A study on bluetongue virus infection in Saudi Arabia using sentinel ruminants

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ABSTRACT

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Four sentinel herds comprising cattle, sheep and goats were established at various localities in Saudi Arabia. Maternal bluetongue antibodies were detected in all four sentinel herds but disappeared in 4–6 months, immediately followed by seroconversion in all. Serological results indicated that the animals were recently exposed to BT virus serotypes 10, 12, 15 and 20. The epidemiology of the disease in Saudi Arabia is discussed.

Keywords: Bluetongue virus, Saudi Arabia

INTRODUCTION

Bluetongue (BT) is an infectious, non-contagious arboviral disease of ruminants caused by an *Orbivirus* of the family Reoviridae. Twenty-four virus serotypes are presently recognized. The virus is vectored by several species of *Culicoides* midges.

The existence of BT and related *Orbivirus* activity in Saudi Arabia and surrounding countries has been evidenced by both seroconversion (Hafez & Taylor 1984; Herniman, Gumm, Owen, Taylor & Sellers 1980; Taylor, Al-Busaidy & Mellor 1991; Afshar & Kayvanfar 1974; Al-Busaidy & Mellor 1991) and virus isolation from overt clinical disease (Soliman, Hafez & Ozawa 1972; Abu Elzein & Tageldin 1985; Abu Elzein, Gameel, Al-Afaleq & Hassanien 1992).

This paper reports on a comprehensive investigation of the epidemiology of BT in ruminants in Saudi Arabia by making use of sentinel herds.

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MATERIALS AND METHODS

Establishment of sentinel herds

Four sentinel herds were established for the study. Two herds were at the Al-Ahsa oasis in the Eastern region (a calf herd and a sheep and goat herd), one sheep herd at Abha (South Western region) and one calf herd at Al-Kharj (Central region).

The reasons behind choosing these localities were that both Al-Ahsa and Al-Kharj are oases with an abundance of water (Al-Ahsa being the largest oasis in the country), and that most of the dairy farms of the Kingdom are located there. In addition, preliminary studies indicated the presence of several species of *Culicoides* midges in these two oases (Hilali, unpublished data 1993). Abha was chosen because of a previous incursion of African horsesickness (Anderson, Mellor & Hamblin 1989) and it was thought that arbovirus-infected *Culicoides* might be blown from the horn of Africa into this area.

Al-Ahsa sentinel calf herd

Forty-four sentinel calves were used in the study. All were born from Holstein-Friesian parents. The parents themselves were born and reared on the King

Faisal University Farm at Al-Ahsa. The sentinel calves were born on different dates between January 1993 and June 1994 and were located on this farm.

Al-Kharj sentinel calf herd

This herd was established at a private dairy farm. It comprised 23 calves which were born in April 1993.

Al-Ahsa sentinel sheep and goat herds

Forty-eight lambs born between October 1992 and January 1993 at King Faisal University Farm at Al-Ahsa were used.

Abha sentinel sheep herd

This herd consisted of 15 sheep and goats located at a private farm at Abha. Each sentinel animal was bled monthly for 12 months in order to test for maternal BT antibodies and seroconversion (Mohamed & Taylor 1987).

All the sentinel animals were placed under close clinical observation for signs of BT infection.

Preliminary sero-surveillance for BT virus antibodies on the farms where the sentinel animals were kept

In order to establish what the BT situation was on the farms where the sentinel animals were housed, sera were collected from adult cattle, sheep and goats from the farms at Al-Kharj, Al-Ahsa and Abha. The sera were inactivated at 56°C for 30 min and stored at −20°C until tested in the agar gel immunodiffusion (AGID) test using the method of Mohamed & Taylor (1987). The test was conducted on microscope slides coated with 2 mℓ of 1,2 % Noble agar in borate buffer pH 9,2. Soluble BT virus (BTV) antigen (obtained from Pirbright Laboratories, UK) was added to the central well and alternating peripheral wells received test and positive control serum respectively. Negative control antigen (obtained from Pirbright Laboratories, UK) prepared from uninfected baby hamster kidney (BHK)-21 cells was used. Slides were incubated in a humid chamber at room temperature and examined daily for 3 d when final reactions were recorded.

Detection of BTV group-specific antibodies in sera collected from sentinel animals

The standard micro AGID test was used as described above (Mohamed & Taylor 1987). Controls were included using BHK-21 non-inoculated cell cultures as mock antigen. Sheep hyperimmune serum prepared against the BT type 4 virus was used as reference antiserum (obtained from Pirbright Laboratories, UK).

Determination of the type-specificity of BTV antibodies in sentinel animals

The sera from sentinel herds which were BTV positive in the AGID tests were examined for BTV type-specificity using serum neutralization tests (SNT). The standard micro SNT method, routinely performed at the OIE World Reference Laboratory for Orbiviruses at Onderstepoort, South Africa, was used to test for the international BTV serotypes 1–24. Two-fold serum dilutions were made in flat-bottomed cell culture microtitre plates in phosphate buffered saline (PBS) pH 7,4 containing 0,2 % (V/V) bovine plasma albumin (PBS-BA) and inactivated at 56°C for half an hour. To each well an equal volume (50 µℓ) of virus diluted in PBS-BA pH 7,4 to contain $10^{3,0}$ TCID$_{50}$/mℓ was added.

The plates were then sealed and incubated for 1 h at 37°C and overnight at 4°C. The following morning BHK cells suspended in Glasgow modified Eagle's medium, supplemented with 10% tryptose phosphate broth and 2% bovine serum, were added to each well in 0,05 mℓ volumes containing $2,5 \times 10^4$ cells. Plates were resealed and incubated at 37°C for a further 5 d. Microscopic readings were made at 3 and 5 d, and titres scored.

RESULTS

Clinical observations on the sentinel animals and their herd-mates

Al-Ahsa sentinel cattle herd

Adult cattle and sentinel calves did not show any clinical signs suggestive of bluetongue infection. However, calves born from Friesian dams, showed malformations of their feet, manifested by straight or outwardly bent lower limbs (Fig. 1). This deformation was evident in all of them. Fourteen calves were born dead and had the same type of deformation. The calves from local zebu parents (Hasawi) were born normal. Two abortions were recorded on this farm during the period of study.

Al-Kharj sentinel cattle herd

No clinical signs suggestive of BTV infection were seen in the sentinel calves or their adult herd-mates. Of the 30 calves, 26 were normal, three were born with deformities of all four feet and one abortion was recorded.

Sentinel sheep herds at Al-Ahsa and Abha

No clinical signs suggestive of BTV infection were observed in the sentinel animals at Al-Ahsa or Abha. All the newly-born lambs or kids were normal, except one lamb, in the Al-Ahsa sheep herd, which showed malformations of all four feet.

Results of the preliminary sero-surveillance for BTV antibodies on the farms where the sentinel herds were established

Table 1 summarizes the results of the preliminary survey for BTV antibodies in the non-sentinel adult cattle, sheep and goats resident with the sentinel animals on the respective farms. At the Al-Ahsa farm 100 % of the animals were positive whereas at Al-Kharj 63,4 % of the cattle and at Abha 35,5 % of the sheep tested positive.

Seroconversion in sentinel animals

The maternal immunity in the Al-Ahsa sentinel sheep disappeared between 2 and 6 months after birth and seroconversion was detected in all animals during the following month (Table 2).

The calves in the Al-Ahsa sentinel herd were born sporadically throughout the period between January 1993 and June 1994. All calves received maternal colostral antibodies which was lost in 3–5 months. Seroconversion was observed in all cases within 1 month after the disappearance of maternal antibodies (Table 2).

All calves in the Al-Kharj sentinel herd were born in April 1993. Fifteen of these received antibodies from their dams (65 %); and eight were seronegative (35 %). These eight calves seroconverted in June 1993 (at the age of 2 months) and remained so until the end of the study on that farm. Of the 15 calves which received maternal antibodies, two lost their antibodies by the age of 3 months (July 1993) and immediately seroconverted in August 1993. The remaining 13 calves lost their maternal antibodies at the age of 6 months (October 1993) and immediately seroconverted in November 1993 (Table 3).

In the Abha sentinel sheep herd all the animals had maternal antibodies. Waning of the maternal antibodies was detected in 80 % of the animals by the sixth month, and seroconversion was achieved within the following month by each animal. The others continued to be seropositive for the whole period of the study.

Detection of BTV type-specific antibodies

Serum samples from the sentinel calves, lambs and kids which were seropositive in the AGID test, were sent to the OIE World Reference Laboratory at Onderstepoort for serotyping.

The cumulative results confirmed the presence of antibodies against BTV serotypes 1–7, 10–13, 15 and 17–24; and complete absence of antibodies against BTV serotypes 8, 9, 14 and 16 (Table 4). As can be seen, only antibodies against BTV serotypes 1, 4, 12, 15, 18, 20 and 22–24 could be detected in the sera of Abha sentinel sheep. In Al-Ahsa sentinel sheep, only antibodies against the BTV serotypes 5,

6, 10, 12 and 20–24 were detected. In Al-Ahsa cattle, antibodies against BTV serotypes 1–6, 10–13, 15 and 17–24 were detected. At Al-Kharj, only antibodies against BTV serotypes 2, 4, 6, 7, 10, 12, 15 and 20–24 were detected in the sentinel calves.

Fig. 2 illustrates the prevalence in cattle sera (Al-Ahsa and Al Kharj farms) of type-specific antibodies against the different BTV serotypes. The highest prevalence was seen for types 20, 12 and 10 followed by serotype 15. No antibodies to BTV serotypes 8, 9, 14 and 16 were detected in cattle sera and a low prevalence of antibodies was seen for the rest of the virus serotypes.

Fig. 3 illustrates the distribution of type-specific BTV antibodies in sheep sera (Abha and Al-Ahsa). No antibodies against BTV serotypes 2, 3, 7–9, 11, 13, 14, 16, 17 and 19 were detected in sheep sera. Again the highest prevalence was seen in antibodies against BTV serotypes 20, 15, 10 and 12, with a low prevalence of antibodies against BTV serotypes 1, 4–6, 18 and 21–24.

At Al-Ahsa, monospecific reactions were detected against BTV serotypes 10, 12, 15 and 20 in calves and against BTV serotypes 10, 12 and 20 in sheep. In the Al-Kharj cattle herd monospecific reactivity was found against BTV serotypes 2 and 20; while Abha sheep showed monospecific reactivity against BTV types 15, 20 and 22 (Fig. 4).

DISCUSSION

The clinical examination of the sentinel animals and their herd-mates, throughout the period of study, did not reveal signs of BTV infection. However, deformities were continuously seen in all the Friesian sentinel calves born at Al-Ahsa and to a lesser extent at Al-Kharj. The deformities were identical in all cases, involving mainly the limbs from the knee joint and downwards. Interestingly, all the calves born during the period of study from native zebu parents at Al-Ahsa farm did not show such abnormalities. This might indicate that exotic cattle breeds are more prone to BT infection than the native cattle kept under the same conditions.

Developmental anomalies such as arthrogryposis, hydranencephaly and vertebral column abnormalities (Bowne, Luedke, Jochim & Metcalf 1968; Luedke, Jochim, Bowne & Jones 1970; Leindo & Castro 1981) were not seen in any of the sentinel calves or lambs or their herd-mates in this study. However, some abortions and still-births were seen at a low frequency at Al-Ahsa and Al-Kharj in the cattle, but not in the sheep herds.

According to the veterinarians of Al-Ahsa sheep farm (Dr Gasim, personal communication 1991), the farm records did not reveal any malformations during the

previous 5 years. However, one lamb of the Hijazi breed was born deformed during the study period. A BTV was isolated from it (and the event will be recorded in a separate publication). Since the sheep kept at Al-Ahsa and Abha were of local breeds, such breeds might have innate resistance against clinical BTV infection (Abu Elzein & Taylor 1987; Taylor *et al.* 1991).

The ecological picture at Al-Ahsa and Al-Kharj is very similar. Both farms are situated at an oasis with many irrigation canals, boggy land, ponds, small drainage ditches, muddy areas in pastures, leaks from irrigation pipes, small seepages and diverse agricultural activities, all the year round. These factors, together with the hot weather, are quite favourable for the continuous breeding of the *Culicoides* midges (Mellor & Pitzolis 1979; Kline & Greiner 1985).

It is evident from the high incidence of the BT antibodies in cattle and sheep and the continuous seroconversion of the sentinel animals that BTV infection is endemic in the Al-Ahsa area. Supporting evidence is the high incidence of malformations in Friesian calves and the isolation of BTV from each deformed calf as well as from *Culicoides* midges caught in the area (Abu Elzein *et al.* to be published). If it is true that calves that become infected *in utero* can become carriers of the BT virus for life (Leindo & Castro 1981), and that those infected post-natally can elicit a long BT viraemic phase (Luedke, Jochim, Bowne & Jones 1970), Al Ahsa could be a reservoir of BTV infection from where it could spread to other localities in the Kingdom provided that a competent insect vector is available. It has been confirmed that, following wind dispersion, single BTV-infected female *Culicoides* can establish a BT "hot-spot" (Hugh-Jones, Taylor,

FIG. 1   Malformation of the lower legs of sentinel calves born from Friesian cows

TABLE 1   Prevalence of BTV antibodies in adult comparisons of the sentinel animals

Locality and animal breed	Number tested	No. positive	% positive
Al-Ahsa cattle	150	150	100,0
Al-Ahsa mixed sheep and goat farm	210	210	100,0
Al-Kharj cattle	54	34	63,0
Abha mixed sheep and goat farm	63	22	35,0
Total	477	416	87,2

TABLE 2 Loss of BTV maternal antibodies and seroconversion in Al-Ahsa sentinel herds

Date of birth	No. of sheep	Date of loss of maternal antibodies	Age at loss of maternal antibodies (months)	Date of seroconversion
Oct 1992	11	Mar/Apr 1993	5–6	Apr/May 1993
Nov 1992	18	Apr 1993	5	Apr/May 1993
Dec 1992	6	Feb/Mar 1993	2–3	Mar/Apr 1993
Jan 1993	13	Mar/May 1993	2–4	Apr/June 1993
	No. of calves			
Jan/Feb 1993	4	Apr/May	3–4	May/June 1993
Apr 1993	3	Aug 1993	4	Sept 1993
Jun/Jul 1993	3	Dec 1993	5	Jan 1994
Aug/Sept 1993	4	Jan/Feb 1994	4–5	Feb/Mar 1994
Nov 1993	5	Mar 1994	4	Apr 1994
Dec 1993	6	Apr 1994	4	May 1994
Jan 1994	3	May 1994	4	June 1994
Feb 1994	3	Jun 1994	4	July 1994
Mar 1994	7	Jul/Aug 1994	4–5	Aug/Sept 1994
Apr 1994	2	Aug 1994	4	Sept 1994
May 1994	1	Sept 1994	4	Oct 1994
Jun 1994	3	Sept 1994	3	Oct 1994

TABLE 3 Loss of BT maternal antibodies and seroconversion in the Al-Kharj sentinel calf herd

Date of birth and total no. of animals	Maternal antibody status	Date of loss of maternal antibodies	Date of seroconversion	Remarks
April 1993 (23 calves)	(15) with antibodies in April 1993	Two calves in July 1993	August 1993	Remained seropositive until end of experiment
		Thirteen calves in October 1993	November 1993	Remained seropositive until end of experiment
	(8) with no maternal antibodies	No maternal antibodies	June 1993	Remained seropositive until end of experiment

TABLE 4 BTV type-specific antibodies in the sera of sentinel cattle, sheep and goats

Sentinel herd	No. of sera examined	No. of sera giving neutralizing antibodies against the 24 BTV serotypes											
		1	2	3	4	5	6	7	8	9	10	11	12
Al-Ahsa calf herd	60	2	2	1	6	1	3				12	2	18
Al-Ahsa sheep herd	40					2	2				6		7
Al-kharj calf herd	30		5		1		3	1			10		4
Abha sheep herd	30	1			1								1
Cumulative	160	3	7	1	8	3	8	1	0	0	28	2	30
Percentage		1,9	4,4	0,6	5	1,9	5	0,6	0	0	17,5	1,3	18,8

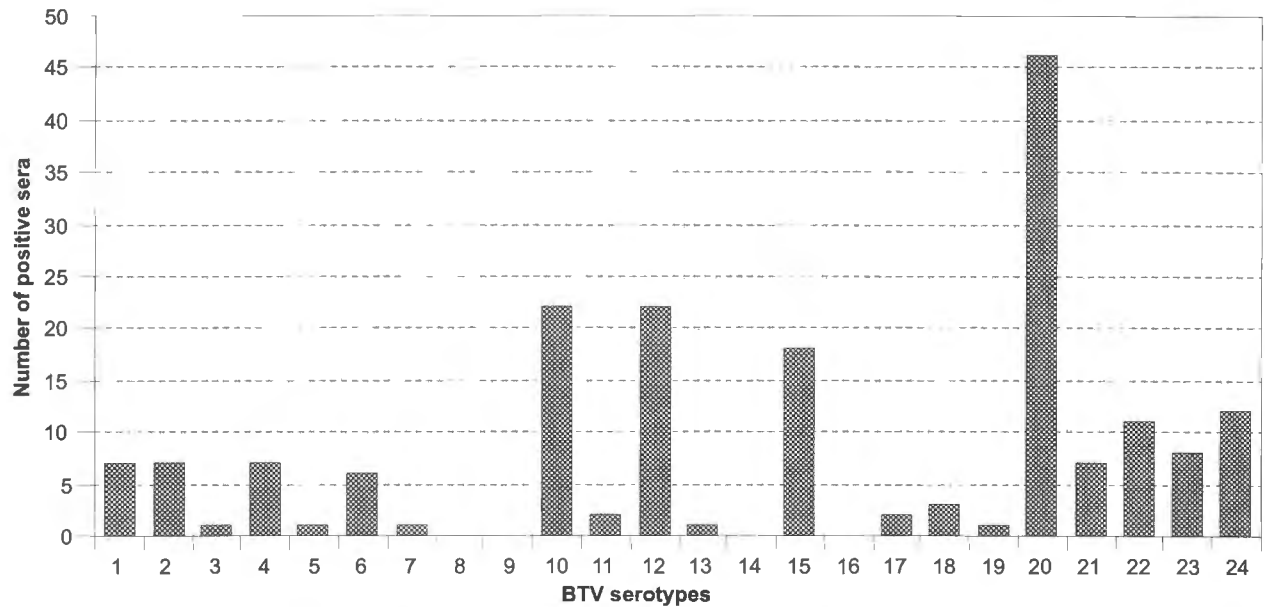| Sentinel herd	No. of sera examined	No. of sera giving neutralizing antibodies against the 24 BTV serotypes											
		13	14	15	16	17	18	19	20	21	22	23	24
Al-Ahsa calf herd	60	1		10		2	3	1	26	4	7	4	9
Al-Ahsa sheep herd	40								19	1	1	2	1
Al-kharj calf herd	30			8					20	3	4	4	3
Abha sheep herd	30			19			1		18		2	9	6
Cumulative	160	1	0	37	0	2	4	1	83	8	14	19	19
Percentage		0,6	0	23,1	0	1,3	2,5	0,6	51,9	5	8,8	11,9	11,9

FIG. 2 Prevalence of serotype-specific antibodies to BTV in sentinel cattle
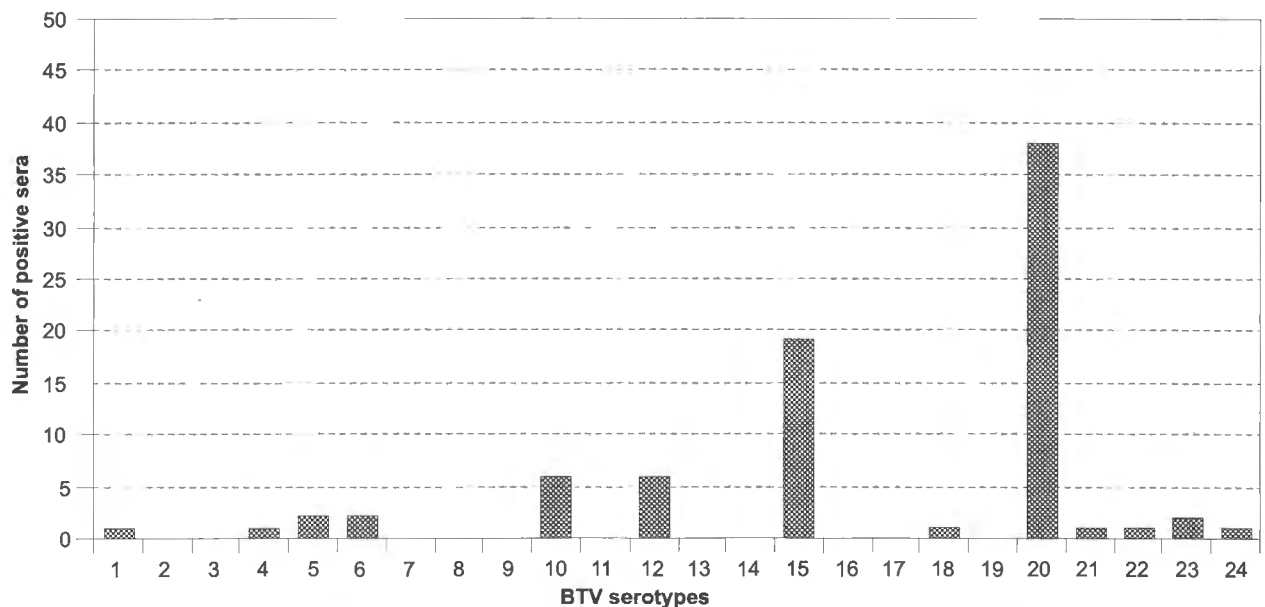

FIG. 3 Prevalence of serotype-specific antibodies to BTV in sentinel sheep

Jones, Luther, Miller, Karns & Hoyt 1989; Taylor *et al.* 1991).

The finding that calves lose their maternal antibodies by the age of 3–5 months and sheep between 4–6 months, corresponds well with similar studies performed elsewhere (Mohamed & Taylor 1987; Mohamed & Mellor 1990; Al-Busaidy & Mellor 1991). The fact that seroconversion occurred throughout the months of study indicate continuous BTV activity during that period, as well as the presence of *Culicoides* vectors.

A lower percentage of non-sentinel cattle were seropositive for BT, fewer sentinel calves received colostral antibodies and the incidence of congenital malformations were lower, suggesting a lower BTV activity. This could be due to the continuous use of insecticides on the farm, resulting in a reduction of the *Culicoides* population, but with a few surviving *Culicoides* maintaining the BT virus activity, confirming previous reports (Abu Elzein 1986; Taylor *et al.* 1991) that a few competent *Culicoides* midges can maintain a "hot-spot" of BTV activity.
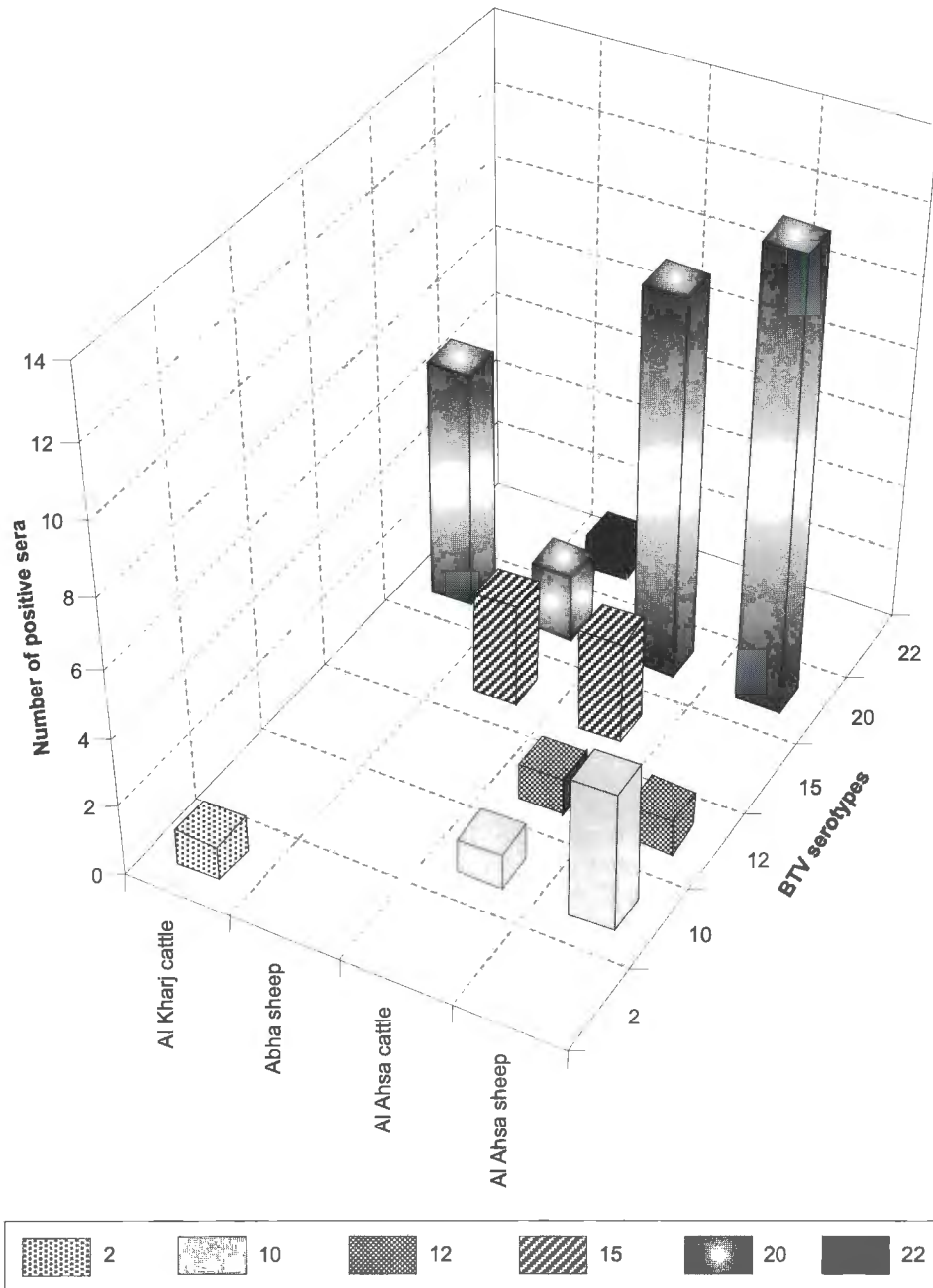
FIG. 4   Number of sera from the sentinel herds showing monospecific reactivity against BTV serotypes

The pattern of BT seroconversion in sheep at Abha indicated that there was also continuous BT virus activity in that area. Though conditions at Abha are not well suited for the breeding of *Culicoides*, it is possible that some wind-borne *Culicoides* can be blown from the horn of Africa into Abha.

The results of the serotyping of antibodies present in the sera of cattle and sheep in the study are of great interest. Antibodies against BTV serotypes 1–7, 10–13, 15 and 17–24 were detected. Of these, antibodies

against BTV serotypes 1–5, 7, 10–13, 15 and 21–24 were detected for the first time in Saudi Arabia.

In a previous publication, Hafez & Taylor (1984) tested the sera of 30 sheep and one goat for BTV serotypes 1–22. These sera were collected between 1977 and 1982. Their results indicated the presence of antibodies against BTV serotypes 6, 14, 17, 19 and 20 only. The present study suggests a radical change in the prevalence of BTV serotypes since then. Antibodies to BTV serotypes 10, 12, 15 and 20 were

predominant in cattle, sheep and goats, indicating that these four virus serotypes had recently been circulating in the field. This conclusion is supported by the detection of monospecific antibodies against them at high frequency (Fig. 4). Of the five type-specific antibodies reported earlier in Saudi Arabia against BTV types 6, 14, 17, 19 and 20 (Hafez & Taylor 1984), we could not detect any antibodies against serotype 14 and only rarely against serotypes 17 and 19. Only serotype 20 is still circulating in the field at high frequency, and serotype 6 to a lesser extent.

From the foregoing it is clear that Saudi Arabia has been exposed to the introduction of new BTV serotypes probably through importation of live animals and embryos or through BTV-infected midges blown into the country from the surrounding countries in which BT is endemic. Live animals are imported from countries of the Middle East, East Africa, Australia and the USA. Two interrelated ecosystems in the Middle East were suggested by Al-Busaidy & Mellor (1991), the first including countries of the Arabian peninsula, the second countries further north including Turkey, Jordan, Syria, Israel and Cyprus. In the first group of countries monospecific reactions were originally detected against BTV serotypes 3, 6, 16, 17, 19 and 20, with serotypes 4 and 22 later added for Oman (Al-Busaidy & Mellor 1991). The present study adds serotypes 10, 12, 15 and 20 as monospecific reactors in Saudi Arabia, of which only type 20 has previously been detected in the country. From the second group of countries serotypes 2–4, 6, 10 and 16 were reported.

Serotypes 1–17, 20, 21 and 22 were recorded in Africa (Mohammed & Taylor 1987; Davies 1978; Soliman *et al.* 1972). In the USA serotypes 17, 2, 10, 11 and 13 were reported (Godfrey, Blue, Taylor & Shotts 1985; Barber 1979; Gibbs, Griener, Taylor, Barber, House & Pearson 1983; Jessup, Work, Bushnell, Sawyer & Osburn 1990). Serotypes 1 and 20 were recorded in Australia. The 20 serotypes recorded for Saudi Arabia in the present study could, therefore, have originated in any or in a combination of these sources.

The economic significance of BT in Saudi Arabia has not been evaluated. Some years ago abortions, stillbirths and foetal malformations occurred on some large dairy farms on which artificial insemination of their cows with imported semen is practiced, but unfortunately no virus isolation was attempted and BT was not identified as the cause. A devastating outbreak of BT-related virus disease with high mortality rate was, however, reported in deer in Saudi Arabia (Abu Elzein *et al.* 1992), and confirmed by BT-related virus isolation, and recently BTV was isolated on several occasions from indigenous sheep which showed a mild disease characterized by fever, oedema of the face, mild stomatitits, coronitis and dullness. Studies on these outbreaks will be reported later.

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