Passage of Chicken Egg Yolk Antibody Treated with Hydroxypropyl Methylcellulose Phthalate in the Gastrointestinal Tract of Calves

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ABSTRACT. Two types of chicken egg yolk antibody samples for oral passage trials in calves were prepared; (1) hydroxypropyl methylcellulose phthalate (HPMCP) antibody powder (HAP) — a powder produced by spray-drying a supernatant obtained after precipitation of lipids from egg yolk with HPMCP and (2) control antibody powder (CAP) — a powder produced from an antibody solution without HPMCP. Antibody activity and pattern of distribution of both antibody preparations in the gastrointestinal tract of calves were compared by enzyme-linked immunosorbent assay. At 2 hr post administration, anti-K99 fimbrial antibodies from both the CAP and the HAP were detected in the abomasum of calves with titers of 1:128 and 1:256, respectively. However, at 4 hr, anti-K99 fimbrial titers of the CAP and the HAP were reduced to 1:2 and 1:64, respectively, due to digestion in the abomasum. These results indicated that the egg yolk antibody powder with HPMCP was more resistant against gastric juice in the stomach, thereby, ensuring a transfer of functional antibodies to the small intestine of calves after oral administration. — KEY WORDS: antibody, gastrointestinal-tract, HPMCP.

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The calf is born almost devoid of serum immunoglobulin (Ig) and depends on colostrum for virtually all of its humoral passive immunity [3]. Calves not receiving colostrum or receiving inadequate amounts are disposed to septicemia or enteritis. Serum Ig concentration of the calf is important for prevention of septicemia. However, antibodies in serum generally appear to be of less value in protection against enteropathogens [9] whereas passive immunity associated with ingestion of colostrum or milk containing specific antibodies against these enteropathogens has proven more effective. IgG is the predominant Ig in bovine colostrum and is expected to be the predominant passively acquired Ig isotype in the intestine in passive immunity. However, IgG is not stable under acidic conditions and easily decayed in the stomach. The problem must be resolved to achieve protective antibody activity in the intestine. There are few reports about the activity and distribution of antibodies in the gastrointestinal tract of calves fed with milk containing antibodies. In a previous study, we have achieved protection against enterotoxigenic *Escherichia coli* (ETEC) infection in calves by treating the animals orally with chicken egg yolk antibodies [4]. Our antibody powder contained hydroxypropyl methylcellulose phthalate (HPMCP), an enteric coating substance for drugs [2, 12]. HPMCP-coated drugs are resistant to gastric juice and dissolution of the drugs in intestinal fluid is pH-dependent. The objective of the present study was to evaluate the antibody activity and passage of chicken egg yolk antibodies treated with HPMCP in the gastrointestinal tract of calves.

ETEC strain 431 (O101: K30; K99; F41. NM, ST+) grown in minca broth was used. The fimbriae fraction was detached from bacterial cells by heating at 60°C for 30 min as described previously [4]. The K99 fimbriae were purified by a combination of gel filtration and ion exchange chromatography [6]. Purified fimbrial vaccine, containing 0.5 mg of the fimbrial antigen in emulsion oil mixed with 5% mannide monooleate, was injected intramuscularly in the breast muscles of ten 5-month-old white Leghorn hens (strain Hyline W36). Six weeks after the initial injection was given, the hens were boosted in the same manner, and the collection of eggs started 2 weeks later. Egg yolks were separated from the egg whites, mixed, and then diluted with 7 volumes of distilled water. A solution of 5% HPMCP (Shinetsu Chemical, Tokyo, Japan) was added to the diluted yolk material to a final concentration of 0.5% for lipid precipitation as described previously [4]. The water-soluble fraction was collected and passed through a 0.45 μm membrane filter. The filtered material was applied to a spray-dry machine to produce the HAP. For production of the CAP, the eggs were treated in the same manner without HPMCP.

Twelve 7 day-old colostrum-fed Holstein calves from ETEC-free farms were used in this study. Upon transfer to the laboratory, calves were started on a commercially available ready-to-feed milk formula for 3 days. Calves were then distributed randomly into 2 groups consisting of 6 calves fed either the CAP or the HAP, per 1.5 liters of milk per calf. The amount of antibody powder was adjusted to provide an antibody titer of 1:2,560 per m*l* of milk. Collection of gastrointestinal specimens was done separately at different times (2, 4, and 6 hr) after antibody administration. Two calves in each group were killed under pentobarbital anaesthesia and necropsy was done immediately to remove the entire gastrointestinal tract. Gastrointestinal contents were taken from abomasum, duodenum, the upper, middle, and lower regions of jejunum, ileum, cecum, colon, and rectum. The each specimen was then homogenized in 9 volumes of phosphate-buffered saline by thoroughly mixing. After centrifugation, the supernatants were filtered through a 0.45 μm membrane filter. The distribution of antibodies in the gastrointestinal tract was determined by enzyme-linked immunosorbent assay (ELISA) using anti-K99 fimbrial antibody.

The ELISA was performed as described previously with slight modifications [4]. Microplates were coated with 5 μg/m*l* of K99 purified fimbrial antigen. The plates were

blocked with bovine serum albumin and washed three times. The wells were filled with 100 μl of serial two-fold dilutions of the samples (10% working solution) and plates were incubated at 25°C for 1 hr. Rabbit anti-chicken IgG (Fc) (Bethyl Laboratories, Montgomery, TX, U.S.A.), diluted 1:6,400, and then monoclonal rat anti-rabbit IgG conjugated with peroxidase (Zymed Laboratories, San Francisco, CA, U.S.A.), diluted 1:1,600, was applied and incubated at 25°C for 1 hr; *o*-phenylenediamine dihydrochloride and hydrogen peroxide were added as substrate. Optical density (OD) values were obtained in a microplate reader (MR 650, Dynatech Laboratories, Chantilly, VA, U.S.A.) at 490 nm. The ELISA antibody titer was determined as the reciprocal of the highest dilution of samples with 0.1 OD value. A duplicate assay of each sample was done on different occasions.

The anti-K99 fimbrial antibody was detected in the abomasum of calves when gastrointestinal contents were assayed by ELISA 2 hr post administration (PA) of the CAP (Table 1). The ELISA antibody titers in the abomasum of 2 calves were 1:64 and 1:128, lower than the antibody titer of 1:256 detected upon administration. The antibodies had reached the distal segment of jejunum with a titer of 1:1,024, 4 times higher than the initial antibody titer detected at time of feeding. At 4 hr PA, the antibody titers decreased markedly to 1:2 with also low antibody titers detected in the duodenum, the upper and middle region of the jejunum. The antibodies had already moved distally to the ileum and proximal portion of the large intestine. Only traces of the antibodies were detected in the small intestine at 6 hr PA. However, the antibody titer in the large intestine was 1:1,024, indicating accumulation of antibodies in this region.

In the calves administered the HAP, the anti-K99 fimbrial antibody was also detected in the abomasum at 2 hr PA with a titer of 1:256, retaining the initial antibody titer detected at time of feeding (Table 1). About this time, the antibodies had only reached the middle region of the jejunum. At 4 hr PA, the remaining antibodies in the abomasum had a titer of 1:64. There was a difference in the antibody titers in the abomasum between the CAP group and the HAP group. Apparently, the HAP contained antibodies which were more resistant to digestion by gastric juice at low pH, a protective advantage afforded by the coating property of HPMCP. Perhaps, spray-drying of egg yolk antibodies with HPMCP resulted in a kind of binding or protective coating of antibodies which enabled them to withstand acidic condition better than non-HPMCP treated antibodies. Results indicate that with HPMCP, antibodies with intact structure and function could reach the small intestinal tract.

The absorption of colostral Ig and the function and half-life of serum Ig of calves are well known [1, 10, 11]. However, there is little data about the course of orally administered Ig from colostrum and milk in the gastrointestinal tract of calves. In this study, antibodies derived from the CAP were assumed to have almost the same fate along the gastrointestinal tract as antibodies derived from and milk after oral administration. The pH of the abomasum is 1 to 2 before milk feeding, increasing to 6 after feeding, and then, as the rate of acid secretion increases, the pH value decreases slowly and reaches pre-feeding values after about 5 hr [8]. The antibody titers of the CAP in the abomasum of calves, 2 hr PA, were 1:64 to 1:128, slightly lower than initial titer upon feeding. At 4 hr PA, the antibody titer decreased markedly to 1:2 through digestion in the abomasum. This indicated that Ig in milk could maintain their antibody activity steadily in the abomasum for 2 hr upon administration, after which time a slow decrease of the abomasal pH ensues and enzymatic digestion of antibodies can occur. The overall rate of release of abomasal content is half the volume every 2 hr and transit time through the small intestine is about 3 hr [7]. In this study, the antibodies were detected in the intestine and had already traveled to the lower region of the jejunum at 2 hr PA and reached the proximal portion of the large intestine at 4 hr PA. The transit time of the antibodies through the small intestine is about 3 hr as mentioned above. Based on these results, we could expect optimum antibody activity within 2 hr PA in the target areas of the small intestine without marked intestinal enzymatic digestion of the antibodies. Beyond this period, more antibodies were detected in the large intestine as shown by ELISA titers in

Table 1. Anti-K99 fimbrial antibody titers of chicken egg yolk antibody in the gastrointestinal tract of each calf administered the control antibody powder or the HPMCP antibody powder

Gastro-intestinal tract	Anti-K99 fimbrial antibody titer ($\log_2$)					
	Control antibody powder Hours post administration			HPMCP antibody powder Hours post administration		
	2	4	6	2	4	6
Abomasum	7, 6	1, <1	<1, <1	8, 8	6, 5	<1, <1
Duodenum	7, 7	1, <1	<1, <1	7, 7	5, 4	<1, <1
Jejunum						
upper	7, 7	1, <1	<1, <1	8, 7	6, 5	<1, <1
middle	9, 8	5, 1	<1, <1	7, 7	6, 6	1, <1
lower	10, 10	7, 4	1, <1	2, 2	8, 7	4, 2
Ileum	<1, <1	10, 9	3, 2	<1, <1	9, 9	9, 9
Cecum	<1, <1	9, 9	10, 10	<1, <1	8, 9	8, 10
Colon	<1, <1	9, 9	11, 10	<1, <1	4, 2	9, 10
Rectum	<1, <1	<1, <1	4, 5	<1, <1	<1, <1	3, 2

this region which is 4 to 8 times higher than those initially detected at feeding. One explanation for this result is that reabsorption of water from the intestinal contents occurs in the large intestine and this may lead to a higher concentration of antibodies.

The course of the antibodies derived from the HAP in the gastrointestinal tract of calves differed slightly from that derived from the CAP. In addition to the above difference in antibody titers in the abomasum of calves at 4 hr PA, the antibody transit time was longer with these antibodies as depicted by the ELISA titers at various parts of the intestine at different time intervals. It was likely that the antibodies derived from the HAP had coagulated with casein and fat of milk and could have impeded their movement along the tract.

Many organisms, e.g. ETEC and rotavirus, predominantly proliferate in the small intestine and are the major cause of diarrhea and death in calves. Antibodies must pass safely through the stomach and exert their function in the small intestine in order to prevent a disease in a successful passive immunization. In case of a short-lived passage of antibodies to the small intestine, a constant antibody activity in the small intestine may not be maintained for a long time allowing for the organisms to grow in the small intestine. The antibodies derived from the HAP had elicited antibody activity against bacterial antigens in the abomasum and small intestine of calves for 4 hr PA. The longer staying power of antibodies in the gastrointestinal tract of calves seemed to be attributed to the coating effect with HPMCP. Effective enteric-coating technique for antibodies needs to be established for a successful passive immunization in animals.

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