Arch. Inst. Razi, 1975, 27, 21-35

THE ISOLATION AND IDENTIFICATION OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN IRAN (*)

by

A. HAZRATI and A.R. AMJADI

INTRODUCTION

The first isolation of Infectious Bovine Rhinotracheitis (IBR) virus in Iran from nasal secretions of imported pregnant cows with an acute respiratory disease was recently reported (8). The present communication describes an investigation of another outbreak of the disease among imported cattle and presents the results of tests undertaken to characterize the IBR virus strains so far isolated in this country.

MATERIALS AND METHODS

Source of materials. – Materials for isolation of the virus were obtained from three outbreaks of moderate to severe respiratory disease with some mortality and abortion among pregnant cows imported from United Kingdom and France into 2 farms in Tehran and 1 farm at Mohammad-abad, Esfahan, in September 1973, April and May 1974, respectively.

Specimens for Virus isolation. – Nasal and conjunctival secretions from infected animals were collected by using sterile cotton swabs. The swabs were immediately placed into screw-capped bottles containing 2 ml. of ELY medium with antibiotics and were transported to laboratory in a thermos flask filled with ice cubes.

Specimens from lung and tracheal mucosa of a dead animal and a piece of placenta from an infected cow with abortion were also collected and brought to the laboratory under the same condition.

The secretion adsorbed to the cotton was extracted into the ELY medium

(*) Bull. off. int. Epiz., 1974, 81 (9–10), 847–863

from the swabs by pressing them with a pair of pincers. The extracts were then centrifuged at 10,000 r.p.m. for 25 minutes and the supernatant fluid was kept at – 70°C until being used as inoculum for virus isolation.

Similarly, the extracts from tissues were prepared from a 10 percent suspension from each tissue specimen in ELY, and were kept at – 70°C until used for virus isolation.

Virus isolation. – Isolation of the virus was attempted by inoculation of primary or secondary culture of calf kidney cells with the above prepared inoculums.

0.2 ml of each inoculum was inoculated into each of 4 cell culture tubes. The inoculated cells were examined microscopically every day for the appearance of cytopathic effect (CPE). Further passages were also made in calf kidney cell cultures.

Cell cultures. – Primary monolayer calf kidney cell cultures were prepared by trypsinization of Dulbecco and Vogt as modified by Youngner (12). Cells were grown in a ELY medium containing 10 percent inactivated calf serum, 100 units of penicillin, and 100μ g of streptomycin/ml.

Secondary cultures of cells were prepared by adding 0.25 percent trypsin solution to monolayers of cell cultures and growing the obtained cell suspension in the culture growth medium.

Experimental transmission. – A 2 year-old native cow was used in the exposure trial. The animal was inoculated intranasally (2 ml of virus fluid per nostril) and intraconjunctivally (0.5 ml of virus fluid per eye). The inoculum was the fluid harvested from BK cell culture previously infected with the newly isolated Esfahan strain. The cow was examined daily and body temperature was recorded for 10 days. Nasal and conjunctival secretions were colledted for virus isolation during the observation period. Pre and post exposure sera were also collected to check the antibody response of the animal.

Infectious Bovine Rhinotracheitis virus. – Strains American, Oxford and Aberdeen of IBR virus (5,6), at their 8th, 15th, and 5th passage levels in bovine kidney cells, respectively, were supplied by the Central Veterinary Laboratory, Weybridge, England. A few additional passages in calf kidney cell cultures were made from each strain before being used in this study.

Antiserum preparation. – Homologous antisera against American, Esfahan and Tehran strains were prepared in rabbits. The animals received 6 intraperitoneal and intravenous injections of 3 to 4 ml of cell culture propagated virus fluid at 4 days intervals. Sera were collected 10 days after the last inoculation and were kept at – 20°C until required.

Antisera against Oxford and Aberdeen strains were received from the Central Veterinary Laboratory, Weybridge.

Neutralization test. – 0.3 ml of each of serial 10 fold dilutions of the virus was mixed with an equal volume of inactivated antiserum. For the control, normal rabbit serum was mixed with the virus dilutions. The virus serum mixtures were incubated for 2 hours. Then each mixture was tested for virus infectivity by inoculating 4 cell culture tubes per mixture. The extent of neutralization was expressed as neutralization Log-Index, which is the difference between the virus titres of virus serum mixtures and the control series.

Ether and chloroform sensitivity tests. – The sensitivity of the new isolates to ether and chloroform was tested by the methods of Andrewes (2), and Feldman and Wang (7) as described previously (9).

Study of cytological changes. – Cells grown on coverslips in Leighton tubes, were infected, and then at appropriate time intervals the coverslips were fixed with Carnoy fixative and stained with Harris haematoxylin eosin stains before being studied microscopically for cytologic changes.

RESULTS

Clinical features of the outbreaks. – The appearance and clinical aspects of all three outbreaks, reported in this communication, were essentially the same as those observed in the outbreak of the disease in Mohammad-abad, Esfahan.

This outbreak appeared in a group of 99 imported cows. The animals. were transported from France to Tehran by air and then immediately to Esfahan by lorries, and they were vaccinated against Rinderpest within 48 hours.

The infection was first observed 6 days after the animals were introduced in the farm and spread rapidly to other individuals so that 70 percent of animals were found to be affected within a period of about 5 to 7 days.

Rhinitis and conjunctivitis were the first noticeable signs of infection, which were accompanied or followed by high temperature (40.5 to 41°C), coughing, depression, dispnea, and serous to mucopurulent nasal and lachrymal discharges (Fig. 1.).

Post-mortem examination on 2 dead cases showed a severe tracheitis and bronchopneumonia. The nasal and tracheal mucosa were congested and covered by an excessive amount of fibrinous mucopurulent exudate (Fig. 2).

15 cases of abortion were also recorded but unfortunately no aborted foetus was submitted to the laboratory for any investigation.

Isolation and properties of the virus. – 24 to 48 hours after inoculation of

primary calf kidney cell cultures with different tissues of respiratory system in fatal cases or with nasal and conjunctival swabs from affected animals, a cytopathic change was observed which was reproduced more evidently in further serial passages in the same cell cultures. The isolated agents in each outbreak were soon found to be very similar to each other in that they readily grew in BK cell cultures producing indistinguishable CPE. Thus only two strains, subsequently designated Tehran and Esfahan strains as the representatives of the causative agents of the outbreaks, were chosen for further studies.

The cytopathic effects of the strains in BK cells resembled those of IBR virus (3,11). The first noticeable changes consisted of rounding, shrinking and clumping of infected cells. The infection spread rapidly and affected the whole cell sheet which resulted in the complete destruction of the cell monolayer. Acidophilic intranuclear inclusion bodies were also observed in a large proportion of the infected cells. The inclusions mostly seemed to fill the nucleus as a homogenous mass. In some nuclei, however, inclusions with thin clear halo and nucleoli and chromatin margination at the nuclear membrane were also observed (Fig. 3, 4).

The virus strains were found highly sensitive to ether and chloroform (Table 1).

Cross neutralization tests between the strains and the American, Oxford, and Aberdeen strains of IBR virus and their respective antisera indicated that both new isolates were antigenically identical to the known strains of IBR virus (Table 2).

Exposure of a cow to Esfahan strain resulted in a clinical and serological response which was comparable with mild form of IBR infection.

The exposed animal developed a temperature response and exhibited some mucoid nasal and lachrymal discharges. The ocular discharge was more severe and particularly noticeable by causing a narrow strip of matted hair on both sides of the face (Fig. 5).

The virus was recovered from nasal discharge of the exposed animal from the first to the 8th day, and from conjunctival secretion, from first to the 7th day, post exposure (Fig. 6).

The virus stimulated the production of antibodies in both naturally and experimentally infected animals. Neutralizing antibodies against American and Esfahan strains of IBR virus were demonstrated in the experimentally exposed cow and in 79 percent of 19 affected and in-contact cattle in the affected herd of Mohammad-abad (Table 3).

DISCUSSION

Infectious Bovine Rhinotracheitis virus has been reported as the cause of Infectious Bovine Rhinotracheitis, Infectious Pustualr Valvovaginitis, Infectious Pustular Balanoposthitis, conjunctivitis, fatal disease of new born calves, Mastitis, Bovine Epizootic Abortion and Encephalitis from different parts of the world (10, 11).

In Iran, the IBR virus infection has not been thoroughly investigated. The results of a serological test, showing the presence of IBR virus neutralizing and precipitating antibodies in 19.3 percent and 4.9 percent of 281 native cattle sera respectively, however, indicated that the virus infection existed among bovine population in this country (1,4).

The isolation of IBR virus from a natural outbreak of IBR in Iran was first reported by Hazrati in 1973 (8). Since then 10 isolations of the virus were made from 2 other outbreaks reported among imported cattle.

The identification of the virus was based on *in vitro* and *in vivo* experiments. The virus multiplied readily in calf kidney cell cultures producing cytopathic alteration and intranuclear inclusion bodies almost identical to those produced by IBR virus. The virus strains were shown to be highly sensitive to lipid solvents and immunologically identical to American, Oxford, and Aberdeen strains of IBR virus.

The clinical and serological responses of a cow experimentally exposed to the protype Esfahan strain of the virus were similar to a mild manifestation of the natural disease.

The clinical picture of the infection is described and it is shown that the virus stimulated the production of IBR antibodies both in naturally and experimentally affected and incontact cattle.

It was difficult to trace the source of infection, as the animals were not in contact with others and no native cattle were introduced into the herd. The reappearance of a previous infection in some or one of the cows, as a result of too much stress from transportation, and its spread among the susceptaible individuals must not be however overlooked.

SUMMARY

The virus of Infectious Bovince Rhinotracheitis (IBR) has been isolated from three outbreaks of moderate to severe respiratory disease, with some mortality and abortion, among pregnant cows imported into 3 farms in Iran.

The isolations, designated as Tehran and Esfahan strains, were identified

as IBR virus on the bases of their cytopathogenicity to cell culture, lability to lipid solvents, and their antigenic similarity to American, Oxford, and Aberdeen strains of IBR virus. Exposure of a cow to Esfahan strain resulted in clinical and serological responses which were similar to those of a mild form of IBR infection.

* * *

ACKNOWLEDGEMENTS

We wish to thank Dr. Kaveh, General Director of the Razi Institute for his advice and support, and Dr. Khoshmanesh, Dr. Farhangfar, and Dr. Forouzesh for reporting the outbreaks and for their cooperation in this study

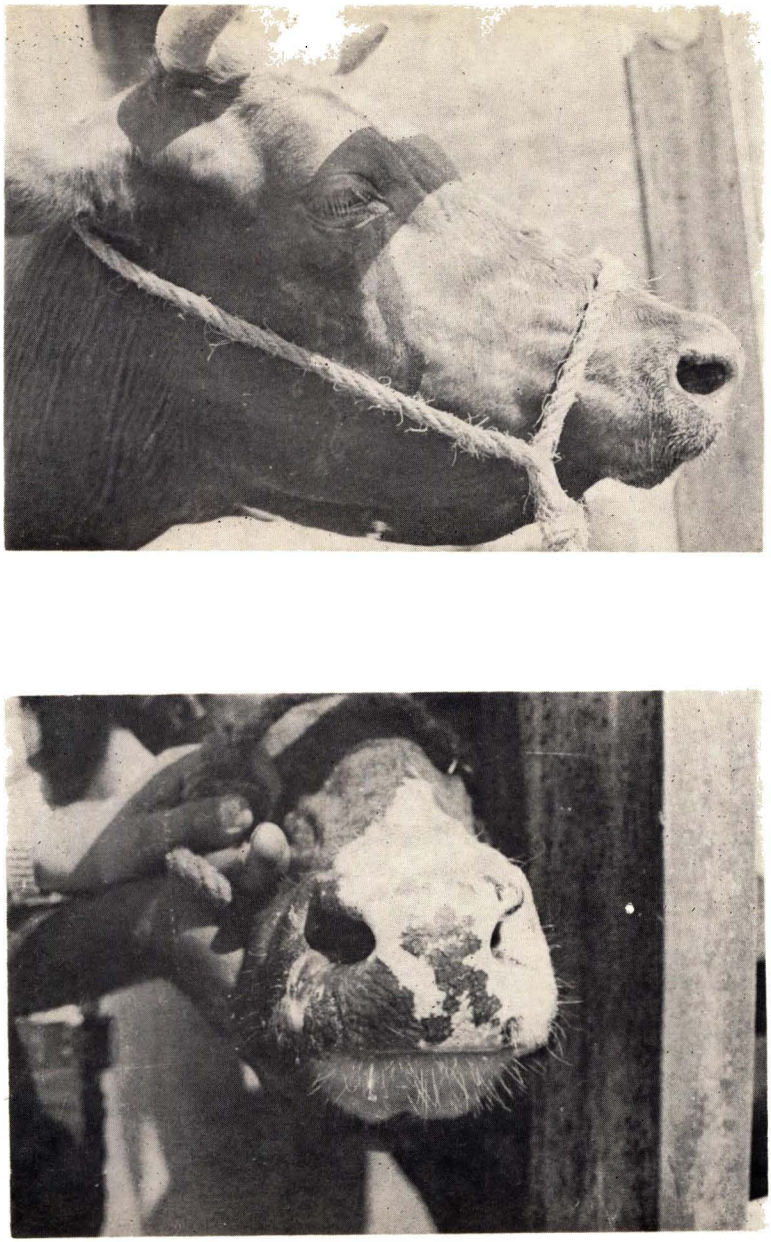


Fig. 1. – Animals from Mohammad-abad, Esfahan, in early stage of the disease, showing depression, nasal and lachrymal discharges.

A

B

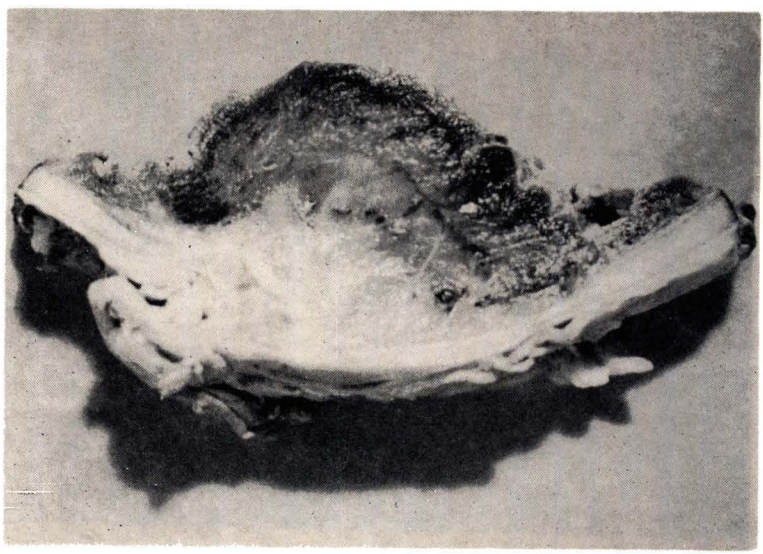
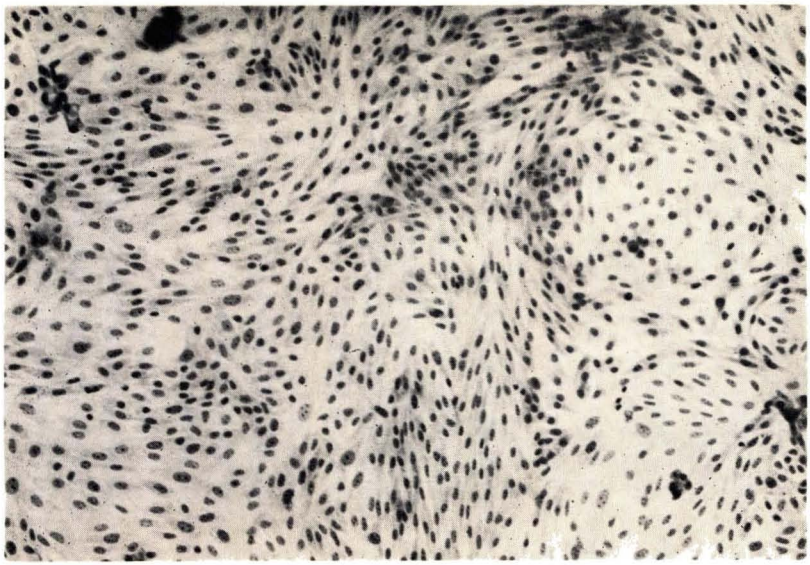


Fig. 2

(A) Trachea of a cow from Mohammad-abad affected with IBR. Note the pseudomembrano-tracheitis.
(B) Horizontal section of trachea from same case. Note the thickness of mucosa and submucosal region.

A



B

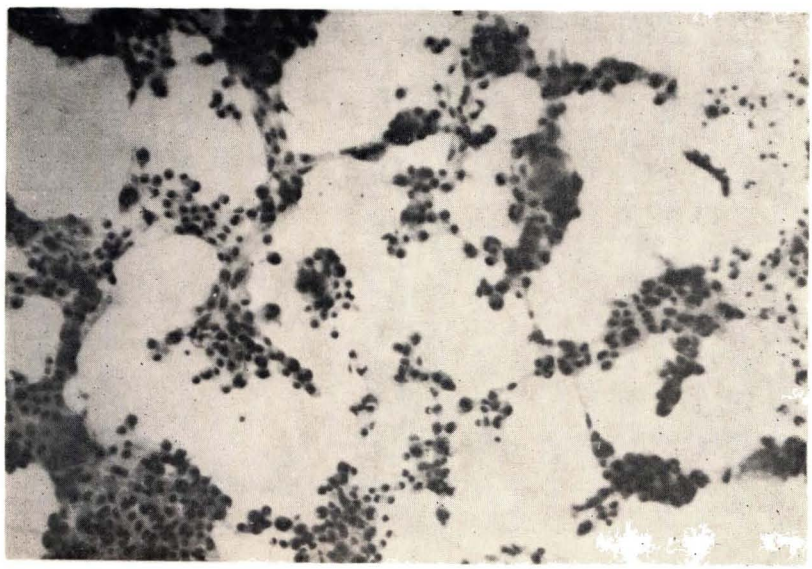
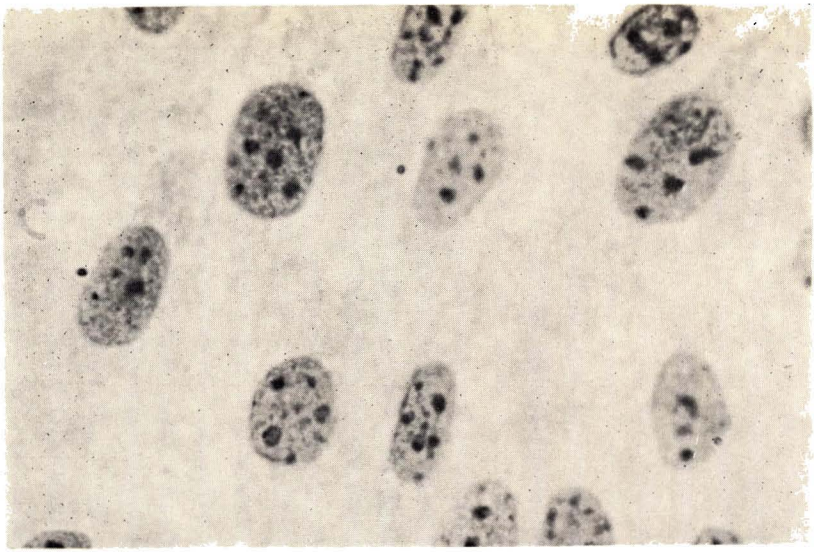


Fig. 3. – Non-infected calf kidney cells (A) and cells infected with Esfahan strain of IBR virus (B).

A



B

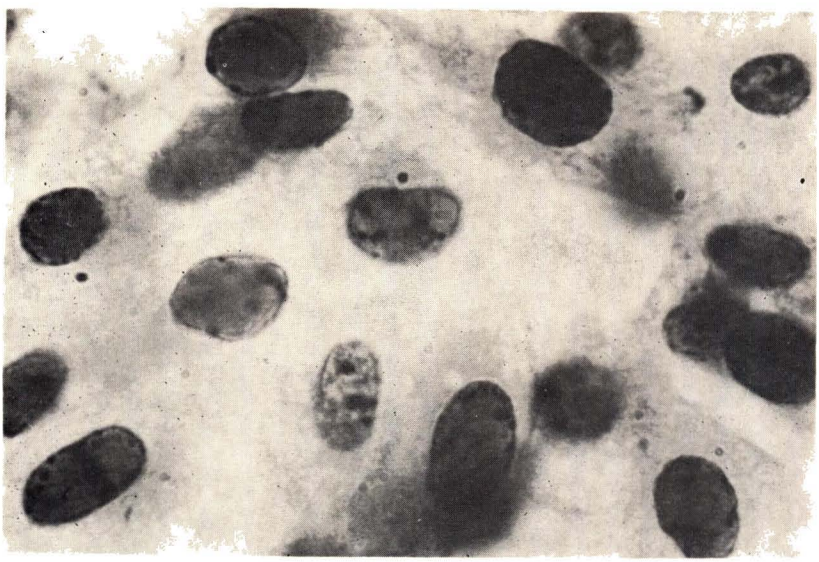


Fig. 4. – Non-infected calf kidney cell culture (A) and cells infected with Esfahan Strain of IBR virus. Note chromatin and nucleoli margination and inclusions in infedted nucleus (B)

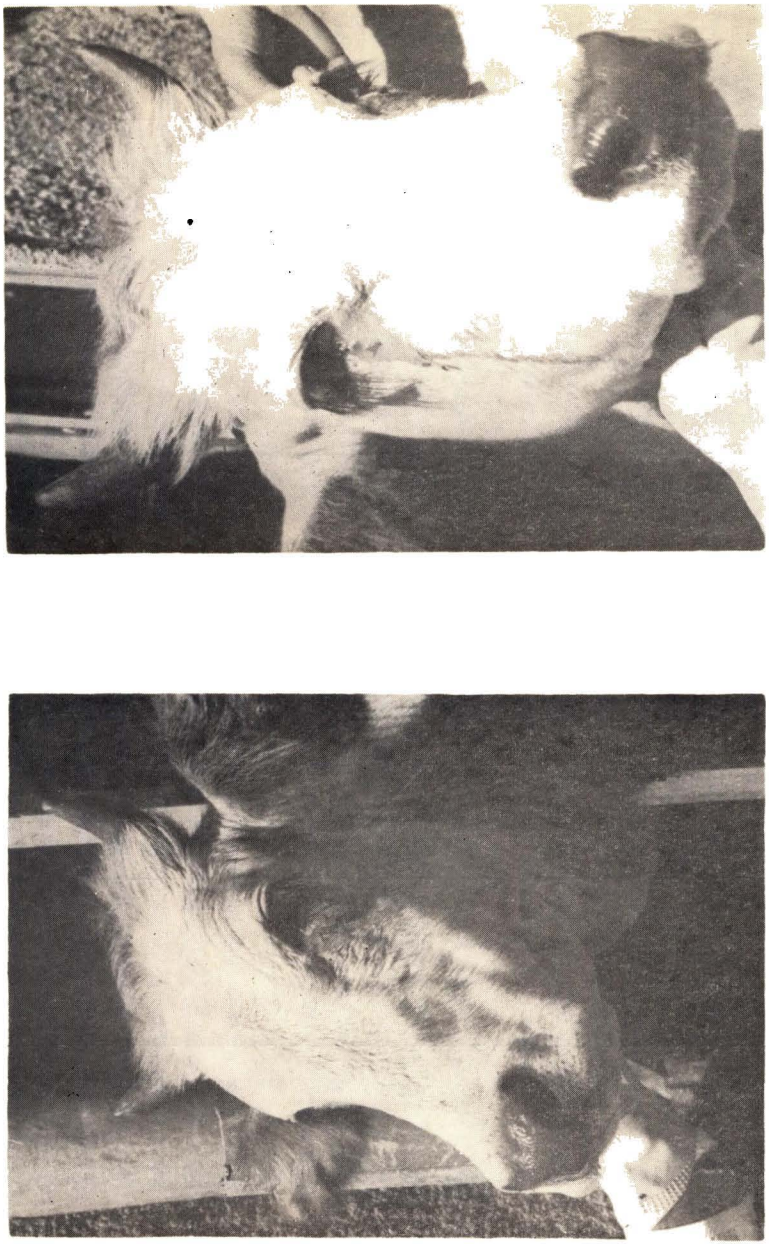


Fig. 5. – Conjunctivitis in a cow exposed to Esfahan strain of IBR virus.

TABLE 1. – *Sensitivity of Tehran and Esfahan strains of IBR virus to lipid solvents.*

	– Log virus suspension dilution						
	0	1	2	3	4	5	6
Esfahan st. control	+	+	+	+	+	+	0
Esfahan st. + ether	0	0	0	0	0	0	0
Tehran st. control	+	+	+	+	+	+	0
Tehran st. + ether	0	0	0	0	0	0	0
Esfahan st. control	+	+	+	+	+	+	0
Esfahan st. + chloroform	0	0	0	0	0	0	0
Tehran st. control	+	+	+	+	+	+	0
Tehran st. + chloroform	0	0	0	0	0	0	0

+ = Virus positive.

0 = Virus negative.

TABLE 2. – *Cross neutralization tests between IBR virus strains isolated in Iran and American, Oxford, Aberdeen strains of IBR virus and their respective antisera.*

Antiserum	Virus strains				
	Tehran	Esfahan	American	Oxford	Aberden
Tehran antiserum	>5.0*	>5.0	>5.0	>5.0	>5.0
Esfahan antiserum	5.0	>5.0	>5.0	5.0	>5.0
American antiserum	>5.0	>5.0	>5.0	>5.0	>5.0
Oxford antiserum	5.0	5.0	5.0	5.0	5.0
Aberdeen antiserum	5.0	5.0	5.0	5.0	5.0

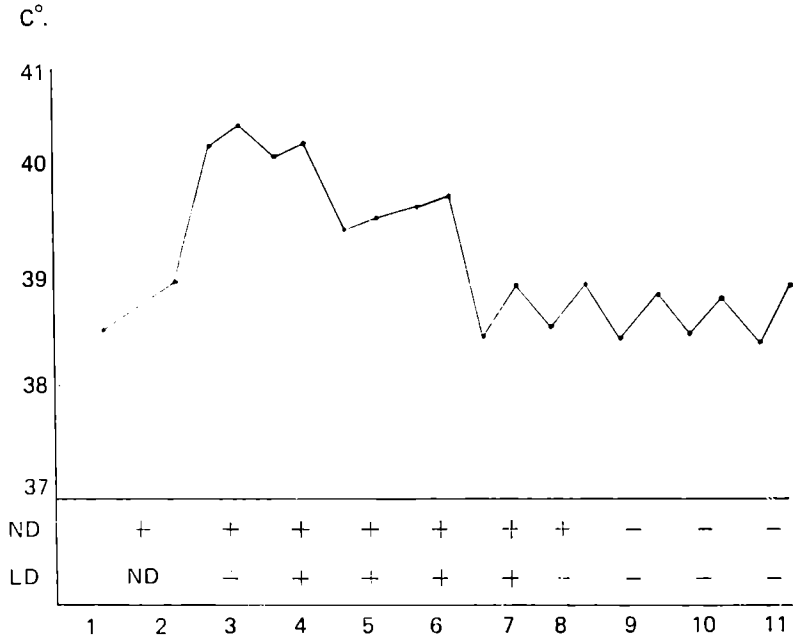
* = Neutralization Log-Index.

Table 3. — Estimation of IBR neutralizing antibody in an exposed cow and in cattle sera from Mohammad-abad, Esfahan. Sera were collected 2 to 3 weeks after exposure or first appearance of IBR infection in the herd.

Serum	Log. Dilution, Esfahan st.					Log. Dilution, American st.				
No.	0	1	2	3	4	0	1	2	3	4
Normal	+	+	+	+	+	+	+	+	+	+
1	0	0	0	—	—	+	0	0	—	—
2	0	0	0	—	—	0	0	0	—	—
3	0	0	0	—	—	0	0	0	—	—
4	+	+	+	—	—	+	+	+	—	—
5	+	0	0	—	—	0	0	0	—	—
6	0	0	0	—	—	0	0	0	—	—
7	+	+	+	—	—	+	+	+	—	—
8	0	0	0	—	—	0	0	0	—	—
9	0	0	0	—	—	0	0	0	—	—
10	+	0	0	—	—	+	0	0	—	—
11	0	0	0	—	—	0	0	0	—	—
12	0	0	0	—	—	0	0	0	—	—
13	+	0	0	—	—	+	0	0	—	—
14	0	0	0	—	—	0	0	0	—	—
15	0	0	0	—	—	0	0	0	—	—
16	+	0	0	—	—	0	0	0	—	—
17	+	0	0	—	—	0	0	0	—	—
18	+	+	+	—	—	+	+	+	—	—
19	+	+	+	—	—	+	+	+	—	—
20/1	+	+	+	—	—	+	+	+	—	—
20/2	0	0	0	—	—	0	0	0	—	—

\+ = CPE. i.e. virus was not neutralized.
0 = No CPE. i.e. virus neutralized.
— = Not done.

20/1 and 20/2 = Pre and post-exposure sera from experimentally exposed animal.



ND = Nasal discharge.
LD = Lachrymal discharge.
ND = Not done.
\+ = Virus isolated.
— = Virus was not isolated.

Fig. 6. — *Temperature response and reisolation of virus from a cow experimentally exposed to Esfahan strain of IBR virus.*

REFERENCES

1. AFSHAR (A.) & TADJBAKHSH (H.). — Occurrence of precipitating antibodies to bovine herpesvirus (Infectious Bovine Rhinotracheitis) sera of farm animals and man in Iran. *J. Comp. Path.*, 1970, **80,** 307–310.
2. ANDREWES (C.H.) & HORSTMANN (D.M.). — The susceptibility of viruses to ethyl ether. *J. Gen. Microbiol.*, 1949. **3,** 290–297.
3. CHEATHAM (W.J.) & CRANDELL (A.). — Occurrence of intranuclear inclusions in tissue cultures infected with virus of Infectious Bovine Rhinotracheitis. *Proc. Soc. Exp. Biol. N.Y.*, 1957, **96,** 536–538.
4 DARAKHSHAN (H.). — Occurrence of IBR virus antibody in sera of cattle from Tehran area. D.V.M. Thesis, No. 793, Faculty of Veterinary Medicin, Tehran University, 1968.
5. DARBYSHIRE (J.H.) & SHANKS (P.L.). — The isolation of Infectious Bovine Rhinotracheitis virus in Scotland. *Vet. Rec.*, 1963, **36,** 897–899.
6. DARBYSHIRE (J.H.), DAWSON (P.S.), PATERSON (A.B.) & LOOSEMORE (R.M.). — The isolation of Infectious Bovine Rhinotracheitis virus in the United Kingdom. A preliminary report. *Vet. Rec.*, 1962, **47,** 156–157.
7. FELDMAN (H.A.) & WANG (S.S.). — Susceptibility of various viruses to chloroform. *Proc. Soc. Exp. Biol. and Med.*, 1961, **106,** 736–738.
8. HAZRATI (A.). — First isolation of IBR virus from nasal secretion of imported cattle in Iran. Annual report, Razi Institute, 1973–74, 78.
9. HAZRATI (A.), AMJADI (A.R.) & DAYHIM (F.). — An outbreak of abortion associated with equine Rhinopneumonitis viral infection in Iran. *Archives de l'Institut Razi*, 1974, **26.**
10. McKERCHER (D.G.). — Infectious Bovine Rhinotracheitis. *Advance in Veterinary Science*, 1959, **5,** 299–328.
11. McKERCHER (P.D.). — A comparison of the viruses of IBR, IPV and Rinderpest. Part 1. Studies of antigenic relationships. *Canad. J. Comp. Med. & Vet. Sci.*, 1964, **28,** 77–88
12. YOUNGNER (J.S.). — Monolayer tissue culture. 1. Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells. *Proc. Soc. Exp. Med. N.Y.*, 1954, **85,** 202–206.