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NOTE

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Antibody response to equine coronavirus in horses inoculated with a bovine coronavirus vaccine

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ABSTRACT. A vaccine for equine coronavirus (ECoV) is so far unavailable. Bovine coronavirus (BCoV) is antigenically related to ECoV; it is therefore possible that BCoV vaccine will induce antibodies against ECoV in horses. This study investigated antibody response to ECoV in horses inoculated with BCoV vaccine. Virus neutralization tests showed that antibody titers against ECoV increased in all six horses tested at 14 days post inoculation, although the antibody titers were lower against ECoV than against BCoV. This study showed that BCoV vaccine provides horses with antibodies against ECoV to some extent. It is unclear whether antibodies provided by BCoV vaccine are effective against ECoV, and therefore ECoV challenge studies are needed to evaluate efficacy of the vaccine in the future.

KEY WORDS: bovine coronavirus, equine coronavirus, horses, vaccine

Equine coronavirus (ECoV) belongs to the species *Betacoronavirus 1* in the genus *Betacoronavirus* which includes bovine coronavirus (BCoV) and dromedary camel coronavirus HKU23 [1, 8, 17]. Clinical symptoms of fever, anorexia, lethargy, leucopenia and digestive problems were seen in horses affected by ECoV in several outbreaks in the United States [12, 13] and Japan [7, 10, 11], and in an experimental challenge study [9]. About 20 to 30% of draft horses kept at a racecourse in Japan were affected in one ECoV outbreak [10, 11]. Those results indicate that ECoV is a highly contagious virus. Although most infected horses recovered, ECoV occasionally led to fatal symptoms like necrotizing enteritis and hyperammonemic encephalopathy in the United States [2, 3]. Vaccination is one of the most important ways of minimizing the symptoms of infectious viral diseases, but a vaccine against ECoV is so far not available anywhere in the world.

BCoV belongs to the same species as ECoV, and it has been reported that bovine and rabbit anti-sera against BCoV cross-react with ECoV to some extent [4, 11]. These results indicate that BCoV is related to ECoV both genetically and antigenically. An inactivated BCoV vaccine is available in Japan [6, 14] and it might also induce antibodies against not only BCoV but also ECoV in horses. This means that the BCoV vaccine could possibly become a surrogate ECoV vaccine. In this study, we investigated the antibody response to ECoV in horses inoculated with the BCoV vaccine.

The BCoV vaccine used in this study was CattleWin BC (Kyoto Biken Laboratories, Kyoto, Japan). This vaccine contains aluminum hydroxide gel as an adjuvant and formalin-inactivated BCoV strain No. 66/HL. Original strain No. 66 was isolated in Japan in 1977 from the feces of a naturally infected calf [15]. Strain No. 66/H is the strain that sequentially cultured the original strain in bovine kidney cell cultures, BEK-l cells and HAL cells [14]. Additionally, vaccine strain No. 66/HL is strain No. 66/H that has been propagated in HmLu-1 cells. The manufacturer's instructions indicate that 1 m*l* of the vaccine is to be intramuscularly administered to cattle twice, about 1 month apart.

Six 1-year-old Thoroughbred horses were randomly divided into two groups of three, each group receiving either 1 or 2 m*l* of the vaccine. Horses were vaccinated intramuscularly twice, 28 days apart. Clinical examinations were performed daily for 3 days after each vaccination, and rectal temperatures were measured once daily during this study. Horses with rectal temperatures exceeding 38.5°C were defined as having significant pyrexia. The experimental protocol and all animal procedures were approved by the Animal Care Committee of the Equine Research Institute of the Japan Racing Association.

The virus neutralization tests for BCoV No. 66/H and ECoV NC99 were performed on serum samples collected at 0, 7, 14, 28, 42 and 56 days post first inoculation (dpi) as described previously [11]. ECoV strain NC99 is a reference strain that was

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Table 1. Virus-neutralizing antibody titers against bovine coronavirus and equine coronavirus in individual horses immunized twice with either 1 or 2 m*l* of bovine coronavirus vaccine

Virus strain	Horse No.	Vaccine volume (m*l*)	Antibody titers at each day post first inoculation					
			0 [b]	7	14	28 [b]	42	56
Bovine coronavirus	1	1	<8 [c]	<8	32	128	256	256
(strain No. 66/H)	2		<8	<8	32	128	1,024	512
	3		<8	8	32	64	1,024	256
	GMT [a]		4	5	32 ** [d]	102 **	645 **	323 **
Bovine coronavirus	4	2	<8	128	256	128	256	256
(strain No. 66/H)	5		32	128	256	256	512	1,024
	6		<8	256	512	256	512	512
	GMT		8	161 **	323 **	203 **	406 **	512 **
Equine coronavirus	1	1	<8	8	16	16	32	32
(strain NC99)	2		<8	<8	16	32	64	64
	3		<8	8	32	32	32	64
	GMT		4	6	20 **	25 **	40 **	51 **
Equine coronavirus	4	2	<8	16	16	8	32	32
(strain NC99)	5		<8	16	64	64	128	128
	6		<8	16	32	32	64	64
	GMT		4	16	32 **	25 *	64 **	64 **

a) Geometric mean titer. b) Horses were inoculated with the bovine coronavirus vaccine at 0 and 28 days post first inoculation. c) For calculation of GMT, titers of <8 were taken to be 4. d) Statistical significance as compared with the antibody titers at 0 days post first inoculation is shown by asterisks (*P<0.05, **P<0.01).

first isolated in the United States in 1999 [4, 17]. Two-fold serial dilutions of serum were mixed with an equal volume of viral suspensions containing two hundred 50% tissue culture infective doses per 0.1 m*l* and incubated for 60 min at 37°C. Then, 0.1 m*l* of each mixture was applied to HRT-18G cells on 96-well plates and incubated for 6 to 7 days. Virus-neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that inhibited viral cytopathic effects.

Statistical analysis was carried out using Ekuseru-Toukei 2012 (SSRI, Tokyo, Japan). Logarithmic transformations of the reciprocal antibody titers were made to stabilize variances. Antibody titers after logarithmic transformation were analyzed by one-way ANOVA with Dunnett's multiple comparison *post hoc* test using the antibody titers at 0 dpi as control. A P-value of <0.05 was considered statistically significant.

The virus-neutralizing antibody titers of horses inoculated with 1 or 2 m*l* of the BCoV vaccine are shown in Table 1. In horses inoculated with 1 m*l* of vaccine, the geometric mean antibody titers against BCoV at 0, 7, 14, 28, 42 and 56 dpi were 4, 5, 32, 102, 645 and 323, respectively, and the geometric mean antibody titers against ECoV were 4, 6, 20, 25, 40 and 51 (Table 1). Compared with the antibody titers at 0 dpi, the antibody titers against both BCoV and ECoV significantly increased at 14, 28, 42 and 56 dpi. In horses inoculated with 2 m*l* of vaccine, the geometric mean titers against BCoV were 8, 161, 323, 203, 406 and 512, respectively, and the geometric mean titers against ECoV were 4, 16, 32, 25, 64 and 64 (Table 1). The antibody titers against BCoV significantly increased at 7, 14, 28, 42 and 56 dpi, and the antibody titers against ECoV significantly increased at 14, 28, 42 and 56 dpi in comparison with the antibody titers at 0 dpi. This study showed that in all horses inoculated with the BCoV vaccine antibody titers against ECoV increased from 14 dpi, although the antibody titers against ECoV were lower than those against BCoV. Maximum antibody titers against ECoV in each horse ranged from 32 to 128. An experimental inoculation study conducted earlier also showed that neutralizing antibody titers against ECoV strain NC99, which is the same strain as used in this study, were 32 to 128 in three horses at 14 days after their inoculation with ECoV-positive feces [9]. However, in horses naturally infected by ECoVs in the 2009 and 2012 outbreaks in Japan [10, 11], the geometric means of neutralizing antibody titers were 304.4 (6 horses) and 348.4 (9 horses), respectively. Thus, the antibody titers of horses inoculated with the BCoV vaccine were similar to the titers of the experimentally infected horses but were lower than the titers of horses naturally infected in actual outbreaks. An experimental challenge study using cattle inoculated with inactivated strain No. 66/H showed that inoculated cattle possessing neutralizing antibody titers of more than 640 showed no clinical signs after challenge with a virulent BCoV, whereas in contrast, inoculated cattle possessing neutralizing antibody titers of less than 160 developed watery diarrhea and fever [15]. Needless to say, the animal species, strain of challenge virus, and method of virus neutralization test in that study are different from our present study. Nevertheless, the antibody titers of all vaccinated horses in the present study were no more than 128, and we therefore consider that the BCoV vaccine will have limited efficacy against ECoV infection in horses. To clarify this, ECoV challenge studies in horses inoculated with the BCoV vaccine will be needed to evaluate the efficacy the vaccine.

The three horses inoculated with 1 m*l* of the vaccine did not exhibit any adverse reaction during this study. In contrast, two out of the three horses inoculated with 2 m*l* of the vaccine exhibited swelling at the inoculation site after the second vaccination. None of the horses developed a fever after the vaccinations. Administration of more than 2 m*l* of the vaccine to horses would likely increase the risk of adverse reactions. As described above, a significant increase in antibody titers against ECoV was observed from

14 dpi irrespective of whether 1 or 2 m*l* was administered. Additionally, the differences in antibody titers against ECoV at each dpi from 14 dpi were less than twofold between horses inoculated with 1 and 2 m*l*. These results suggest that inoculation of 1 m*l* is suitable for horses as well as for cattle.

Although horse No. 5 had no detectable antibodies against ECoV before vaccination, the horse already had antibodies against BCoV (Table 1). That horse was born and had been kept at a farm that reared cows before coming to our facility. In Saudi Arabia, dromedary camel coronavirus HKU23 was detected in apparently healthy horses kept at facilities that reared camels, sheep, goats, and chickens [5]. HKU23, which is closely related to BCoV, is endemic in camels of the Middle East [16] and the HKU23-positive horses frequently came into contact with camels and other animals [5]. HKU23 may have been transmitted from infected camels to those horses. Horse No. 5 in the present study may have also been in contact with infected cows, and BCoV may have been transmitted from infected cows to the horse. However, because there is no epidemiological information, it is unknown whether there were in fact BCoV-infected cows at the farm or whether horse No. 5 had shown any clinical signs.

This study showed that a BCoV vaccine provides horses with antibodies against ECoV to some extent. It is unclear whether the antibodies provided by the BCoV vaccine are sufficient to be effective against ECoV, and therefore ECoV challenge studies in horses are needed to evaluate the efficacy of the vaccine in the future.

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