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Short Report

Immunogenicity of BHK-Rabies Vaccine in Cattle

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ABSTRACT

In this study, the immunogenicity of a rabies vaccine that was used in one of the Isfahan cattle farms is reported. This vaccine was produced by replication of the Pasteur virus (PV) strain on BHK-21 monolayer cell culture. The virus suspension was inactivated by ß-propiolactone treatment and the harvested pool showed sterility and safety. Thus, the obtained vaccine was tested on mice and a satisfactory immune response was observed. Thereafter, the vaccine was tested on 34 cattle divided into 2 groups. Each group was received a different dose of the vaccine intramuscularly. Sera were taken 2 months after rabies vaccination and were analyzed by the rapid fluorescence focus inhibition test (RFFIT) to evaluate the titer of the rabies-neutralizing antibody. Both groups under study showed a sufficient seroconversion. *Iran. Biomed. J. 4: 129-131, 2000*

Keywords: Rabies, Anti-rabies vaccine, BHK-21 cell, Cattle immunization

INTRODUCTION

Rabies virus, the etiological agent of rabies that attacks the central nervous system, is of a major importance in human and veterinary medicine. According to the recommendations of World Health Organization (WHO), the important challenge of the prevention and the control of the rabies in the world will require international efforts to increase the availability and the use of high quality cell culture rabies vaccines for man and animal. An important aspect of the activities to ensure such availability is transfer of technologies to the developing countries for the production of such vaccines [1]. Today, a variety of cell substrates are used to prepare the human or veterinary rabies vaccines, including primary cell cultures and cell lines from a number of species [2]. The main purpose of this project is to produce a cell culture rabies vaccine, and to study its potency based on the Habel test [3]. The presence of the rabies-neutralizing antibody in cattle and the comparison of the neutralizing antibody titers following the injection of 1 and 2 ml of vaccine, are also investigated.

MATERIALS AND METHODS

Rabies vaccine production. The PV-PARIS/BHK virus [4] was used for the production of the rabies vaccine and the BHK-21C13 cell line (ATCC:CLL10) [5] was used for virus propagation in the culture flasks and was mycoplasma free [6]. Briefly, the minimum essential medium (MEM), containing 300 µg of glutamine and 30 µg of gentamicin supplemented with 10% fetal calf serum (FCS) was used for cell culture. The cells were multiplied by successive passage until the appropriate quantity of the cells needed for producing a batch of vaccine was obtained. The cell cultures were inoculated by adding the seed virus with a titer of 10^7 focus forming unit per ml (FFU/ml) [7], shaken at 34°C for an hour and then dispensed into culture flasks at the rate of 100 ml per flask.

The quantity of the added viral inoculum was calculated in order to obtain the optimum multiplicity of the infection (1 FFU/ per 10 cells) [8]. The flasks were incubated at 37°C for 24 hours in a stationary position allowing infected cells to become confluent. After the cell monolayer became confluent, the growth medium was replaced with a

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maintenance medium. MEM maintenance medium, containing the above amount of gentamicin together with 0.3% bovine serum albumin (BSA) instead of FCS, was used after 24 hours of cells infection [9]. Then, the culture flasks were incubated at 34°C for 48 hours to obtain the maximum yield of virus in the maintenance medium. The incubated medium containing the cultured virus was harvested after 72 hours and was clarified by centrifugation at 1,000 g for 10 minutes. The resulting medium, with the pH maintained above 7, was inactivated by β-propiolactone at a concentration of 1:4,000 [10, 11]. Sodium merthiolate (0.01%) was added as preservative and aluminium hydroxide (equivalent to 0.025 g of dry weight of aluminium per liter of vaccine) was used as adjuvant [12]. Following the addition of aluminium hydroxide, the pH was adjusted to 8.0. Before inactivation by ß-propiolactone, the titer of the virus was 8×10^6 FFU/ml, indicating that 1 ml of this vaccine contained approximately 8×10^6 of rabies virus.

Virus titration. Rabies virus titer was determined using BHK-21 cells in a 96-well microplate. Cells (5×10^4) were distributed in each well and infected with 50 µg of varying dilution of PV-PARIS/BHK virus. After 24 hours, the microplate was washed with PBS (phosphate buffer saline supplemented with Mg^{+2}, Ca^{+2}) and fixed with cold acetone. The cells were stained with fluorescein-labeled anti-rabies-nucleocapside immunoglobulin. The foci of the virus-infected cells were counted using fluorescence microscope and the titer of the virus was determined [7].

Potency test. The potency of the vaccine was determined according to the method of the Habel [3]. Mice were inoculated intraperitoneally with the vaccine followed by challenge with the Challenge Virus Standard (CVS) strain of the fixed rabies virus. The immunization procedure was based on inoculation of 60 mice with 0.25 ml of the vaccine intraperitoneally on Saturday, Monday and Wednesday for 2 weeks (a total of six doses). Thirty mice were isolated and used as controls. At the time of challenge, CVS rabies strain was diluted ten-fold (10^{-1}-10^{-7}), using 2% heat-inactivated horse serum in distilled water. Groups of 10 vaccinated mice were challenged intracerebrally with 0.03 ml of the 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} dilutions of CVS. Then, the control mice were inoculated with the 10^{-7}, 10^{-6} and 10^{-5} dilutions of the virus in order to determine which dilution represents the mean lethal dose (LD_{50}). After the challenge, the mice were screened each day for the symptoms of the rabies for a period of 14 days. Any death occurring after 5 to 14 days was recorded. The 50% end-point dilutions in the vaccinated and the control mice were determined by the method of Reed and Meunch [3]. By subtracting the log of the 50% end-point dilutions in the vaccinated animals from the log of the 50% end-point dilutions in the control group, the log of the "LD_{50} of protection" of the vaccine was obtained. To meet the minimum required potency, the difference should be 3 (log 10^3), indicating that the vaccine confers protection against 1,000 LD_{50}.

Vaccination and serum samples. The subjects of this study were 34 Holstein dairy cattle with approximately the same age. The cattle were divided into 2 groups. None of them had ever been previously vaccinated against rabies.

Group I, 17 subjects were vaccinated with 1 ml of the inactivated BHK-rabies vaccine intra-muscularly. Group II, 17 subjects were vaccinated by 2 ml injection of the same vaccine intramuscularly. To evaluate the titer of the rabies-neutralizing antibody, sera of the both groups were kept as controls before injection and were compared with the sera of the same groups following 1 and 2 ml of the injection after 2 months.

Neutralization test. The titers of rabies-neutralizing antibody in the sera were evaluated by the rapid fluorescence focus inhibition test (RFFIT) [13]. The dilutions of heat-inactivated serum were incubated at 37ºC for an hour with a fixed amount of CVS strain of the rabies virus, which was adapted to cell culture. Residual virus infectivity, based on the foci of virus-infected cells, stained with fluorescein-labeled anti-rabies-nucleocapside, was then detected using fluorescence microscope. The titer of the antibody in the test serum (IU/ml) was obtained by the comparison with the titer of national reference standard included in each test.

RESULTS AND DISCUSSION

According to the Habel test, the determination of the vaccine potency showed that the potency of this vaccine was $10^{4.8}$. This indicates that the potency of the vaccine meets the requirements of the WHO [6]. Before injection of the vaccine, the titers of the rabies-neutralizing antibody were zero, and after 2

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months following the injection of 1 and 2 ml of vaccine, the subjects of group I had a satisfactory seroconversion with a good mean antibody titer of 3.4 IU/ml. The subjects of group II also showed a similar seroconversion with a 30% higher mean antibody titer (4.3 IU/ml) than group I (Table 1). None of the cattle showed less than 0.5 IU/ml titer of the rabies-neutralizing antibody by the RFFIT test. According to the WHO, the minimum titer of the rabies-neutralizing antibody must not be less than 0.5 IU/ml in humans or animals after immunization against rabies [1, 6]. It indicates that the manufactured vaccine was qualified to induce virus-neutralizing antibody against rabies. The obtained results showed that 1 ml injection in comparison with 2 ml injection of the BHK-rabies vaccine was adequate and more economic to induce a high protection against rabies in cattle. At this point, the dairy farm was contaminated by 6 cases of cattle rabies (tested and confirmed by the WHO Collaborating Center for References and Research on Rabies at the Pasteur Institute of Iran). Following this rabies vaccine, no further rabies cases have been reported to date on this farm.

Table 1. Rabies-neutralizing antibody titers (IU/ml) in group I and II two months after vaccination of cattle. The titer of antibody before vaccination in both groups was zero.

Number of cattle	Titer of antibody Group I	Titer of antibody Group II
1	0.8	0.8
2	1.0	2.5
3	13	8.2
4	1.1	2.5
5	2.4	2.5
6	1.2	13
7	0.9	3.4
8	0.5	3.2
9	4.3	4.3
10	1.4	2.4
11	2.7	1.4
12	4.3	2.5
13	4.5	10
14	8.2	3.2
15	4.5	1.2
16	3.2	2.5
17	4.3	2.6

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