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Serological Evidence for the Occurrence and Prevalence of Bluetongue Among Ruminants in Saudi Arabia

S.M. Hafez, A.I. Radwan, S.I. Bekairi and A.A. Al-Mukayel

Animal Production and Health Section, Regional Agriculture and Water Research Center, Ministry of Agriculture and Water, P.O. Box 17285, Riyadh 11484, Saudi Arabia

ABSTRACT. The agar gel double diffusion technique was used to test for bluetongue precipitating antibody in serum samples collected from ruminants in Saudi Arabia. Of 560 sheep serum samples, 61 goat serum samples, 112 cattle serum samples and 3 camel serum samples from 6 different localities in the Kingdom, 336 (60%), 26 (43%), 20 (18%) and 2 (67%) samples, respectively, contained bluetongue antibody. This is the first report on the occurrence of bluetongue in Saudi Arabia.

In October 1980, a syndrome similar to bluetongue (BT) was reported among Najdi Sheep in Al-Kharj area near Riyadh (Chang Chen and Asmar 1980). However, no virus could be isolated from samples which were collected from sick animals and sent to the Animal Virus Research Institute, Pirbright, United Kingdom. No convalescent sera could be collected from the surviving animals in 1980 for serological diagnosis because of owner resistance.

This paper describes preliminary results concerning the detection of BT precipitating antibody in serum samples from sheep, goats, cattle and camels from various locations in Saudi Arabia (Fig. 1).

Material and Methods

Antigens

The BT precipitating antigen was prepared from African Green Monkey Kidney (VERO) cells infected with type 10 BT virus. The antigen was harvested and concentrated by freezing and thawing the infected cell sediment and precipitating the proteins of the supernatant fluid with ammonium sulphate as previously de-

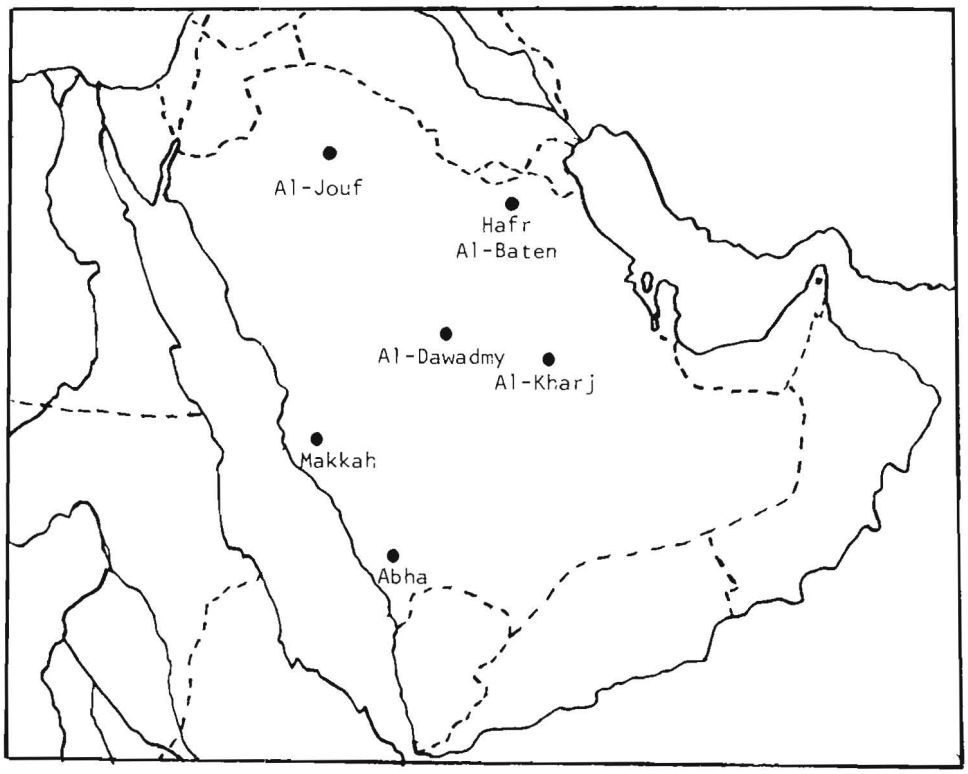

Fig. 1. A map of the Kingdom of Saudi Arabia illustrating the locations from which serum samples were collected.

scribed for porcine adenovirus antigen (Hafez and Liess 1980). Control negative antigen was prepared from non-infected VERO cell cultures in the same manner.

Reference and Test Sera

Limited amounts of BT hyperimmune sheep serum and normal sheep serum free from antibody against BT virus were provided by the Animal Virus Research Institute, Pirbright, United Kingdom, and the National Animal Disease Laboratory, Ames, Iowa, U.S.A.

Test serum samples were collected between November 1977 and March 1982 for a serological survey of brucellosis in Saudi Arabia (Radwan *et al.*, 1983) and were kept frozen at −20°C. The serum samples were used for immunodiffusion tests without heat inactivation. Table 1 shows the location and animal species from which the sera were collected, the dates of collection and the number of samples tested. Figure 1 shows the places from which serum samples were collected.

Table 1. Detection of bluetongue precipitating antibody in serum samples of sheep, goats, cattle and camels collected from different localities in Saudi Arabia.

Place	Date of Collection of Samples	Animal species											
		Sheep			Goats			Cattle			Camels		
		No. of Samples Tested	Positive Samples		No. of Samples Tested	Positive Samples		No. of Samples Tested	Positive Samples		No. of Samples Tested	Positive Samples	
			No.	%		No.	%		No.	%		No.	%
Makkah[1]	Nov. 1977	129	116	90	37	22	59	22	14	64	2	2	100
Abha	Nov. 1982	94	3	3	1	0	0	–	–	–	–	–	–
Al-Jouf	May 1981	78	32	41	22	4	18	–	–	–	–	–	–
Al-Dawadmy	Jan. 1982	99	79	80	1	0	0	–	–	–	–	–	–
Hafr Al-Baten	Dec. 1981	100	78	78	–	–	–	–	–	–	–	–	–
Al-Kharj	June 1978	60	28	47	–	–	–	39[2]	6	15	1	0	0
	March 1982	–	–	–	–	–	–	51[2]	0	0	–	–	–
TOTAL		560	336	60	61	26	43	112	20	18	3	2	67

1. Serum samples were collected from animals sacrificed during the Hajj Season which included animals imported from Africa and Australia.
2. Serum samples were collected from dairy cows imported from U.S.A.

Immunodiffusion Tests

The tests were carried out in Petri dishes of 10 cm diameter. Each plate contained 25 ml of diffusion medium consisting of 1% Agarose A (Pharmacia Fine Chemicals, Denmark) in physiological saline with 0.01% sodium merthiolate as a bacteriostat. Four sets of wells, 6 mm in diameter, were punched in every plate. Each set consisted of a central well and 6 peripheral wells 3 mm apart. Antigen was placed in the central well and test sera were placed in the peripheral wells. Plates were incubated at 20°C temperature in a humidified atmosphere and examined daily for precipitin lines using indirect lighting in a dark room. All positive sera were retested against negative control antigen.

Results

Testing the Precipitating Antigens

Precipitin lines produced by the cell associated and extracellular antigens were convergent with each other when tested in adjacent wells against immune serum. No lines were formed when these antigens were tested against negative control serum or when the negative antigens were tested against the BT immune serum.

Serological findings

Complete precipitin lines of varying intensity were formed between the wells containing the BT precipitating antigen and some test sera. Other sera did not form complete lines and only clear spurs originating from the ends of the precipitin lines produced by adjacent sera were seen. The lengths of the spurs varied between 1 and 3 mm. These serum samples which formed only spurs were considered positive also. None of the reacting sera formed any precipitin lines when retested with the negative control antigen. The numbers and percentages of positively reacting sera are shown in Table 1. Reactors were detected in all 6 test sites in widely scattered locations in Saudi Arabia and in all 4 of the domestic ruminant species examined (sheep 60%, goats 43%, cattle 18% and in 2 of 3 camels).

Discussion

Since we did not have sufficient quantities of reference positive serum, it was not included for the examination of field serum samples as described in previous studies (Hafez and Ozawa 1973, Hafez 1978). Under such conditions, it was impossible to strengthen the reaction of field sera by the recruiting effect of positive serum placed in adjacent wells. However, this phenomenon was occasionally observed when a strongly positive field serum sample was examined in a well adjacent to a weakly reacting serum. The number of positive sera in this study would probably be increased if each field serum sample were to be placed in a well adjacent

to a positive reference serum. Nevertheless, the numbers and percentages of positive sera detected in this study are sufficient to confirm the occurrence of BT in Saudi Arabia and give an indication of its prevalence. The absence of any vaccination program against BT in Saudi Arabia suggests that precipitating antibody among Saudi animals had arisen as a consequence of either natural infection or of passive immunization with maternal antibody.

The occurrence of BT has been reported in Jordan and Iraq (Hafez 1978), Iran (Afshar and Kayvanfar 1974), Oman and Yemen (Anon 1980), Sudan (Eisa *et al.* 1979), Egypt (Hafez and Ozawa 1973) and Palestine (Howell 1963), eight countries surrounding Saudi Arabia. In addition, *Culicoides* spp., the arthropod vectors of BT virus, have been detected in Saudi Arabia (W. Büttiker, personal communication). Accordingly, the serologic confirmation of the occurrence of BT in Saudi Arabia is not surprising.

Importation of animals to Saudi Arabia for breeding, the dairy industry and meat consumption has increased considerably in recent years. Most dairy cattle are imported from the U.S.A., while slaughter animals are usually imported from the East African countries, India and Australia. Bluetongue occurs in all the above countries. The BT viremic phase is quite long, even in the presence of humoral antibody (Luedke 1970). It could be possible that some of the imported animals might be viremic upon their arrival to Saudi Arabia and if exposed to bites by *Culicoides* or other vector, the virus serotypes they are carrying will be distributed. Therefore, there are continuing opportunities for introducing new BT virus strains having biological and antigenic characters different from the local strains. In this connection, it is worthy of note that 34 serum samples collected from Merino sheep imported from Australia and slaughtered in Makkah in 1977 were all positive for BT precipitating antibody.

The use of type specific serologic tests will help to identify locally prevalent serotypes; these can be compared with the BT virus serotypes present in countries from which animals are currently imported into Saudi Arabia. In addition, studies on the epizootiology and pathogenesis of BT infection among different animal species and breeds in Saudi Arabia are needed.

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الإثبات السيرولوجي لوجود وانتشار مرض اللسان الأزرق في المملكة العربية السودية

سمير محمد حافظ، أحمد إبراهيم رضوان، سعد البكيرى وعبد الله المقيل

قسم إنتاج وصحة الحيوان - المركز الإقليمي لأبحاث الزراعة والمياه - وزارة الزراعة والمياه - الرياض - المملكه العربية السعودية

لقد تم استخدام اختبار الانتشار الترسيبي في الآجار للكشف عن الأجسام المناعية لفيروس اللسان الأزرق في عينات السيرم المجموعة من الحيوانات المجترة بالمملكة العربيه السعودية. وتم فحص ٥٦٠ عينة سيرم مجموعة من أغنام، ٦١ من ماعز، ١١٢ من أبقار، ٣ من جمال من ٦ مناطق مختلفة بالمملكة ووجد أن ٣٣٦ (٦٠٪)، ٢٦ (٤٣٪)، ٢٠ (١٨٪) ٢ (٦٦٪) من هذه العينات على التوالي تحتوى على أجسام مناعية. وهذه الدراسة تعتبر الأولى لأثبات وجود مرض اللسان الأزرق بالمملكه العربية السعودية.