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Field evaluation of chicken egg yolk immunoglobulins specific for bovine rotavirus in neonatal calves

Brief Report

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Summary. The oral efficacy of chicken egg yolk immunoglobulins (yIg) specific for bovine rotavirus (BRV) serotypes G6 and G10 in protecting neonatal calves was examined in a herd of cattle under field conditions. In one of the three trials, yIg-treated calves tested under high relative humidity (RH) showed a significantly increased mean body weight ($P < 0.05$) and a decrease in number of calves shedding high titer of BRV (G6) in stool compared to control calves ($P < 0.01$), suggesting that our yIg product was effective in a field condition with an epidemic outbreak of BRV diarrhea.

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Group A bovine rotavirus (BRV) is a major cause of neonatal calf diarrhea [6, 15]. Passive immunization by feeding BRV immune-bovine colostrum to calves has been reported [14, 18]. Passive protection by anti-rotavirus chicken egg yolk immunoglobulins (yIg) against experimental rotavirus infections in mice [2, 8, 20] and neonatal calves [9] has also been documented. It has been proposed that the use of anti-BRV yIg as a supplement to feed for calves within the immediate post-natal period may be a clinically amenable option for controlling BRV disease [9] because BRV is highly immunogenic in poultry [8] and mass production of yIg using bioengineering methods is highly feasible [4, 19]. To our knowledge, there has been no report on the practical evaluation of yIg in protecting calves against BRV infection under field conditions. The present study aims to evaluate the clinical efficacy of a sustained post-natal oral feeding of anti-BRV yIg to neonatal calves in a herd of cattle under field conditions. Specifically, the efficacy of yIg administration was gauged according to body weight gain, mortality, decrease of BRV titer in stool and fecal score.

Shimane (serotype G6, P type undefined) [16] and KK-3 (serotype G10, P11) [13] strains of BRV were kindly supplied respectively by Dr. K. Sato and Dr. Y.

Murakami, National Institute of Animal Health, Tsukuba, Japan. Virus cultivation in MA-104 cells was done with Eagle's Minimum Essential Medium containing 1μg/ml of trypsin (Type III, Sigma, St. Louis, MO). Virus titration of 10% homogenates of diarrheic stool samples was performed with roller tube cultures [5]. Virus infectivity titers were expressed by median tissue culture infective dose ($TCID_{50}$) as determined by the appearance of cytopathic effect in MA-104 cells. The virus neutralization test was conducted using the same medium as described previously [5]. The virus neutralizing antibody titer of yIg powder was expressed as the reciprocal of the highest yIg dilution that showed complete inhibition of cytopathic effect of 200 $TCID_{50}$ of homologous BRV.

Each of one thousand BRV-seronegative (titer, <10) White Leghorn hens were immunized with formalin-inactivated Shimane or KK-3 antigen containing 10^9 $TCID_{50}$. Anti-Shimane or anti-KK-3 yIg powder was prepared from eggs derived from the immunized hens using hydroxypropylmethylcellulose phthalate as described previously [7, 9]. The anti-KK-3 and anti-Shimane yIgs were integral components of our bivalent yIg trial product.

Detection of BRV was done by inoculation of stool homogenate (10%, wt/vol) collected at the onset of severe diarrheal episodes onto MA-104 cells and examination for the appearance of cytopathic effect. BRV antigen in the medium of MA-104 cells was detected by the use of a commercial latex agglutination test kit (Serodirect-Rota, Eiken Chemical Co. Ltd., Tokyo, Japan). Serotyping of BRV isolates was done by virus neutralization tests using hyperimmune chicken anti-Shimane (titer, 20 480) or anti-KK-3 (titer, 20 480) yIg solution prepared by saturated ammonium sulphate precipitation as described previously [8].

A herd of Japanese Black beef calves housed in a high-roofed 50-cow capacity calving barn in Atsuta village near Sapporo City, Hokkaido, Japan, was selected for the field trials because the herd had neonatal calf diarrhea and pneumonia for the three years immediately prior to the field trials. Some of the dams were transferred for this study from other prefectures. Weather conditions, diarrhea outbreaks and BRV shedding in stools of calves during the pre-trial period were investigated in the herd from April to September, 1993. Thereafter, a series of three field trials to evaluate yIg efficacy in calves were conducted sequentially in 1994 (February 21 to March 29, April 2 to May 12, and May 1 to 29) in the same barn. Ten Japanese Black calves were randomly assigned to yIg-treated or control group in each trial. All of the calves in the treated groups were orally administered a total of two grams of combined anti-Shimane and KK-3 yIg (each with homotypic titer of 12 800) three times a day for two weeks after birth. The yIg solution reconstituted with 50 ml of distilled water was delivered orally via a 50 ml syringe. A total of 30 head of control calves were not treated with yIg. All the treated calves were left in contact with the control calves in order to evaluate the efficacy of yIg in preventing natural BRV transmission. The calves in both groups were nursed by their dams for the whole duration of the trial including the suckling of their colostrum. All dams were given a commercial vaccine for enterotoxigenic *Escherichia coli* K99.

Body weight gain of surviving calves was expressed as body weight at two weeks over body weight on day of birth. Body weight gain of dead calves was expressed as percentage weight gain from birth until the day they died. Fecal score was determined numerically every morning as follows:

0 = normal feces, 1 = soft feces, 2 = mild diarrhea, 3 = severe diarrhea. Cumulative fecal scores were determined daily for each calf for two weeks after birth. Detection of BRV was done daily by the same method as in the pre-trial period using MA-104 cells and a latex agglutination test.

All calves in the first trial, but not in the second and third trials were bled once at 24 h of age for evaluation of serum total protein (TP), anti-Shimane and anti-KK-3 neutralizing antibody titer. TP was measured by a commercial kit (Protein Assay Kit, Bio-Rad, Richmond, Ca). A commercial fetal calf serum (Gibco Lab., Grand Island, NY., lot no. 49K0290) was used as control serum for evaluation of serum TP, and anti-Shimane and -KK-3 neutralizing antibody titer.

Clinico-pathologic findings for calves that died the year before the field trials (April in 1992) were collected from the Ishikari Livestock Hygiene Service Center. Detection of enterotoxigenic *E. coli* K99 and K88 was done using specific antisera by the public officers of the same livestock service center.

The means of air temperature, daily relative humidity (RH) and precipitation in the Sapporo area during the field trials (February to May, 1994) were calculated from data issued by the Japan Weather Association.

Body weight gains and cumulative fecal scores were compared between the treated and control calves by Welch test. The number of calves shedding BRV between these groups was compared by Fischer exact test. The percentage of days associated with rain or snow, mean of RH (%), air temperature, and precipitation (mm/day) in the first trial were compared with those of the second or the third trials by Fisher exact and Welch tests. The means of serum TP levels in the treated group were compared with those in the control group in each trial by Student t-test. The mean serum TP level of the surviving calves in both treated and control groups were compared with those of dead calves using the same test.

Mortalities of calves were found to be associated with diarrhea and pneumonia aside from accidental causes in the farm during the pre-trial period (April, 1992 to January, 1994). The pathogenic agent associated with a serious outbreak of diarrhea in September, 1992 was enterotoxigenic *E. coli* K99. Since October, 1992, the farm workers began to administer a commercial vaccine for enterotoxigenic *E. coli* K99 to dam. Since then, there was a total of 18 mortalities associated with diarrhea (in February, August, and December, 1993). The pathogenic agent inducing these diarrhea in 1993 was confirmed to be BRV as described below. The results of BRV detection from feces of the 21 heads of calves during the pre–trial survey for BRV incidence in 1993 are summarized in Table 1. The number of BRV positive calves associated with diarrhea was 14 out of 21 diarrheic calves (66.7%). BRVs isolated from 14 calves were all identified as belonging to serotype G6 according to the criterion of more than 20-fold difference in neutralizing antibody titer (Table 1).

Table 1. Pre-trial survey for BRV isolation from calves associated with diarrhea in April to September, 1993

BRV detection by isolation test						
BRV positive (n = 14)				BRV negative (n = 7)		
Calf no.	Date of birth	Appearance of diarrhea[b]	G serotype[a] of isolates	Calf no.	Date of birth	Appearance of diarrhea[b]
664	Apr. 9, '93	7	G6	669	Apr. 14, '93	16
667	23	7	G6	672	20	12
975	24	14	G6	673	20	8
680	25	11	G6	679	24	10
681	26	11	G6	974	May 2	8
684	27	13	G6	700	6	1
686	30	13	G6	827	Aug. 4	13
691	May 2	6	G6			
784	Jul. 5	32	G6			
814	26	11	G6			
821	30	13	G6			
837	Aug. 10	7	G6			
850	20	6	G6			
875	Sep. 16	0	G6			
		Mean ± SD				Mean ± SD
		10.8 ± 7.2				9.7 ± 4.8

[a]Serotyping of BRV isolate was done by virus neutralization test using hyperimmune chicken anti-Shimane (G6) or anti-KK-3 (G10) yIg prepared by saturated ammonium sulphate precipitation [8] according to the criterion of more than 20-fold difference in neutralizing antibody titer

[b]Given in days after birth

The monthly means of daily RH (%) from April through September, 1993 were 62, 68, 77, 70, 76 and 72, respectively (Mean ± SD, 70.8 ± 5.5%). The mean RH (%) on the days of birth of BRV-positive and -negative calves were 69.0 ± 11.4 and 59.7 ± 10.8, respectively.

The percentage of total rainy and snowy days, mean of RH, and mean precipitation during the first trial (73%, 68%, and 5.2 mm, respectively) were significantly higher than the means of the second or third trial (46%, 58%, and 1.6 mm respectively in the second, 35%, 58% and 1.8 mm, respectively in the third, $P < 0.05$). Mean maximum and minimum air temperature during the first trial (2.8°C, −3.3°C, respectively) were significantly lower than the means of the second or third trial (12.0°C and 4.7°C in the second, 18.2°C and 9.0°C in the third, respectively, $P < 0.01$).

From 100 liters of yolk supernatant, 350 g of anti-Shimane and anti-KK-3 yIg spray-dried powder was prepared. The homologous neutralizing antibody titers of anti-Shimane and anti-KK-3 yIg spray-dried powder was 128 000/g for each strain. Data on body weight gain, BRV detection and mortalities in the three field trials are summarized in Table 2. In the first trial, the yIg-treated calves, compared with control calves, showed a significant increase in body weight ($P < 0.01$) as well as a significant decrease in the number of calves excreting high titer ($> 10^4$ $TCID_{50}$/g of feces) of BRV ($P < 0.01$). The number of dead calves in the treated and control calves were 2 and 3, respectively. BRV was detected from the 2 dead calves (No. 119 and No. 125) but not from surviving calves in the treated group. In contrast, BRV was detected from all calves except one (dead calf No. 126) in the control group (Tables 2, 3). There were no significant differences between treated and control calves in both the second and third trials regarding body weight gain and virus excretion (Table 2). In these two trials, BRV was not detected from dead calves coming from treated or control groups. The main causes of accidental death were fracture and impaired health of the dam.

The mean total fecal scores of treated groups in the first, second, third and all trials combined were 8.2 ± 4.4, 7.9 ± 3.7, 5.6 ± 3.1, and 7.2 ± 3.8, respectively. The same scores for control groups in the first, second, third, and all trials combined were 8.8 ± 4.9, 6.3 ± 3.7, 6.8 ± 3.7, and 7.3 ± 4.1, respectively. There was no significant difference in fecal scores between the treated and control groups in each trial.

Table 2. Summary of results in three field trials

No. of trial	Treatment group (n)		Body weight gain (Mean ± SD)		No. of BRV-positive calves	
			kg	%	$10^2 - 10^4$[a]	$> 10^4$[b]
First	yIg	(10)	3.5 ± 5.2[c]	11.1 ± 17.0	0	2[d]
	con[e]	(10)	-0.5 ± 2.5	-1.4 ± 9.3	0	9
Second	yIg	(10)	2.9 ± 4.5	9.3 ± 14.6	0	0
	con	(10)	3.0 ± 5.6	10.9 ± 20.3	0	1
Third	yIg	(10)	2.7 ± 5.4	8.3 ± 18.1	4	0
	con	(10)	1.8 ± 3.4	8.0 ± 13.4	4	1

[a] Infectious virus titer; 10^2 to 10^4 $TCID_{50}$/gram of feces
[b] Infectious virus titer; over 10^4 $TCID_{50}$/gram of feces
[c] $P < 0.05$, compared with control group of the first trial by Welch test
[d] $P < 0.01$, compared with control group of the first trial by Fisher exact test
[e] Control as non-treated group

Table 3. Summary of results in the first trail

Group	Survived or died (n)	No. of calf	Sex	Cumulative fecal score[a]	BRV detection	Infectious virus titer ($TCID_{50}$/gram of feces)
yIg-treated	Survived (8)	113	♀	12	−	$< 10^2$
		115	♂	11	−	$< 10^2$
		117	♂	16	−	$< 10^2$
		121	♂	11	−	$< 10^2$
		123	♂	2	−	$< 10^2$
		127	♂	10	−	$< 10^2$
		129	♂	5	−	$< 10^2$
		131	♀	5	−	$< 10^2$
	Died (2)			(Time of death)[b]		
		119	♀	5 (8)	+	$> 10^4$
		125	♂	5 (17)	+	$> 10^4$
Control	Survived (7)	114	♂	13	+	$> 10^4$
		118	♀	2	+	$> 10^4$
		120	♂	10	+	$> 10^4$
		124	♂	3	+	$> 10^4$
		128	♀	13	+	$> 10^4$
		130	♂	13	+	$> 10^4$
		132	♂	5	+	$> 10^4$
	Died (3)			(Time of death)[b]		
		116	♀	14 (11)	+	$> 10^4$
		122	♀	3 (7)	+	$> 10^4$
		126	♀	12 (10)	−	$< 10^2$

[a] For 2 weeks in survived calves
[b] Days after birth

The TP and neutralizing antibody titers of serum samples from calves used in the first trial are shown in Table 4. The mean TP level (g/dl) in the surviving and dead calves were 5.6 ± 0.9 and 4.5 ± 0.6, respectively. The mean TP levels in the surviving calves in the first trial were significantly higher than those of the dead calves ($P < 0.05$) in this trial. The neutralizing antibody titer against either Shimane or KK-3 BRV was not detected from calf No. 125 (< 10). The titer in a commercial fetal calf serum was also < 10 (negative) against both Shimane and KK-3 strains and its serum TP concentration was 3.8 g/dl. The mean TP levels detected in all calves used were 5.4 ± 0.9 g/dl (Table 4). The mean of anti-KK-3 serum neutralizing antibody titer in all groups was higher than the mean of anti-Shimane titer. This may have been due to transfer of serum antibody specific

Table 4. Serum total protein and virus neutralization antibody titers to Shimane and KK-3 strains of BRV in 24 h sera of calves in the first trial

Calf group	Survived or died (n)	Calf no	BRV detection	Total protein (g/dl)	Neutralizing antibody titer against	
					Shimane	KK-3
yIg-treated	Survived (8)	113	−	4.8	80	160
		115	−	6.5	80	320
		117	−	4.2	80	160
		121	−	5.0	1280	320
		123	−	5.4	80	320
		127	−	4.8	80	160
		129	−	6.5	320	320
		131	−	7.8	160	640
			Mean ± SD	5.6 ± 1.2	270 ± 417	300 ± 159
	Died (2)	119	+	4.2	80	320
		125	+	4.4	0	0
			Mean ± SD	4.3 ± 0.1	40 ± 57	160 ± 226
Control	Survived (7)	114	+	5.7	320	640
		118	+	5.8	160	640
		120	+	6.1	160	320
		124	+	5.9	1280	640
		128	+	4.8	40	640
		130	+	5.5	40	320
		132	+	5.5	80	320
			Mean ± SD	5.6 ± 0.4[a]	297 ± 444	503 ± 171
	Died (3)	116	+	5.1	80	640
		122	+	5.2	320	640
		126	−	3.8	80	320
			Mean ± SD	4.7 ± 0.8	160 ± 139	533 ± 185
All	Survived (15)		Mean ± SD	5.6 ± 0.9[b]	283 ± 414	395 ± 190
	Died (5)		Mean ± SD	4.5 ± 0.6	112 ± 121	384 ± 268
	Total (20)		Mean ± SD	5.4 ± 0.9	240 ± 368	392 ± 204

[a] $P < 0.05$, compared with dead calves in the control group
[b] $P < 0.05$, compared with all dead calves in this trial

for BRV G6 to the intestinal lumen during epidemic outbreaks of BRV G6 as observed elsewhere [3, 10].

In this study, the efficacy of BRV specific yIg in protecting neonatal calves under field conditions was gauged according to body weight gain, the number of calves that died after treatment, BRV titer in stool and fecal score. In the first trial, oral yIg administered daily to neonatal calves for 2 weeks after birth greatly enhanced ($P < 0.05$) the rate of body weight gain and markedly reduced BRV titer in stools as well as the number of calves shedding the virus in stools ($P < 0.01$). In this trial, dead calves in treated and control groups were undernourished (low serum neutralizing antibody titer, and had low serum TP) which may have predisposed them to a host of other complications [17] and thus could not be considered in the overall efficacy evaluation. The second and third trials did not show significant yIg efficacy due to low grade infection seen during these trial. Some factors may help explain these findings. Firstly, there was an unusually prolonged low RH and precipitation recorded in Japan during these trials (summer of 1994). It had been observed that low RH adversely affects rotavirus infectivity [1, 11, 12]. Secondly, there may have been a decline in the quantity of infectious virus shed into the environment due to yIg treatment in the first trial resulting in lesser virus load for succeeding trials in the same premises.

yIg treatment prevented viral shedding in some but not all calves. However, there was no relationship observed between BRV stool titer and presence or severity of diarrhea (data not shown) indicating that under field conditions, host and environmental factors are critical factors in induction of diarrhea. We used the stool titer to gauge efficacy since reduction or prevention of excretion and virus spread is an important factor in the control of BRV transmission cycle. Within the two-week post-natal period, the overall reduction in viral excretion was marked and most clearly seen in trial 1 when environmental conditions were conductive to BRV transmission as shown by stool titers (Table 2).

The observation that yIg-treated calves had a marked advantage in overall body weight gain and virus excretion profile led us to conclude that the efficacy of our bivalent (G6, G10) trial product derived from specific reactivity between the field strain and our yIg specific for G6 serotype which was isolated in the farm. Although we used colostrum-fed calves as preferred by the farmers for their livestock in this trial, the effect of yIg would probably have been more dramatic in a dairy herd in which calves received no colostrum or milk but yIg plus artificial milk replacer.

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