

## *Note*

### **The occurrence of bovine virus diarrhoea/mucosal disease in Iran**

by

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#### **Summary**

*Sera were collected from cattle throughout Iran and were screened for neutralizing antibodies to the NADL strain of bovine virus diarrhoea, using monolayers of primary calf kidney cells. It was shown that while northern provinces around the Caspian Sea and the northern region of the Persian Gulf were almost free from the disease, in the central part of the country the rate of infection was 16 to 23%, and in western and eastern provinces a high percentage of positive cases (32 to 69%) were noticed.*

Since 1966, cross-bred cattle of two dairy farms in the Karadj district near Tehran, as well as several native herds in the same area, have suffered from a disease manifesting itself sporadically with low morbidity but high mortality. The disease closely resembles mucosal disease and on the basis of pathology and clinical observations it was diagnosed as the first case of bovine virus diarrhoea/mucosal disease (BVD-MD) in Iran (V. Sohrab - unpublished). In May 1969 a severe outbreak occurred in cattle herds around Tehran and at the same time in 3 provinces Isfahan, Kerman and Khorassan. Because of the severity and type of lesions, rinderpest was suspected. Although this diagnosis was confirmed by rinderpest virus isolation (Mirchamsy, Shafyi and Bahrami 1970) there was some suspicion of the possibility of a mixed infection of rinderpest and BVD-DM existing at least in some areas. It then became apparent that information about the incidence of this disease throughout the country should be collected. The present report indicates the results of a serological survey undertaken in an attempt to provide necessary information about the occurrence of BVD-MD in the whole country.

#### **Materials and Methods**

##### **Cattle sera -**

Blood samples were collected from provincial slaughterhouses by district veterinary officers or by staff of the Razi Institute. The serum was separated and forwarded

to the Institute where all samples were clarified and inactivated at 56° C for 30 minutes. Sera were stored at -20° C until used.

### **Cell culture -**

Primary and secondary cell cultures of calf kidney (CK) were grown in standard cell-culture tubes. Growth medium consisted of Melnick medium supplemented with 10% calf serum free from BVD-MD neutralizing antibodies (NA) and the usual concentration of Penicillin-Streptomycin. A confluent cell sheet was normally available 4 days after seeding. Maintenance medium was similar to the growth medium but without calf serum.

### **Virus -**

Strain NADL of BVD-MD, producing distinct cytopathogenic effect (CPE) in CK cells (Fernelius and Ritchie 1966), kindly supplied by Dr A.L. Fernelius, was used in these studies. Virus stock had undergone 3 passages in CK cells and was stored at -65° C.

### **Serum-neutralization test -**

The samples screening test developed by Taylor and Rampton (1968) was used throughout this study. The technique adopted in our laboratory is briefly as follows. Equal volumes of virus suspension diluted in growth medium to yield  $10^{3.0}$  TCID<sub>50</sub> per ml, and undiluted serum, were mixed and held for 2 hours at 4°. 0.2 ml of mixture was inoculated into each of four tubes containing CK monolayers. All tubes were examined after 3 to 4 days. Tubes with CPE were discarded. Final readings were made on the seventh day after infection. If 2 or more tubes of CK cells were protected, the animal was considered to have experienced BVD-MD virus infection. A virus titration was included in each test. Known hyper-immune and susceptible sera were also included in each group of tests. Uninoculated CK cell cultures of the same test were maintained as a check on the presence of contaminant viral agents. Only one stock of NADL virus was used. This stock was titrated 40 times in 5 months. It always showed a titer of  $10^6$  TCID<sub>50</sub> per ml and variation in titre was negligible.

## **Results**

Forty batches of 60 samples were titrated.

The number of samples from each province and the percentage of positive sera are listed in table 1. While bovine sera of western provinces (Azerbaijan and Kermanshah) or eastern provinces (Khorassan, Kerman and Baluchestan) showed a high percentage of positives (32 to 69%) the rate of infection in central provinces Isfahan, Shiraz and Lorestan was much lower (16 to 23%). It is interesting to note that two northern provinces Gilan and Mazandaran, located in south of Caspian Sea as well as southern region Bandar Abbas, in north of Persian Gulf are almost free from the disease. In these areas percentage of positive sera was not exceeding 2.5 to 4%.

Table 1 - The distribution of neutralizing antibodies to  
BVD - MD Virus in the provinces  
of IRAN

Province	Samples Collected	Positive	
		Number	Percentage
Western (Azerbaijan)	285	171	60
Capital (Tehran)	331	190	57.40
Eastern (Mashhad, Kerman and Baluchestan)	390	157	40.25
Western (Kermanshah and Khuzestan)	626	233	37.22
Central (Isfahan, Fars and Lorestan)	477	93	19.49
Southern (Bandar Abbas)	78	3	3.84
Northern (Gilan and Mazandaran)	207	7	3.38
Total	2394	854	35.67

### Discussion

Neutralising antibodies against BVD-MD virus were demonstrated in 854 out of 2394 sera tested. The average incidence of positive cases was 36%. The incidence in cattle approximates countries where the disease has been studied approximates our finding. This was for example 45% in Italy (Scatoza et al 1968), 43% in Belgium (Wellmans and Leunen 1966), 44% in north Germany (Bögel 1966) and 47% in native cattle of Kenya (Taylor and Rampton 1968). Based on the incidence of antibodies the country can be divided into 3 sections. As shown in Fig. 1, all eastern and western boundary provinces are heavily infected. The central section is less contaminated, and finally the Caspian area in the north and Bandar-Abbas in the south, both isolated by chains of mountains from the central plateau, are almost free from the disease. From the present antibody pattern we can assume that the disease is most probably introduced into Iran via the eastern and western frontiers. This is supported by the fact that there is a continuous importation of cattle and sheep from both sides. The imported animals are mostly directed to Tehran slaughterhouse. There are, on the other hand, many natural pasture lands in our eastern and western frontiers where cattle from both sides of the frontier graze during day and are brought back to their respective villages at night. It is in practice, thus, very difficult to differentiate animals of each country during the day in the grazing grounds. The central part of Iran is in the same way contaminated because it is not uncommon to see that dealers sometimes prefer to send the imported cattle to central areas where they are sold to local dealers who distribute them throughout the whole region. It is worth mentioning that Gilan and Mazandaran are rich in grazing grounds and are one of the main centers of cattle breeding in Iran. These provinces also export cattle to Tehran and to southern areas without receiving

imported animals. Bandar-Abbas and other southern districts in Persian Gulf are isolated from the central part of the country by a chain of high mountains. Their bovine population is not important, just covering local need. These areas are also excluded from imported cattle.

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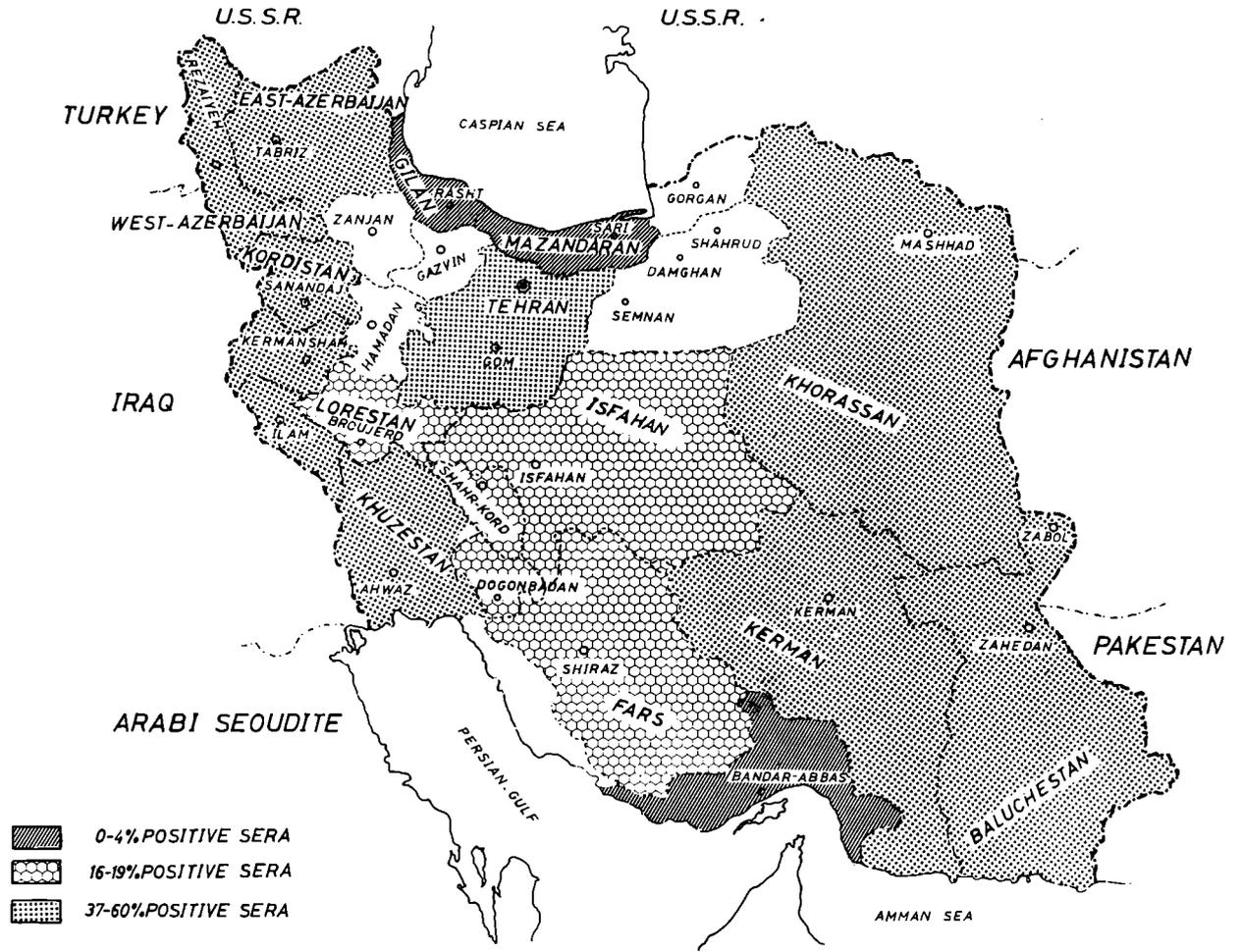


Fig. I-Distribution of bovine diarrhoea-mucosal disease in IRAN