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SEROLOGICAL SURVEY FOR ANTIBODIES AGAINST INFECTIOUS BOVINE RHINOTRACHEITIS AND PARAINFLUENZA 3 VIRUSES AMONG CATTLE IN IRAN.

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Summary.

Nine hundred and twenty three bovine serum samples, collected from various parts of Iran, were tested for neutralizing antibodies against infectious bovine rhinotracheitis (IBR) and parainfluenza 3 (PI3) viruses.

Of the total sera examined 314 (34.02per cent) had antibody against IBR virus and 588 (62.2 per cent) contained antibody against P13 virus

The results obtained indicated that IBR and P13 virus infections are widely disseminated among the bovine population throughout the country.

Introduction

Since the isolation of IBR virus, in 1956 (7) and P13 virus, in 1959 (9), from cattle, these two viruses have been incriminated in the etiology of bovine respiratory diseases complex. Both viruses were shown to produce frank and inapparent infections among cattle almost all over the world.

IBR virus has recently been isolated from cases of infectious bovine rhinotracheitis among imported cattle in Iran (5,6), and it was also indicated that a high percentage of cattle population in this country have haemagglutination inhibition (HI) antibodies to PI 3 virus (1).

The present communication records the occurrence of neutralizing antibodies to IBR and P13 viruses in bovine serum samples, and provides further evidences on the presence and distribution of these two viruses among Iranian cattle population.

Materials and Methods

Cell-culture. - Monolayer cultures of primary or secondary bovine embryonic kidney (BEK) cells were used for virus propagation and neutralization tests. Cells were grown in an ELY medium (Earle's balanced salt solution, lactalbumin hydrolysate, and yeast extract) containing 10% inactivated calf serum, 100 units penicillin, and 100 μg of streptomycin per ml.

Viruses. - Esfahan strain of IBR and T1 strain of P13 were used in these studies.

Esfahan strain was isolated from a natural case of infectious bovine rhinotracheitis in this laboratory and was used at its 5th passage level in BEK cells (6).

The Tl,a bovine strain of P13 virus, was supplied by The Central Veterinary Laboratory, Weybridge, and was used after a few additional passages of the virus in BEK cells(4).

To prepare the virus stocks BEK cells were inoculated with the desired virus and incubated at 37 C. for 2 hours. After this adsorption period the infected cells were overlaid with ELY medium containing 2 per cent foetal calf serum and incubated until the cytopathic effect was almost complete. The infected fluid along with cells were then harvested after they were frozen and thawed. Cell debris were sedimented by low-speed centrifugation and the supernatant virus suspension was stored at -70 C. until used.

Sera. - Blood samples were obtained from apparently healthy cattle in various parts of the country. The sera were separated from blood samples as usual, and were stored at - 20 C. until required. Serum samples were heated at 56 C. for 30 minutes prior to testing.

Neutralization tests. - To detect the IBR antibody, four-fold dilutions in ELY of the inactivated serum smples were mixed with equal volumes of virus suspension containing 40 to 100 TCID 50 of the virus per 0.1 ml. The virus-serum mixtures were incubated at 37 C. for 2 hours and then each mixture was tested for infectivity by inoculating 2 BEK cell culture tubes using 0.2 ml. of the mixture as inoculum. After an adsorption period of 2 hours, 1.5 ml. of ELY was added to each tube and the cells were re-incubated at 37 C. Virus inoculum was titrated simultaneously. The cultures were examined daily for cytopathic effect (CPE) for 5 days. Highest dilution of serum that completely inhibited CPE

in 50 per cent of the cell culture tubes was taken as the serum titre which was calculated by Reed and Muench method (8).

P13 neutralizing antibody was titrated as follows:

Ten-fold dilutions of the virus in ELY were mixed with equal volume of a 1/2 dilution of the serum. For control normal bovine serum was used simultaneously. The virus serum mixtures were incubated for two hours at 37 C., and then each mixture was tested for infectivity in the same manner as above. The differnce between the virus titre and the titre of the virus in serum mixture was taken as the neutralization index (NI) of the serum.

Results and Discussion

Neutralizing antibody titre of 1:2 and greater against IBR virus has been taken as an index of a positive serum. As shown in Table 1 from a total 923 serum samples tested 314 sera (34.02 per cent) were found to be positive. The percentage of samples having neutralizing antibody varied from 11.9 to 60.7 per cent. Higher percentage of positives were reported in Ahwaz and in Tehran.

The incidence of IBR antibody obtained, however, was in general much higher than those reported previously (2,3), and is an indicative of a relatively wide dissemination of the virus infection among bovine population in this country. Isolation of IBR virus from several clinical cases supported this view (5,6), and further indicates that the virus infection, under favourable condition, could become economically important.

In the case of P13 antibody, neutralization index (NI) equal to or greater than 2 was taken as the evidence of a positive serum sample. Positive P13 neutralization was shown in 62.2 per cent of 923 serum samples tested (Table 2). The lowest percentage of positives (25.4 per cent) was recorded from Mazendaran, where cattle are kept in natural pastures. The highest percentage (87.8 per cent) was recorded in Tehran area, where animals are kept under very intensive dairy farms conditions which favour the distribution of any respiratory infection.

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Table 1. – Distribution of neutralizing antibody to infectious bovine rhinotracheitis (IBR) virus among cattle in Iran.

Localities	No. of sera tested	No. of positive sera	Percentage of positives
Tehran	321	115	35.8
Esfahan	128	31	24.2
Guilan	130	34	26.2
Mazendaran	67	8	11.9
Ahwaz	158	96	60.7
Maragheh	70	20	28.5
Meshed	49	10	20.4
Total	923	314	34.02

Table 2. – Distribution of neutralizing antibody to parainfluenza 3 (P13) virus among cattle in Iran.

Localities	No. of sera tested	No. of positive sera	Percentage of positives
Tehran	321	282	87.8
Esfahan	128	44	34.3
Guilan	130	90	69.2
Mazendaran	67	17	25.4
Ahwaz	158	82	51.9
Maragheh	70	37	52.8
Meshed	49	22	44.9
Total	923	574	62.2

References

1. Afshar, A. - The occurrence of antibodies to parainfluenza 3 virus in sera of farm animals and man in Iran. Br. Vet. J., 125, (1969): 529–533.

2. Afshar, A., and Tadjbakhsh, H. - Occurrence of precipitating antibodies to bovine herpesvirus (Infectious bovine rhinotracheitis) in sera of farm animals and man in Iran. J. Comp. Path., 80, (1970): 307–310.

3. Darakhshan, H. - Occurrence of IBR virus antibody in sera of cattle from Tehran area. DVM Thesis, No. 793, Faculty of Veterinary Medicine, Tehran University (1968).

4. Dawson, P.S., and Cruickshank, J.G. – The isolation of a bovine strain of parainfluenza 3 virus - A preliminary report. Vet. Rec., 75, (1963): 388–389.

5. Hazrati, A. - First isolation of IBR virus from nasal secretion of imported cattle in Iran. Annual report, Razi Institute, (1973–1974): 78.

6. Hazrati, A., and Amjadi, A.R. – The isolation and identification of infectious bovine rhinotracheitis virus in Iran. Bull. Off. int. Epiz., 81 (9–10), (1974): 847–863.

7. Madin, S.H., York, C.J., and McKercher, D.G. – Isolation of the infectious bovine rhino tracheitis virus. Science, 124, (1956): 721–722.

8. Reed, I.J., and Muench, H. - A simple method of estimating 50 per cent end points. Am. J. Hyg., 27, (1937): 493–497.