Seroepidemiology of *Rinderpest* and *Peste des Petite Ruminants* in Sheep and Goats in Iran

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Summary
Serological studies, on sheep and goats sera collected from different parts of the country, were conducted to detect antibodies against rinderpest (RP) and peste des petite ruminants *(PPR). As a general policy, sheep and goats are not vaccinated against rinderpest in Iran and, therefore, these animals should not carry antibodies against the virus. Serum samples were collected both from regions affected and non-affected by RP. Serum neutralisation (SN) test detected the positive sera and the competative ELISA test, using monoclonal antibody developed by Pirbright Research Institute (UK), differenciated between PPR and RP. This communication records, for the first time, the presence of PPR antibodies in sheep and goats in Iran.*

Introduction

During the past few decades expansion of animal husbandary industries and the increase of imports and exports of farm animals between Africa and Asia have resulted in, from time to time, African animal diseases transgress the geographical boundries and spread, particularly, to the Middle East where such exotic viruses cause outbreaks and death in animals. The outbreaks of SAT1 type of foot and mouth disease in 1963 is a good example.

RP or cattle plague can be a calamity for livestock and decimate the whole cattle population of an affected area. Sheep and goats are not usually affected severely by RP virus, although studies in India have shown that severe infection may occur in these animals(1). Outbreaks of RP had

not been reported in Iran for 12 years untill 1982, when unexpectedly the disease flared up, at the same time, in Meshad and Tehran. However, the outbreaks came undercontrol, in no time, by utilising quarantine measures and mass vaccination by cell culture vaccine(2,3,4).

From 1987 to 1989 several minor RP outbreaks occurred in south west and north of the country (Internal reports). These outbreaks were confirmed by cell culture virus isolation and susceptible calf inoculations.

So far, there has been no report, either clinical or serological, to indicate the occurrence of PPR in this country. In our serological survey on RP, the detection of high neutralising antibody titres in sheep and goats sera against RP virus made us suspicious of the presence of *Morbilli* virus in these ruminants. Reports on the presence of PPR like disease in sheep of central Soudi Arabia and Sultanate of Oman helped consolidate our suspicion(5, 6).

The present paper reports the results of serological studies, to detect antibodies to PPR, in sheep and goats in Iran.

Materials and methods

Cell culture: Monolayer of bovine embryo kidney (BEK) cell line was used for virus propagation and neutralisation tests. Cells were grown in an ELY medium (Earles balanced salt solution, lactalbumin hydrolysate and yeast extract) containing 5% inactivated foetal calf serum, 200 units penicillin, 200 mg streptomycin and 50 units Fungizon per l.

Sera: 2536 blood samples were obtained from apparently healthy sheep and goats of various parts of the country. Sera were separated from blood samples under sterile conditions and kept frozen at -20°C until used.

Virus: Rinderpest virus (Plowright strain P:104) was used in these studies. To prepare the virus stock BEK cells were inoculated with the virus and incubated at 37°C for 2 h. After absorbtion period, the infected cells were overlaid with ELY medium, fortified with 2 percent foetal calf serum, and incubated until the CPE was almost complete. The infected fluid along with cells were then harvested. After freezing and thawing, cell debris were sedimented by low speed centrifugation and the supernatant was stored at -70°C until required.

To detect the RP virus, antibody serum neutralisation test was performed. Briefly, two-fold dilutions in ELY of the inactivated serum

samples were mixed with equal volumes of virus suspension containing 100-200 $TCID_{50}$ of virus per 0.1 ml. The virus-serum mixtures were incubated at 4°C overnight 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 absorption period of 2 h, 1.5 ml of ELY was added to each tube and the cells were reincubated at 37°C. Virus inoculum was titrated simultaneously.

The cultures were examined daily for CPE for 5 days. Each serum that completely inhibited CPE in both cell culture tubes was taken to be positive. For confirmation, positive sera were submitted to Pirbright Institute in U.K. for further tests.

Results

Out of 2536 serum samples, 986 sera had titre of $\frac{1}{2}$ and 592 sera had a titre of $\frac{1}{10}$ against rinderpest virus in seroneutralisation test (Table 1). A part of 592 sera which had a titre of 1:10 or higher were tested, at Pirbright Institute (UK) by competetive ELISA test, for presence of antibodies against PPR and RP. The results showed that all samples were negative for rinderpest virus while they revealed positive reaction against PPR.

Discussion

During the 1969-rinderpest-outbreaks in Iran and since then no clinical RP infection has been observed in sheep and goats. In the absence of RP outbreaks, the presence of high serum neutralising antibodies against RP virus in small ruminants that had not been vaccinated were good reasons to justify investigating whether the neutralising antibodies belonged to RP or other related viruses.

Unlike virus neutralisation, the competative ELISA test can detect specific RP virus antibodies without showing a cross reaction with antibodies to PPR virus(7).

Beaton(8) suggested that, in Africa, RP in sheep and goats was rare. Regig et al.(9) have seen rinderpest-like disease in goats and sheep in Saudi Arabia. Also, Lefevre(1) in his publication reported that "Small ruminants are also susceptible to rinderpest virus and may become, from time to time, infected."

It is clear now there is serological relationship between PPR and rinderpest viruses(1). Furthermore, the report from Sudan on outbreaks of a disease in goats during 1971-2, originally diagnosed as rinderpest, is now confirmed to have been *peste des petite ruminants*(2, 4).

Our preliminary study which have shown the presence of antibody to PPR virus in sheep and goats was a lead to suspect the presence of this disease among sheep and goats in Iran.

ELISA, using monoclonal antibodies(7), proved to be an excellent technique to differenciate between antibodies to PPR and RP, because, examined serum samples gave negative results to RP but positive reaction to PPR.

It is worth mentioning that, apparently, Iranian fat-tailed sheep are not so susceptible to PPR virus to manifest clinical symptoms as African races do.

Table 1. Results of seroneutralisation test:

serial no.	Province	sheep & goats sera	positive $\frac{1}{2}$ dilu	positive $\frac{1}{10}$ dilu	percent positive 1:2	percent positive 1:10
1	Fars	149	118	98	79	65
2	Hamedan	514	306	209	60	41
3	Kermanshah	389	226	182	58	47
4	Lorestan	170	11	5	6	3
5	E. Azarbaijan	50	5	2	10	4
6	W. Azarbaijan	50	31	23	62	46
7	Ilam	174	7	2	4	1
8	Khozestan	340	160	54	46	16
9	Zanjan	160	2	0	1	0
10	Boushehr	15	4	1	8	2
11	Gorgan	53	14	9	26	17
12	Blouchestan	43	6	3	14	7
13	Mazandaran	429	96	2	22	0.004
	Total	2536	986	592	39%	13%

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