challenge infection. The challenge ETEC were detected in faeces consistently in Group B and Group C piglets up to 6 days post-infection. Clinical and faecal scores differed

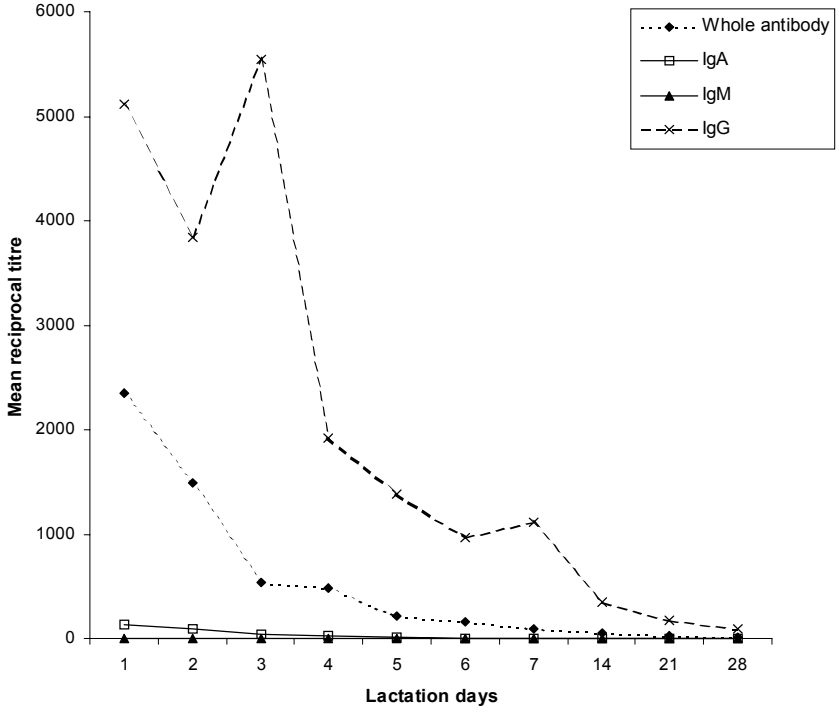

Fig. 1. Mean titre of whole antibody and immunoglobulin isotypes in lacteal secretions of cows vaccinated with K88ac on different lactation days

significantly ($P < 0.05$) between groups A and B, and between Groups B and C, respectively. The challenge infection with $K88ac^+$ ETEC in piglets deprived of passive antibody showed adherence of bacteria to the brush border of enterotcytes. BILLEY et al. (1998) demonstrated multiple receptors on porcine intestinal epithelia for K88 variants of *Escherichia coli*. However, colostrum containing a high concentration of anti-ETEC IgG antibodies could inhibit bacterial adhesion in the intestine of piglets which received passive antibodies. This was in accordance with the observations made by other authors (WESLEY et al., 1988; OHASHI, 1994; SAIF et al., 1994). SAIF (1985) stated that the ruminant IgG_1 might supplement the role of secretory in lactogenic immunity. The proteolytic degradation of IgG in the gut (VAN ZAANE et al., 1986) could also be prevented by the trypsin inhibitors present in cow colostrum (QUIGLEY et al., 1995). However, the dose and frequency of ETEC specific colostral antibodies to protect neonates from ETEC infection need to be standardized. The influence of continuous feeding of non-maternal antibodies in the development of active gut immunity also requires careful study.

Acknowledgements
We wish to express our gratitude to Dr. Jan Naessens, ILRAD, Kenya, for providing antibovine monoclonal antibodies, and to the Assam Agricultural University for providing the necessary facilities.

References

BILLEY, L. O., A. K. ERICKSON, D. H. FRANCIS (1998): Multiple receptor on porcine intestinal epithelial cells for the three variants of Escherichia coli K88 fimbrial adhesin. Vet. Microbiol. 59, 203–212.

CORDLE, C. T., J. P. SCHELLER, T. R. WINSHIP, E. L. CANDLER, M. D. HILTY, K. L. SMITH, L. J. SAIF, E. M. KOHLER, S. KRAHOWSKA (1991): Passive immune protection from diarrhoea caused by rotavirus of E. coli: An animal model to demonstrate and quantitate efficacy. In: Immunology of milk and the neonate (Mestecky, J., C. Blair, P. L. Ogra, Eds.). Plenum Publishing Co., New York. pp. 317–327.

FAIRBROTHER, J. M. (1992): Enteric colibacillosis. In: Diseases of Swine (Lemon, A. D., B. E. Straw, W. L. Mengelling, S. D. Allaire, D. J. Taylor, Eds.). 7th ed. Iowa State University Press, Iowa. pp. 489–497.

HUMSKI, A., V. BILIĆ, D. HAJSIG, B. HABRUN (1998): Serotyping of enterotoxigenic isolates of *Escherichia coli* from suckling and weaned piglets with colibacillosis. Vet. arhiv 68, 191–198.

LANZA, I., D. I. SHOUP, L. J. SAIF (1995): Lactogenic immunity and milk antibody isotypes to transmissible gastroenteritis virus in sows exposed to porcine respiratory corona virus during pregnancy. Am. J. Vet. Res. 56, 739–747.

NAGY, B., P. Z. FEKETE (1999): Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. Vet. Rec. 30, 259–284.

OHASHI, S. (1994): Studies on bovine enterotoxigenic *Escherichia coli* bacterins containing K99 antigens. Tohoku J. Vet. Clin. 17, 65–75.

QUIGLEY, J. D., K. R. MARTIN, H. H. DOWLEN (1995): Concentration of trypsin inhibitor and immunoglobulin in colostrum of Jersey cows. J. Dairy Sci. 78, 1573–1577.

SAIF, L. J. (1985): Passive immunity to coronavirus and rotavirus infections in swine and cattle: enhancement by maternal vaccination. In: Infectious diarrhoea in the young (Tzipori, S. Ed.). Elsevier Science, Amsterdam. pp. 456–466.

SAIF, L. J., J. L. VAN COTT, T. A. BRIM (1994): Immunity to transmissible gastroenteritis virus and porcine respiratory coronavirus infections in swine. Vet. Immunol. Immunopathol. 43, 89–97.

SPIRA, W. M., R. B. SACK, J. L. FROEHLICH (1981): Simple adult model for *Vibrio cholerae* and enterotoxigenic *Escherichia coli* diarrhoea. Infect. Immun. 32, 739–747.

VAN ZAANE, D., J. IJZERMAN, P. W. DE LEEUW (1986): Intestinal antibody response after vaccination and infection with rotavirus of calves fed colostrum with and without rotavirus antibody. Vet. Immunol. Immunopathol. 11, 45–63.

WESLEY, R. D., R. WOODS, I. CORREA, L. ENJUANES (1988): Lack of protection in vivo with neutralizing monoclonal antibodies to transmissible gastroenteritis virus. Vet. Microbiol. 18, 197–208.

Received: 29 April 2001
Accepted: 20 December 2001

BARMAN, N. N., D. K. SARMA, B. N. NEOG: Uklanjanje enterotoksigenih sojeva bakterije *Escherichia coli* u pokusno inficirane prasadi pasivnom imunizacijom goveđim kolostrumskim protutijelima. Vet. arhiv 71, 381-387, 2001.

SAŽETAK

Ako prasad nije posisala kolostrum od krmače može se pasivno imunizirati kolostrumskim protutijelima podrijetlom od krava. Steone krave bile su vakcinirane intramuskularno i intramamarno inaktiviranim enterotoksigenim sojem bakterije *E. coli.* U njihovu kolostrumu dokazana je visoka razina specifičnih protutijela razreda IgG i IgA. Jednodnevna prasad koja je dobila kolostrum s visokim titrom protutijela IgG preživjela je izazivačku infekciju, dok je 75% prasadi koja je dobila kolostrum od nevakciniranih krava uginulo od izazivačke infekcije. Uginula je i sva prasad koja nije dobila kolostrum.

Ključne riječi: enterotoksigena *E. coli*, kolostrumska protutijela, prasad

---