Arch. Inst. Razi, **36, 37**: 19-29 (1986)

STUDIES ON THE RESPONSES OF CALVES TO AN ATTENUATED RINDERPEST VACCINE VIRUS

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Summary: Ten calves susceptible to rinderpest were each injected with 1 ml of attenuated rinderpest vaccine virus, at its 110th passage in calf kidney cells.

Multiplication of the virus in the calves and the immunological response of the animals to the virus were investigated during the 15 days of the experiment.

The virus was isolated neither from the buffy coat cells of live animals nor from tissue samples of those slaughtered at various intervals from 4 to 15 days after inoculation.

Neutralizing antibody to the virus was demonstrated in serum samples from 8 days after inoculation, but no precipitating antigen was found in the tissue samples collected from slaughtered calves.

Infection of calves with virulent strains of rinderpest virus usually results in production of precipitating antigen, detectable in properly harvested tissues from infected animals by the gel immunodiffusion test. The results of the present experiment on the failure of calves to produce any demonstrable precipitinogen to the attenuated rinderpest virus, show that the presence of rinderpest-precipitating antigen in any tissue samples of a rinderpest suspected case would be attributable to infection of the subject by a virulent virus, and could be considered as a further confirmation of the value of application of the gel diffusion test in the diagnosis of rinderpest.

KEY-WORDS: Calf - Cattle diseases - Immune response - Immunoprecipitation tests - Rinderpest virus - Vaccines.

Rinderpest is a contagious viral disease of cloven-hoofed animals, particularly of cattle and buffaloes. In newly infected

Reprinted from: Rev. sci. tech. Off. int. Epiz., 1985, 4 (4), 803-810.

regions, mortality rates are usually high (up to 90%) although, in enzootic regions, they are generally low (as low as 10%) (2).

During the past 16 years, the disease has spread twice from eastern neighbouring countries to Iran. In the 1967 outbreak, it was responsible for the loss of some 20 thousand cattle and buffaloes. The disease was finally eradicated in 1970 (6). New outbreaks of the disease were reported from Tehran and Khorasan provnces in 1982. Country-wide vaccination, quarantine and sanitary measures (including the stamping-out policy) brought the disease to an end nearly forty days with less than 1,000 deaths (12).

Rapid diagnosis is of great importance in the control and disease to an end nerly forty days with less than 1,000 dedouble diffusion test to the study of antigen-antibody reactions by Ouchterlony in 1948 (8), the technique has been used extensively in diagnosis by detecting rinderpest virus soluble antigen. This test has the advantages of ease, rapidity and reliability.

When using the gel diffusion test for diagnosing rinderpest, it is very important to ascertain that the detected soluble antigen is related to a avirulent rinderpest virus, and not to that of the vaccine virus. This report describes the results of tests for soluble viral antigen after vaccination of susceptible cattle against rinderpest, in order to further evaluate the gel diffusion test for the diagnosis of the disease.

Materials and Methods

Rinderpest virus

The 110th passage of the cell-culture attenuated Plowright strain of rinderpest virus was used in the experiment.

Cell culture

Calf kidney primary cell cultures were used as the host system for isolating virus from the collected tissue samples.

Precipitating antigen

Lymph nodes from cattle killed by virulent rinderpest virus were used as positive precipitating antigen.

Rinderpest precipitating antiserum

Rabbit rinderpest antiserum was used as positive rinderpest precipitating serum. It was prepared by a series of 5-weekly intramuscular inoculations of 5 ml lapinized rinderpest virus, Nakamura strain. Serum was obtained one week after the final inoculation.

Animals

Ten young unvaccinated calves, free from rinderpest antibody, were used. The animals were inoculated subcutaneously with $10_{4.5}$ TCID50 of the virus in 1 ml volume.

Serum and blood samples

Coagulated and defibrinated blood samples were collected immediately before inoculation and thereafter at 24-hour intervals until the last day of the test. Sera were separated, centrifuged and stored at —20°C before being tested for rinderpest antibodies.

Tissue samples

The calves were slaughtered at various intervals as shown in Table 1. The slaughtered animals were autopsied immediately. A post-mortem examination was performed and specimens of mesenteric, prescapular and popliteal lymph nodes, tonsils, epithelial tissues from oesophagus, abomasum, duodenum, ileum, jejunum, caecum and rectum were collected and kept at - 70°C before being tested for rinderpest virus and precipitating antigen.

Preparation of the inoculum for virus isolation

Defibrinated blood samples as well as tissue samples were used for virus isolation. The blood specimens were spun at 1,000 rpm 15 minutes in a refrigerated centrifuge and the buffy coat of each was collected by aspiration and suspended vigorously to the original volume of ELY medium (Earle's balanced salt solution, lactalbumin hydrolysate, and yeast extract) containing 100 units of penicillin and 100 mg of streptomycin per ml. A 10% suspension of each of the frozen tissue samples was made

in ELY medium. The suspension was centrifuged at 3,000 rpm for 30 minutes in an international refrigerated centrifuge with 8 tubes. The supernatant fluid was used for virus isolation.

Virus isolation

0.4 ml of the prepared buffy coat suspension was dispensed into four tubes of calf kidney cell culture from which the maintenance medium had just been discarded and washed two times with PBS. The inoculated tubes were incubated at 37°C for one hour followed by addition of 1.5 ml of the medium to each tube.

Neutralization test

Serum samples were tested against attenuated rinderpest vaccine strain for neutralizing antibody. Serial tenfold dilutions of the virus were prepared using ELY medium. 0.3 ml of each dilution was mixed with an equal volume of 1/2 dilution of either normal serum or serum samples inactivated at 26°C for 30 minutes. The virus-serum mixtures were incubated at 37°C for 60 minutes, and then 2 tube cultures used for inoculation, each receiving 0.1 ml of the virus-serum mixture. Infected cultures were incubated for 60 minutes, then nutrient medium was added, and the cultures were again incubated for 7 days and checked for CPE every day.The titre of virus against each serum sample was calculated. The difference between the virus titre and the titre of each virus-serum mixture was taken as the neutralization index (NI) of tested serum sample.

Agar gel diffusion test

Agar gel containing 1% of Difco special agar in PBS and 1:2,000 thiomersal was used. A pattern of holes was stamped in the agar gel, using a specially constructed cutter designed to cut all the wells simultaneously. The agar plugs were removed and the floors of the wells were sealed with melted agar. The tissue samples were placed in the upper right and left lateral wells, as well as the lower ones. The known positive rinderpest control antigen was distributed into the top and bottom wells. The central well was filled with rabbit rinderpest hyperimmune serum. The gel was left at 37°C for 24 hours before being examined for the presence of precipitin lines.

RESULTS

Pathological changes

No visible pathological changes were observed in any test animal.

Virus isolation

All cell cultures inoculated by either buffy coat preparations or tissue sample suspensions were examined daily for 14 days. No cytopathic change was observed. A blind passage was made of each sample, and no virus was isolated.

Precipitating antigen

No precipitin formation was observed between the positive antiserum and any of the tissue samples. However, a visible precipitin line formed between positive antiserum and positive (control) tissue samples in each set.

Neutralizing antibody

The results of neutralization tests with the blood samples collected from the calves during the experiment are summarized

TABLE I

Appearance of neutralizing antibody against rinderpest vaccine virus in cattle inoculated with one ml of $10^{4.5}$ $TCID_{50}$/ml of Plowright strain vaccine virus

Days after inoculation	C.309	C.310	C.311	C.312	C.313	C.314	C.315	C.316	C.317	C.318
−1	0*	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	K-0	0	0	0	0	0	0	0	0	0
5	K	K-0	0	0	0	0	0	0	0	0
6	K	K	K-0	0	0	0	0	0	0	0
7	K	K	K	K-0	0	0	0	0	0	0
8	K	K	K	K	K-2.5	0	0	0	0	0
9	K	K	K	K	K	K-2.5	2.5	2.5	2.5	0
10	K	K	K	K	K	K	K-3.5	3.5	2.5	2.5
11	K	K	K	K	K	K	K	K-3.5	2.5	2.5
12	K	K	K	K	K	K	K	K	K-3.5	3.5
13	K	K	K	K	K	K	K	K	K	K-3.5

* → Numbers indicate the titre of neutralizing antibody to the rinderpest virus, expressed as NI.

K Blood sample was collected immediately before slaughtering the animal. The numbers coming after K show the antibody titre of the sera to the virus before being killed.

in Table I. Circulating neutralizing antibody was demonstrated as early as 8 days after inoculation, and soon reached a high titre which remained stable during the experiment.

DISCUSSION

Rinderpest is exotic to Iranian cattle. It occurs in some Asian and African countries. Because of migration of animals, especially ruminants, near the borders of Iran with neighbouring countries, the possibility of dissemination of the rinderpest virus in the country has always been present. Annual national vaccination has been adopted to give Iranian cattle bood protection against the disease. Despite the vaccination programme, rinderpest may be seen sporadically among illegally imported cattle, and spreading among unvaccinated calves, as was the case with the 1982 outbreak. Protection of the cattle population, and success of any eradication programme in this country, depend largely on quick dianosis of the disease in the infected area. Several reliable methods have been developed for the diagnosis of the disease. Among these methods, detection of precipitation antigen in lymphatic tissues of infected animals by the agar gel diffusion test is one of the easiest to perform, yet has the advantages of rapidity and reliability (14).

Reliability of the immunodiffusion test in diagnosing natural cases of rinderpest depends on the collection of suitable tissue samples at the proper time. Brown and Scott (1) followed the development of rinderpest specific precipitinogens in the prescapular lymph nodes of cattle infected with a virulent strain of rinderpest virus. The precipitating antigen was detected on the first day of fever and persisted until the 8th day, the highest percentage of positive samples occurring on the 3rd, 4th and 5th days of fever. Animals killed on the 10th day or later were always negative. A serious drawback of the agar gel diffusion reaction as a diagnostic test is the direct relationship between the presence of the precipitinogen in the animal and the clinical response. This reaction is independent of the strain of the virus but the possibility of a positive result increases with the severity of the clinical response (16).

In the present experiment, it was demonstrated that an attenuated strain of rinderpest virus,i.e. the Plowright vaccine strain at its 110th passage level in calf kidney cell cultures, failed

to stimulate the production of precipitating antigen when injected in susceptible calves. This finding concurs with that of Provost and Borredon (11), who showed that the naturally attenuated rinderpest virus strains did not stimulate the production of precipitating antigen in the tissues of affected cattle. The inability of attenuated srains of rinderpest virus to stimulate precipitating antigen could be attributed to the fact that attenuated virus attains much lower titres in the tissues of inoculated animals in comparison with the virus (13, 17). Taylor and Plowright (18) found that attenuated culture-adapted virus did not proliferate in the mucosa of the alimentary tract, the nasal mucosa, or in the parenchymatous organs. It was strictly lymphotropic and they suggested that the lack of pathogenicity stemmed from this characteristic. This has been confirmed in the present study in which we were unable to isolate the virus from blood and tissue samples of animals during 15 days after inoculation. The appearance of neutralizing antibody in inoculated calves from the 8th day after vaccination, and its increase in titre during the period of experiment, showed that the virus multiplied enough to stimulate the antibody producing system of the body. The relationship between the presence of circulating antibodies and resistance has been studied by several authors (19, 3, 7). Neutralizing antibodies were detected as early as the fifth day after infection by Scott and Brown (15).

However, high titres are not usually evident until about a week after the onset of illness. Their first appearance, moreover, is related to the dose of infecting virus, beign earliest after high doses (10). Peak titres were found two to four weeks after the onset of illness by Mac Owan (5), Plowright (9) and Johnson (4).

Although rinderpest is not an endemic disease in Iran, the country has always been at risk from neighbouring countries. For this reason, an annual vaccination is practised, and any mortality among cattle with alimentary tract lesions, especially in an area at risk, is investigated for rinderpest.

The gel diffusion test, a rapid and easy test to detect precipitinogen in the lymph nodes of infected animals, is usually performed, by a virus isolation procedure for further confirmation of the diagnosis.

Gel diffusion has proved to be a very reliable test for the

diagnosis of rinderpest, providing the samples used for the test are collected properly and at the right time.

On the other hand, in areas where vaccination with an attenuated virus strain is practised, any doubt should be eliminated from the potential ability of vaccine virus to stimulate the production of precipitating antigen in vaccinated animals. The results of the present investigation showed that the Plowright vaccine strain did not stimulate the production of precipitating antigen in vaccinated calves. Thus, even in areas where animals are annually vaccinated with this vaccine, a positive gel diffusion test in any rinderpest suspected case would be attributable to infection of the subject with natural rinderpest virus.

ACKNOWLEDGEMENTS

We are very grateful to Dr. A. Hazrati for his interest in the subject and his valuable revision of this article.

We should also like to thank the staff of the Animal Virus Department of Razi State Institute for technical assistance during the course of this work.

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ETUDE SUR LA REPONSE DES VEAUX A UN VACCIN ATTENUE CONTRE LA PESTE BOVINE. — M.H. Roustai, M. Hessami, B. Ghaboosi et T. Ephtekhari.

Resume: Dix veaux sensibles a la peste bovine ont ete chacun soumis a l'injection d'un ml d'un vaccin contre la peste bovine, attenue par 110 passages sur cellules de rein de veau.

La multiplication du virus chez les veaux et leur reaction immunitaire ont e te observes au cours d'une periode de 15 jours.

Le virus n'a ete isole ni dans les leucocytes des animaux vivants, ni dans les prelevements tissulaires des animaux abattus a differentes dates apres l'inoculation (entre 4 et 15 jours).

Les anticorps neutralisants one ete mis en evidence dans le serum 8 jours apres l'inoculation, mais aucun antigene precipitant n'a ete trouve dans les tissus preleves sur les veaux apres abattage.

L'infection des eaux avec des souches virulentes de virus

bovipestique se traduit en general par la production d'antigene precipitant, detectable par une epreuve d'immunodiffusion en gelose dans des tissus preleves correctement sur des animaux infectes.

Dans cet essai, les auteurs one constate que les veaux etaient incapables de produire des anticorps precipitants vis-a-vis du virus bovipestique attenue, ce qui prouve que la presence d'un antigene precipitant bovipestique dans des prelevements tissulaires issus d'un animal suspect de peste bovine peut etre attribuee a une infection par un virus virulent, et confirme l'interet de l'epreuve d'immunodiffusion en gelose dans le diagnostic de la peste bovine.

MOTS-CLES: Epreuves d'immunoprecipitation - Maladies des bovins - Reaction immunitaire - Vaccins - Veau - Virus de la peste bovine.

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ESTUDIO DE LA RESPUESTA DE LOS TERNEROS A UNA VACUNA ATENUADA CONTRA LA PESTE BOVINA. — M.H. Roustai. M. Hessami, B. Ghaboosi y T. Ephtekhari.

Resumen: Se administro a diez terneros sensitivos a la peste boving una inyeccion de un ml de una vacuna contra la peste bevina, atenuada con 110 pases en celulas renales de ternero.

Se observo la multiplicacion del virus en los terneros y su reaccion inmunitaria durante un periodo de 15 dias.

No fue aislado el virus ni en los leucocitos de los animales vivos, ni en las muestras tisulares de los animales sacrificados en distintas fechas despu s de la inoculacion (entre 4 y 15 dias).

Se descubrieron los anticuerpos neutralizantes en el suero 8 dias despues de la inoculacion, aunque no se encontro ningun antigeno precipitante en los tejidos tomados en los terneros despues del sacrificio.

La infeccion de los terneros con cepas virulentas de virus bovipestoso se suele poner de manifiesto produciendo antigeno precipitante, detectable en tejidos tomados correctamente de animales infectados, con una prueba de inmunodifusion en agar.

Los autores comprobaron en esta prueba que los terneros

no podian producir anticuerpos precipitantes frente al virus bovipestoso atenuado, lo que prueba que la presencia de antigeno precipitante bovipestoso en muestras tisulares en un caso sospechoso de peste bovina se podria atribuir a la infeccion del animal por un virus virulento. Se podria considerar la presencia del antigeno como una confirmacion suplementaria del interes de ia prueba de inmunodifusion en agar para el diagnostic de la peste bovina.

PALABRAS CLAVE: Enfermedades de bovinos - Pruebas de inmunoprecipitacion - Reaccion inmunitaria - Ternero - Vacunas - Virus de la peste bovina.

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