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SEROLOGICAL SURVEY OF SHEEP IN IRAN FOR TYPE SPECIFIC ANTIBODY TO BLUETONGUE VIRUS

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Summary

The results of a serological survey of sheep in different parts of Iran for group and type specific antibody to bluetongue virus (BTV) are reported using the agar gel immunodiffusion test (AGID) and microneutralization tests with the international serotypes 1 to 24. Analysis of the data indicated that during 1983-1987 types 3,7, 20, 22 and also untyped viruses (possibly EHD) or viruses closely related to them were infecting sheep in this region of the world.

Introduction

Bluetongue is an arthropod borne disease that usually affects sheep but has also been recorded in cattle, deer and other ruminants (Verwoerd and others, 1979; Sellers, 1981) in some areas of the world. Generally infection with BTV causes no clinical disease (in Austria, the Caribbean and south America) although the epidemic potential of the virus has been demonstrated on several occasions, for instance in 1956-57 when large number of sheep died in Spain and Portugal due to bluetongue disease. Consequently, severe restriction has been imposed on the international movement of breeding livestock from countries with bluetongue virus (Gibbs, 1981).

To the authors knowledge, bluetongue disease has never been confirmed by virus isolation in Iran but serological evidence indicates that the virus has been present, at least since 1972. At that time a total of 586 serum samples tested by immunodiffusion from different regions showed that 28 per cent of sheep were AGID positive (unpublished data). This event drew our attention to the incomplete knowledge of our information on the epidemiology of bluetongue virus in Iran.

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Several unsuccessful attempts have been made to isolate the causative agent from clinically suspected animals but the isolates were not bluetongue virus. For this reason a serological survey was conducted from 1983 till 1987. The tests used for this survey were the bluetongue AGID test (Pearson and Jochim, 1979) which detects antibody elicited by previous infection with bluetongue virus but gives no information on the serotypes of virus present in the survey area; in addition positive sera were examined with a microneutralization test which can indicate the serotype of the virus circulating and infecting the sheep. In this survey sera collection was done from traditional farms in different regions of the country, except the northern and central areas in which bluetongue infection is very low or negligible.

Materials and Methods

Sera:

920 indigenous sheep sera were collected from different parts of the country between 1983 and 1987. The majority of the sera were from animals aged not older than 18 months. All sera were collected from late spring untill the end of summer corresponding to the insect breeding season.

AGID tests

All sera were examined by the agar gel immunodiffusion test for antibody to bluetongue virus using an antigen prepared from BTV 20 according to the method described by Pearson and Jochim (1979).

Serum Neutralization test:

From 237 AGP positive samples, 59 sera were selected representing almost all suspected counties. Each of the sera was examined in a microneutralization system against international bluetongue virus serotypes 1-24 using the method described by Herniman and others (1983).

Results:

The overall distribution of the test samples and the percentage of positive sera are shown in table 1.

In 1983 twenty two AGID positive sheep sera from western counties (Ilam-Bakhtaran) were selected for microneutralization test against all 22 available bluetongue serotypes. In each instance virtually

no neutralization was obtained suggesting, perhaps, the presence of a new serotype in Iran. Two animals showed some neutralization to type 22, which had previously only been detected in Nigeria with a level of 1:30 in one, which is scarcely significant, or a level of 1:60. As all other sheep are negative we think it is difficult to conclude that either sheep Nos. 11, 14 were infected with type 22. It is more probable that these are cross neutralizing antibodies stimulated by infection with an unknown type (Table 2).

In 1986 antibody levels in 22 selected AGID positive sera from south-western and south-eastern counties were estimated by microneutralization tests using bluetongue serotypees 1-22. Using the cluster technique (Taylor and others, 1985a) the results indicate that serotypes 3, 7, 20, 22 were circulating in south-western counties and serotypes 3, 20, 22 were circulating in south-eastern counties (Table 3). In 1987 fifteen AGID positive sheep sera from north-western and north-eastern areas were examined by microneutralization test against bluetongue serotypes 1-24. Again clusters were found, this time with only types 3 and 23 (Table 4).

Discussion

The prefred method for identifying the various serotypes of bluetongue virus active in an area is to isolate viruses, however this approach is expensive and involves establishing sentinel herds when clinical disease is not occurring regularly in the area. For virus isolation, the best way is to select a group of young sheep or lambs and bleed them every month until they convert from AGID negative to AGID positive allowing the observer to stimate the month in which [illegible] Armed with this knowledge it might then be possible to isolate the causative agent.

At the present time, due to lack of sufficient laboratory facilities in remote areas, isolating the virus is not possible. For this reason and in the absence of clinical outbreaks of disease a serological survey was carried out to indicate the serotypes of bluetongue virus present in Iran.

The result of microneutralization tests on 22 sheep sera from south-eastern and south-western areas reveals that types 3, 7, 20 22 and types 3, 20, 22 were present in south-Western and eastern counties respectively in 1986. By looking at the results in table 3, we would prefer to exclude the results from sheep numbers 26 and 42 as they probably come from animals that have been stimulated to produce more broadly cross-reactive responses than the remainder. If

we then group other sera together, irrespective of region, 17 samples have experienced type 3, type 7 and 9 each of types 20 and 22. The frequency against other types are much lower and it is in contrast with these results that constitute clusters and on which we would base and ascertain that types 3, 7, 20 and 22 have been circulating. At a regional level we think there are clusters to types 3, 7, 20 and 21 in the south-western region and to types 3, 20 and 22 in south-eastern region.

The result of our microneutralization tests from animals in north-eastern and north-western regions in 1987 shows the presence of bluetongue type-speecific neutralizing antibody clusters to types 3 and 23 though not to 7, 20 or 22.

Previous studies have suggested the presence of unknown serotypes of bluetongue in Syria (Taylor et al., 1985b) or Saudi Arabia (Hofez and Taylor, 1985). But in a recent reappraisal of results obtained in Syria (Taylor, 1987) the failure to detect type specific antibodies is ascribed to occasional low responsiveness of sheep sera to BTV coupled to the high threshold level imposed by the test. This, to some extent, explain the failure to detect antibody clusters in the samples collected in 1983. Now, further studies of the epidemiology of bluetongue virus and related orbiviruses in Iran are required to confirm and extend the present observations. Our next step would be to try to isolate the bluetongue virus or any other viruses which could produce apparent clinical symptoms of disease among indigenous sheep in Iran.

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Refernces:

Verwoerd D.W. Huimans H & Erasmus B.J. (1979). Comprehensive Virology Eds. Frrenkel-Conrat and R.R. Wagner New York. Plenum Press. 5.258.

Pearson J.E. & Jochim M.M. (1979) American Association of Veterinary Laboratory Diagnosticians 22nd annual proceedings p. 463.

Sellers R.F. (1981) Virus disease of food animals Ed. E.P.J. Bibbs London Academic Press P. 567.

Gibbs E.P.J. (1981) Advances in Veterinary Sciences and Comparative Medicine 25, 71.

Herniman K.A.J. Boorman J.P.T. & Taylor W.P. (1983) Journal of Cambridge 90, 171.

Taylor, W.P. Gumm, I.D., Gibbs, E.P.J. and Homan, J. (1985a) In "Bluetongue and Related Orbiviruses" ed. Barber, T.L. and Jochim M.M. pub, Alan R. Liss, Inc., New York. pp. 527-530.

Hafez, S.M. and Taylor, W.P. (1985) In "Bluetongue and Related Orbiviruses" ed Barber, T.L. and Jochim M.M. pub. Alan R. Liss, Inc. New York. pp 531-537.

Taylor, W.P. (1987) Bluetongue in Syria and Jordan. (in Press).

Table 1. The distribution of AGID positive sheep sera in different counties of Iran.

Counties	No. of sera.	No. of Positive	Positive Percentage
Western and North Western	443	150	34
Eastern and North Eastern	272	56	20
South Western and South Eastern	205	31	15

Table 2. Neutralizing antibodies to BTV serotypes in positive sera collected from Ilam and Bakhtaran in 1983.

	Serotypes of Bluetongue virus used																					
No sheep	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	* -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* No neutralizing antibody detected

Table 3. Reciprocal antibody titres to serotypes of bluetongue virus found in sheep in south-western and south-eastern counties of Iran in 1986.

		Bluetongue virus serotypes																				
	No. of Sheep	1	2	3 +	4	5	6	7 +	8	9	10	11	12	13	14	15	16	17	18	19	20 +	21	22 +
South Western	23	–	–	40	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–
	24	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25	10	–	30	–	–	–	–	–	–	10	–	–	10	–	–	–	–	–	–	10	–	10
	26	–	–	10	–	–	–	40	–	560	560	560	560	560	560	560	–	–	–	60	–	–	–
	27	–	–	30	–	–	–	10	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	28	–	–	30	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	60	–	–
	29	–	–	30	–	–	–	10	–	10	–	–	–	–	–	–	–	–	–	–	10	–	10
	30	–	–	60	–	–	–	30	–	–	10	–	10	–	–	–	–	–	–	–	–	–	–
	31	–	–	30	–	–	–	10	–	–	–	–	10	–	–	–	–	–	–	–	–	–	10
	32	–	–	40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
	33	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	35	–	–	40	–	–	–	60	10	–	–	–	–	–	–	–	–	–	–	30	40	–	10
South Eastern	36	10	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	37	–	–	60	–	–	–	10	10	30	–	–	–	–	–	30	–	–	–	10	60	–	80
	38	–	–	–	–	–	–	–	–	–	10	30	10	–	–	–	–	–	–	30	10	–	–
	39	–	–	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30
	40	–	–	40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30	–	10
	41	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	30	–	40
	42	–	–	40	–	–	–	30	10	–	–	560	–	530	–	560	–	–	–	40	–	–	80
	43	–	–	30	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	44	–	–	40	–	–	–	–	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–

+ Figures in columns Nos. 3, 7, 20 22 representing the antibody clusters

Table 4. The result of Microneutralization test from north- western and north-eastern counties of Iran in 1987.

	Bluetongue virus serotypes																							
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	-
46	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	-
47	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	30	-
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-
52	-	-	10	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-
53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10
54	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-
58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figures indicates reciprocal antibody titre to bl uetongue virus.