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Antibody response in cattle after vaccination with an inactivated rabies vaccine

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=ABSTRACT=

Rabies is mainly transmitted to cattle by carnivores in Korea. Once clinical symptoms have occurred, the disease is almost invariably fatal but only protective by vaccination. This study is aimed to certify efficacy on cattle of one rabies vaccine(CaniShot® RV-F manufactured by CAVAC) which the efficacy was already certified on cats and dogs. Also, it is purposed to compare with the efficacy on guinea pigs as laboratory animals. Ten guinea pigs among selected 20 guinea pigs were vaccinated once and measured by neutralizing antibodies at 21 DPI with controls. Five calves among 7 were vaccinated once and measured by the same way with 2 controls. As results, the vaccine induced protectable antibodies in guinea pigs as well as cattle with a single dose. Although these animals were not challenged, neutralizing antibodies are largely accepted as evidence of immunity.

Key words : inactivated vaccine, rabies, FAVN, neutralizing antibody

Introduction

Rabies is a zoonotic disease persisting over time and achieving worldwide distribution in a variety of species causing a serious public health problem. It is transmitted only when the virus is introduced into bite wounds, open cuts in skin, or onto mucous membrane from saliva, comes into contact with the victim's mucosa or fresh skin lesions, or on very rare occasions through inhalation of virus-containing aerosol[6, 9].

The virus first binds to receptors on the muscle cells, but is highly neurotropic through the rest of the infection. In general the incubation period is inversely related to the size of inoculums, degree of innervations and proximity of the bite to the central nervous system. After incubation periods, an infected host shows mild fever, pain and paresthesia at the wound site. As the virus spreads in the central nervous system, progressive encephalitis develops. Furious rabies is rapidly fatal brainstem encephalitis characterized by hydrophobia or aerophobia, hyperactivity and fluctuating consciousness. Once clinical symptoms have occurred, the disease is almost invariably fatal. It is only protective by vaccination[7].

Since Louis Pasteur developed the 1st rabies vaccine, the rabies vaccine was often prepared from

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nervous tissue and used for more than 100 years with questionable efficacy to human. These vaccines are of relatively low potency per dose, and those produced on sheep or goat brain are frequently associated with serious adverse events. Furthermore, some sheep brain vaccines are produced in India where 20% of cattle are estimated to be afflicted with scrapie and nervous tissue-derived vaccines also may produce severe neurological complications. Many of the poor populations at risk of contracting rabies still depend on nerve tissue vaccines, whereas in affluent populations, safe and highly efficacious rabies vaccines produced in cell culture have been available for 20~30 years[6].

Rabies is transmitted to cattle by different animals in different regions of the world. In North America, foxes and skunks transmit the disease to cattle; in Europe, it is transmitted by foxes, while in India, dogs are the major source of transmission. In Latin America, the vampire bat is the one. In Korea, like in India, carnivores including dogs are mainly responsible for transmitting the disease to cattle. According to the Livestock Health Control Guidelines(2009) published by the Ministry for food, agriculture, forestry and fisheries, cattle in rabies outbreak regions should be vaccinated to develop antibodies to rabies. Meanwhile, vaccines which are safe, easily produced, and induce high immunogenicity with an extended protection period are needed to cattle in Korea[5, 9].

There is one inactivated rabies vaccine manufactured by a domestic company even if there are a couple of inactivated rabies vaccines by multinational companies. However, the vaccine is not yet studied concerning the efficacy in cattle although the aspect has been proved efficacious in cats and dogs. Therefore, this study was aimed to investigate the efficacy of the domestic inactivated rabies vaccine in cattle and compare it with that of lab animals which are designated for the efficacy test of the inactivated rabies vaccine by the Korean Standard Assay of Veterinary Biological Products (KS-AVBP).

Materials and Methods

1. Vaccine

CaniShot® RV-F manufactured by Choong Ang Vaccine Laboratories was used. It is a rabies vaccine prepared with the PV fixed virus grown in BHK-21 cells, inactivated by binary ethylenimine and freeze-dried with a stabilizer containing trehalose.

2. Animals

Twenty guinea pigs, 350~400g in weight and 7 Holstein calves, 120 kg in weight were used as laboratory animals.

3. Vaccination

Ten guinea pigs were vaccinated subcutaneously with the vaccine, 0.5mL after diluting by 10 times with sterilized saline containing 10% horse serum. The neutralizing antibodies were measured after clinical observation everyday for 21 days, together with non-treated controls. Five calves were vaccinated intramuscularly with 1 mL of the same vaccine previously used in guinea pigs. The neutralizing antibodies were measured in 3 weeks, 4 weeks and 5 weeks post vaccination. Then, the correlation of the efficacy between guinea pigs and cattle was examined.

4. Fluorescent Antibody Virus Neutralization(FAVN) test

The FAVN test is carried out on 96-well microtitration plates. A stable cell culture BHK-21 cultivated in a propagation medium with 10% calf serum was used. After trypsinization, a cell suspension containing 4×10^5 cell/mL was prepared. A standard rabies virus reference strain CVS 11 was passaged in a BHK-21 cell culture being diluted to obtain 100 $TCID_{50}$/mL. The test also included titration of reference positive and reference negative canine OIE sera(AFSSA Nancy, France) as well as a WHO reference serum(Copenhagen, Denmark, 30 IU in an ampoule, dilution of serum used for FAVN

was 0.5 IU/mL).

Every examined serum, including reference WHO serum, reference positive and negative OIE sera, was titrated four times. Two fold dilutions, 1:2, $1:2^2$, $1:2^3$, $1:2^4$, and $1:2^5$ were prepared directly on plates. Then, 50 μL aliquots of CVS 11 virus(100 $TCID_{50}$/mL) were added to the diluted sera in individual wells. After one hour of incubation at 37 ℃, a cell suspension containing 4×10^5 cells/mL was added to each well. After 48 h of incubation at 3 7℃, the medium was poured off and the plates were washed several times with PBS, fixed for 30 min with 80% acetone at room temperature and dried for 1 hour. After adding a fluorescent conjugate, the plates were incubated again at 37℃ for 30 min. The entire surface of each well was evaluated. As results, neutralizing antibody titers were obtained by comparing ED_{50} of the examined serum with ED_{50} of the WHO reference serum diluted to 0.5 IU/mL; If ED_{50} of the examined serum is $<ED_{50}$ of the WHO reference serum, then the titer is <0.5 IU per mL. If ED_{50} of the examined serum is $>ED_{50}$ of the WHO reference serum, then the titer is >0.5 IU per mL.

Result

The results of FAVN test on guinea pigs that received once 0.5 mL of 10-fold diluted vaccine are summarized in Table 1. Before vaccination, they were found without rabies neutralizing antibodies. At 21 days post vaccination, all of 10 vaccinated guinea pigs presented varied levels of serum neutralizing titers≥1:8. On the other hand, non- vaccinated guinea pigs did not present any change in antibody reaction.

The results of the test performed on sera of cattle vaccinated with a single dose are summarized in Table 2. Before vaccination, all animals did not present any detectable levels of rabies neutralizing antibodies. 21 days after receiving a single dose of the vaccine, 3 animals presented serum neutralizing titers 1:8 and 2 animals presented 1:4. 28 days and 35 days after vaccination with a single dose, all 5 animals were found with neutralizing titers≥1:8, displaying a rising tendency. Comparing with neutralizing antibodies of guinea pigs, those of cattle turned out having an increasing tendency at 21 days after vaccination.

Table 1. Serum neutralizing antibodies of guinea pigs vaccinated once with 10-fold dilution

Group	No	Method	Antibody titer	
			Before	21 DPI[2]
Test	1	0.5mL, 10-fold dilution (10% horse serum), SC 21 day clinical observations	<1:2	1:32
	2		<1:2	1:16
	3		<1:2	1:16
	4		<1:2	1:32
	5		<1:2	1:16
	6		<1:2	1:8
	7		<1:2	1:32
	8		<1:2	1:8
	9		<1:2	1:128
	10		<1:2	1:32
	GMT[1]		<1:2	1:22.63
Control	1	No treatment. 21 day clinical observations	<1:2	<1:2
	2		<1:2	<1:2
	3		<1:2	<1:2
	4		<1:2	<1:2
	5		<1:2	<1:2
	6		<1:2	<1:2
	7		<1:2	<1:2
	8		<1:2	<1:2
	9		<1:2	<1:2
	10		<1:2	<1:2

[1] Geometric mean titer, [2] Days post inoculation, [3] Subcutaneously.

Discussions

The effort to find competitive rabies vaccine for cattle in regard of safety, efficacy and cost has been made because cattle also can not avoid rabies infection through infected wild lives as well as domestic animals. Cattle under the risk of rabies are

Table 2. Serum neutralizing antibodies of cattle vaccinated once with a single dose

Group	No	Method	Antibody titer			
			Before	21 DPI[2)]	28 DPI	35 DPI
Test	1	A single dose, IM[3)]	<1:2	1:4	1:8	1:16
	2		<1:2	1:8	1:16	1:16
	3		<1:2	1:8	1:8	1:16
	4		<1:2	1:8	1:8	1:8
	5		<1:2	1:4	1:8	1:8
	GMT[1)]		<1:2	1:6.1	1:9.2	1:12.1
Control	1	No treatment	<1:2	<1:2	<1:2	<1:2
	2		<1:2	<1:2	<1:2	<1:2

[1)] Geometric mean titer, [2)] Days post inoculation, 3) Intramuscularly.

designated to be vaccinated in the guidelines of Ministry for food, agriculture, forestry and fisheries.

Among various rabies vaccines, the specific vaccine formulations are adjusted on particular applications, e.g., attenuated or recombinant virus vaccines for wildlife and inactivated or attenuated virus vaccines for human and domestic animals. However, Fehlner-Gardiner *et al.* reported infected cases with the ERA variant while the bait vaccines containing the live, attenuated rabies virus were distributed for 15 years. Likewise, an inactivated rabies vaccine becomes considered safe more than live one. One domestic inactivated rabies vaccine for cats and dogs is raised and tested on cattle to expand its use[3, 6].

For the test of rabies antibody, FAVN method was used. The principal of this test is an alternative *in vitro* neutralization test that defines the antibody titer as the highest dilution of serum that produces complete neutralization of a constant amount of the challenge virus standard(CVS) strain adapted to cell culture before inoculating cells susceptible to rabies virus: BHK-21 cells. This permits less quantitative and tedious microscopic examination, and eliminates the need for complex statistical estimation of titer. The serum titer is the dilution at which 100% of the virus is neutralized in 50% of the wells. This titer is expressed in IU/mL by comparing it with the neutralizing dilution of a standard serum under the same experimental conditions: WHO standard for rabies immunoglobulin(human) No. 2. This microplate method is an adaptation of the technique of Smith *et al.*, modified by Zalan *et al.*, and by Perrin *et al*[1, 8, 9, 10, 12].

The animals used in this study were guinea pigs, designated as the laboratory animal in the KSAVBP for the efficacy test of the vaccine and the target species of this study, calves, 120 kg in their weight. They all were not found with any detectable antibodies to rabies before vaccination. For guinea pigs the amount of vaccine was adjusted to 0.5 mL of 10-diluted original vaccine. The guinea pigs received the adjusted vaccine once and neutralizing antibodies were examined at 21 days post vaccination resulting in fairly developed. The calves developed protectable antibodies 60% at 21 days post vaccination, 100% at 28 days and 35 days post vaccination displaying a rising tendency. After vaccination, the presence of rabies neutralizing antibodies at a titer $\geq$ 1:5 could be interpreted as a good indicator of the immune response[11].

In this study, a single dose trials were performed

to compare immunogenicity with lab animals and resulted in protectable antibody response was developed after a single dose vaccination. However, the National Association of State Public Health Veterinarians, Inc. designated that regardless of the age of the animal at initial vaccination, a booster vaccination should be administered 1 year later. Also, the supportive data have been investigated. In addition to it, a further point that should be raised is the fact that how often the booster vaccination should be made and whether the dose would be the same with that of cats and dogs[1, 7].

In conclusion, the results confirm that the inactivated rabies vaccine can afford antibodies in guinea pigs as well as cattle with a single dose. Although these animals were not challenged, neutralizing antibody levels is largely accepted as evidence of immunity.

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