

## The distribution of Akabane virus in the Middle East

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### SUMMARY

Serological evidence was used to confirm an outbreak of Akabane disease in cattle in the Turkish Province of Aydin in 1980. Thereafter, serum collections from the Middle East were screened for the presence of neutralizing antibodies to Akabane virus. The results indicate that the virus was present in a number of provinces on the south Turkish coast in 1979 and 1980 but that it probably did not persist into 1981; the virus had also been present on Cyprus in 1980 and on at least one previous occasion. There was also evidence of limited virus transmission in the Orontes river valley in Syria in 1979 and less precise evidence to show that occasional infection occurred in the lower Jordan river valley. The failure of Akabane virus to persist in southern Turkey for more than two years indicates that this area is open to epidemic rather than endemic infection. The presence of neutralizing antibodies in the eastern Turkish Provinces of Gaziantep and Diyarbakir suggests that this might be the route whereby Akabane virus occasionally invades the Middle East region.

### INTRODUCTION

Akabane virus is a member of the Simbu serogroup of the family Bunyaviridae; it is transmitted by both mosquitoes and *Culicoides* midges [1–3]. Among domestic animals, host species include buffalo, cattle, camels, sheep, goats and horses [4]. Clinical disease caused by Akabane virus has been reported from Australia, Israel, Japan and Turkey [5–8]. Serological evidence suggests that the virus also occurs in a number of African countries as well as in Bahrain, Cyprus, Indonesia, India, Kuwait, Malaysia, Nepal, Oman, Pakistan, the Philippines, Saudi Arabia, Singapore, Taiwan, Thailand and Yemen [9–11; Taylor WP and Gumm ID, unpublished observations].

The virus is able to cross the ruminant placenta and should this happen in early pregnancy, a variety of congenital abnormalities including arthrogryposis and hydranencephaly are seen at parturition. In adult animals, however, infection appears to be entirely subclinical and in endemic areas most breeding-age animals will have acquired an active immunity sufficient to prevent the virus from reaching the developing foetus. Consequently, the pathogenic effects of Akabane infection are only seen when the virus exceeds the limits of the endemic area and

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infects susceptible animals in the early stages of pregnancy. Such spread is most likely to occur at the edges of the endemic area [5] and may be due to the movement of either infected hosts or infected vectors.

In the Middle East, Sellers and Herniman [12] found serological evidence for the presence of Akabane on Cyprus in 1968 or 1969 but showed that the virus did not persist there into 1970. These authors noted that Akabane disease had occurred in Israel in 1969 and suggested that both Cyprus and Israel lay at the edge of the endemic area; the absence of reports of Akabane disease in Cyprus, Israel or in any neighbouring Middle Eastern country between 1970 and 1980 is entirely in keeping with this suggestion.

In the spring of 1980, the Aydin Provincial Directorate in western Turkey reported a high incidence of congenital abnormalities in new-born bovine calves. Between 4 and 22 March, 31 cases were reported, 27 with arthrogryposis, 3 with hydranencephaly and 1 with torticollis. An examination of the records of Aydin Animal Hospital showed that dystocia had been associated with the birth of a further 74 arthrogrypotic calves in early 1980 [8]. Finally, on the basis of pathological examinations carried out by the Veterinary Faculty of the University of Ankara, the condition was diagnosed as arthrogryposis/hydranencephaly due to infection with Akabane virus.

In the years between 1979 and 1982 the Institute for Animal Health made a number of serum collections to assist investigations into the distribution of diseases of livestock in the Middle East. Using these resources, the present study reports the prevalence of Akabane virus neutralizing antibodies in sera collected in Turkey, Syria, Jordan and Cyprus and makes a preliminary attempt to rationalize the epidemiology of this virus in the Middle East.

#### MATERIALS AND METHODS

##### *Serum samples*

Samples were collected from cattle, sheep and goats using 10 ml evacuated bleeding tubes. Generally serum was removed from the clotted blood at a local Veterinary Investigation Laboratory within 24 h of collection. However, Syrian samples were processed in the Animal Health Institute, Amman, Jordan up to 96 h after collection. The ages of the animals sampled were determined by examining the eruption patterns of permanent incisor teeth and by consulting the owner. Whenever possible, collections were made early in the year in the belief that cool winter temperatures are likely to depress the transmission of insect-borne viruses, and that such samples should reflect the level of virus activity characteristic of the preceding calendar year.

*Turkish samples.* The origins of most serum samples collected from Turkish livestock have been outlined by Taylor and Mellor [13]. Additional samples were collected during a visit to the Provincial Veterinary Hospital at Aydin in May 1980 where a disease resembling Akabane had been reported.

*Syrian samples.* In June 1979, blood samples were collected from sheep maintained under traditional husbandry systems on the steppes to the south of the river Euphrates and from cattle held on Government farms at Raqqa in the Euphrates river valley and at Hama in the Orontes river valley. In April 1980,

blood samples were collected from sheep only, during visits to the steppes and to the Euphrates and Khabour river valleys. In February and March, 1981, further collections were made from steppe sheep and from cattle at Hama while in February 1982 a small collection was obtained from cattle, sheep and goats living at Jurin, in the Orontes valley.

*Jordan samples.* Samples were collected in the spring months of 1979–81. Sheep were sampled at Fijaje, Hallibat, Khanasrah and Ma'an, to the east of the Jordan river valley; goats were sampled only at Ma'an. Cattle were sampled at Wadi Dulail, near Zerka, also to the east of the Jordan valley, and at Dier Alla, on the floor of the Jordan valley close to Amman.

*Cypriot samples.* In 1980 samples were obtained from sheep in two locations in northern Cyprus and in one location in southern Cyprus. In 1981, samples were collected more extensively in northern Cyprus in March, June and November and in southern Cyprus in March and May.

#### *Neutralization tests*

Sera were inactivated at 56 °C and examined in a microneutralization test against 100TCID<sub>50</sub> of Akabane virus, strain JaGAR39 using BHK-21 indicator cells. At the start of the programme sera were screened at final dilutions of 1/20, 1/40 and 1/80 but later this range was changed to 1/80 and 1/160 dilutions only. Any samples with titres higher than 80–160 were then titrated over a range 80–1280. Details of the test method have been described elsewhere [12].

## RESULTS

### *Turkey*

Two months after the first reports of a disease of calves characterized by arthrogryposis, hydranencephaly and torticollis [8], affected new-born calves were still being brought to the veterinary hospital at Aydin, in western Turkey. Table 1 shows the serological results obtained from animals sampled there in May 1980.

Neutralizing antibody levels of between 110 and 640 in samples 245, 247 and 270 from adult cattle collected at Aydin, provided the first clear indication that there had been a recurrence of Akabane virus in the region. Moreover, 5 of the 6 calves examined had congenital abnormalities consistent with a clinical diagnosis of Akabane disease and had serum neutralizing titres to Akabane virus of between 80 and 480. Although it was possible that the antibodies found in samples 251, 252, 269 and 271 were of maternal origin, in the case of sample 244 the calf had been born to an Akabane-infected mother and had a pre-colostral titre of 80 indicative of an intrauterine infection. In summary, this brief clinical and serological survey supported the conclusion of Urman and colleagues [8], that an epidemic of Akabane disease had occurred in Aydin in early 1980.

The results of screening tests carried out on further serum samples collected in May 1980 are given in Table 2. Although it is possible that low-level virus transmission continued during the winter of 1979/80, the results have been interpreted as reflecting the situation as it was at the end of 1979. From the substantial number of positive results from 1-year-old cattle, sheep and goats, it appeared that there had been transmission of Akabane virus in Adana, Aydin,

Table 1. *The presence of neutralizing antibodies to Akabane virus in the sera of newborn calves and adult bovines from Aydin*

Sample number	Donor	Notes	Antibody titre
245	Brown Swiss, 3-years-old	Mother of 244	640
244	Dummy calf, 1-day-old	Deformed hind limbs	80*
247	Brown Swiss, 8-years-old	Gave birth to deformed calf previous week	110
249	Holstein, 8-years-old	Mother of 250	< 20
250	Holstein calf, 1-day-old	Normal	< 20*
251	Holstein, 36-h-old	Severe arthrogryposis	320
252	Brown Swiss, 24-h-old	Dummy with under shot jaw	480
270	Brown Swiss, 3-years-old	Mother of 269	320
269	Brown Swiss, 10-weeks-old	Arthrogryposis	80
271	Brown Swiss, 4-days-old	Blind hydrocephalic	450

\* Sample obtained before the calf had taken colostrum.

Table 2. *The incidence of neutralizing antibodies to Akabane virus in serum samples collected in Turkey in May 1980*

Province	Species	No. locations sampled	Year of birth		
			1979	1978	< 1977
Adana	Ox	1	5/6*	4/5	5/6
	Sheep	3	3/10	5/6	8/13
	Goat	2	4/7	0/2	7/10
Aydin	Ox	2	—	—	3/5
	Sheep	1	2/5	5/5	4/5
Denizli	Ox	1	0/1	2/2	—
	Sheep	3	1/23	2/12	1/11
	Goat	1	0/3	—	0/2
Diyarbakir	Ox	2	2/12	1/5	3/5
	Sheep	3	2/18	0/3	1/7
	Goat	3	2/4	1/2	0/4
Gaziantep	Ox	2	1/7	2/4	2/4
	Sheep	2	0/17	1/6	0/5
	Goat	2	2/10	1/1	4/6
Ismir	Ox	1	—	1/1	1/1
	Sheep	1	0/2	—	0/5

\* Numerator, number positive samples; denominator, number of animals sampled.

Denizli, Diyarbakir, Gaziantep and Izmir provinces during 1979. In contrast, negative results from 244 yearlings and 2-year olds indicated that Akabane virus had not been transmitted among cattle, sheep or goats in the provinces of Afyonkara-Hisar, Amasya, Ankara, Balekesir, Bursa, Canakkale, Kayseri, Konya, Samsun or Istanbul in 1978 or 1979. Nevertheless, results obtained with samples collected in early 1981 indicate that Akabane virus had infected yearling cattle or yearling goats in Aydin, Denizli and Mugla provinces in 1980 (Table 3). The evidence obtained from three positive samples collected from 2-year-old goats in Antalya province was consistent with the presence of Akabane virus in that province in 1979 rather than 1980. Further, from negative results obtained with

Table 3. *The incidence of neutralizing antibodies to Akabane virus in samples collected in Turkey in April and May 1981*

Province	Species	No. locations sampled	Year of birth		
			1980	1979	1978
Antalya	Ox	3	0/2	0/2	1/22
	Sheep	2	0/10	0/4	0/11
	Goat	2	0/8	3/3	2/7
Aydin	Ox	4	5/55	2/10	16/22
	Sheep	1	0/11	3/3	1/1
Denizli	Ox	5	0/13	1/6	1/24
	Sheep	3	0/32	—	0/3
	Goat	3	1/23	0/11	0/18
Mugla	Ox	2	0/10	0/1	4/10
	Sheep	2	0/23	0/6	2/11
	Goat	2	1/22	0/5	0/6

sera from 356 yearling cattle, sheep and goats collected in 18 different localities, it appeared that there had been no transmission of Akabane virus in 1980 in Afyonkara-Hisar, Ankara, Balikesir, Bursa, Isparta, Izmir or Konya provinces. In addition, survey work undertaken during the autumn of 1981 produced no evidence of Akabane virus transmission in yearling animals in any of the four provinces sampled, including Aydin and Denizli (Table 4).

Sera from 3 of 12 horses bled in Aydin in early 1981 were positive for Akabane neutralizing antibodies with titres ranging between 110 and 160. The positive animals were all more than 8 years old. In the cattle, sheep and goat surveys reported above, individual Akabane neutralizing antibody levels varied between 80 and 1280. In cattle, 73% of the antibody positive animals had titres exceeding 240 and 44% had antibody titres exceeding 480. In sheep and goats the proportions were 90 and 43% and 94 and 63% respectively.

### *Syria*

Between 1979 and 1982, of 344 sheep sera collected from a variety of locations throughout northern Syria, Akabane neutralizing antibodies were found in six samples only. One positive yearling was detected at Raqqa, in the Euphrates valley, and was presumed to have come from an animal infected in 1979. A further four positive sera were detected from sheep over 2 years old collected in the yards of a commercial fattening company at Kamishly, near the Turkish border. Finally, in 1981, one positive sample was detected from a yearling animal bled at Menbes, in the Euphrates valley north of Lake Assad near the Turkish border. Antibody levels in positive sheep varied between 120 and 640. In 1981 no positive samples were found among the 54 yearling sheep sampled at seven locations.

Syrian cattle were bled in 1979, 1981 and 1982 as shown in Table 5. Three positive samples were found among a group of 3–4-year-old cattle collected in 1979 at a Government farm near Hama, on the edge of the Orontes river valley, but 10 calves, 10 yearlings and 4 2-year-old animals from the same collection were negative. When cattle on the same farm were re-sampled in 1981, sera from 3 of 4 animals born in 1978 or 1979 showed evidence of prior infection with Akabane

Table 4. *The incidence of neutralizing antibodies to Akabane virus in samples collected in Turkey in October 1981*

Province	Species	No. locations sampled	Year of birth		
			1981	1980	1979
Konya	Ox	3	0/24	—	—
	Sheep	3	0/54	—	—
	Goat	1	0/5	—	—
Antalya	Ox	3	0/29	0/5	0/11
	Sheep	3	0/31	0/12	0/22
	Goat	2	0/21	0/10	0/38
Aydin	Ox	2	0/48	0/21	2/7
Denizli	Ox	1	0/8	—	—
	Goat	2	0/22	0/11	0/10

Table 5. *The incidence of neutralizing antibodies to Akabane virus in ox serum samples collected in Syria*

Year of sampling	Locations sampled	Year of birth					
		1981	1980	1979	1978	1977	1976
1979	Hama	—	—	0/10	0/10	0/4	3/9
1981	Hama	—	0/10	1/2	2/2	—	—
1982	Jurin	0/3	0/3	1/1	—	2/2	0/3

Table 6. *The incidence of neutralizing antibodies to Akabane virus in ox serum samples collected in Jordan between 1979 and 1981*

Location sampled	Year of sampling	Year of birth				
		1980	1979	1978	1977	< 1976
Deir Alla	1979	—	11/21	—	—	5/5
	1980	1/8	0/20	—	—	—
	1981	0/15	—	—	—	—
Wadi Dulail	1979	—	0/10	—	—	1/21
	1980	—	0/14	—	—	—
	1981	0/14	—	—	—	—

virus but this evidence did not extend to the 10 yearlings sampled at the same time. Lastly, during a 1982 visit to Jurin, a sampling site in the Orontes valley north of Hama, positive samples were obtained from one 3-year-old cow born in 1979 and from two older animals. These findings suggest that Akabane virus was probably present in the Orontes valley during 1979.

#### *Jordan*

No evidence of Akabane infection was found in samples from 269 sentinel sheep and goats in flocks situated to the east of the Jordan river valley in the north and south of the country.

Of the two sites from which Jordanian cattle were sampled during successive years (Table 6), Wadi Dulail is situated in an arid area near Zerqa, to the northeast of Amman, while Deir Alla is situated on the floor of the Jordan river to the west of Amman. Neutralizing antibodies were found in five adult cattle sampled at Deir

Table 7. *The incidence of neutralizing antibodies to Akabane virus in serum samples collected in Cyprus in 1980 and 1981*

Sector	Species	Year/month	Year of birth			
			1981	1980	1979	< 1978
Northern	Sheep	1980 April	—	—	0/21	4/20
	Sheep	1981 March	—	2/95	8/92	9/112
	Sheep	1981 June	—	0/88	0/42	0/46
	Sheep	1981 November	—	3/75	2/20	—
Southern	Sheep	1980 May	—	0/10	0/10	—
	Ox	1981 March	—	2/23	0/5	4/28
	Sheep	1981 March	—	0/24	0/20	1/40
	Sheep	1981 May	—	0/24	0/21	—
	Goat	1981 March	—	0/4	0/3	2/3



Fig. 1. The distribution of Akabane virus in Turkey and the Middle East in 1979. ■, Serological evidence of Akabane virus activity; □, no evidence of Akabane virus activity.

Alla in July 1979 indicating that cattle on this farm had had earlier exposure to Akabane virus. At the same time 11/21 3-month-old calves were also positive for Akabane antibodies. When calves in this group were re-sampled as yearlings, in January 1980, no positive samples were found. Antibody titres in the adults varied between 160 and 1280 while in the calves, 1 sample had a titre of 1280 and the remaining 10 had titres in the range 80–160. When 2 and 3-month-old calves were sampled in April 1980, only 1 out of 8 samples was positive and when this second group of calves was resampled in early 1981 no positive sera were found. At Wadi Dulail one positive adult serum sample was detected from an old animal bled in 1979 but there was no evidence of infection on this farm in either 1979 or 1980.

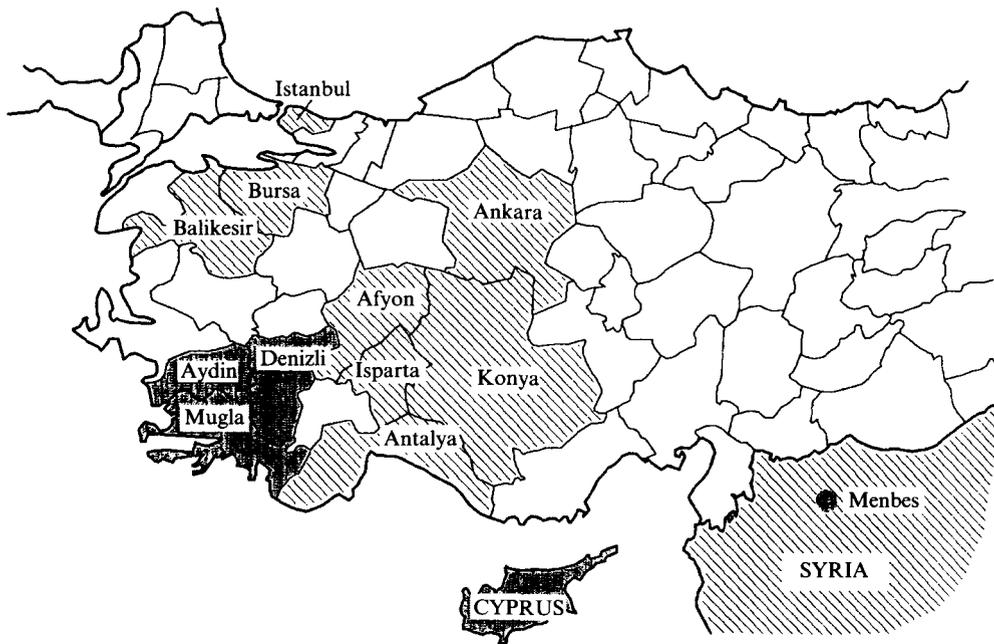


Fig. 2. The distribution of Akabane virus in Turkey and the Middle East in 1980. ■, Serological evidence of Akabane virus activity; ▨, no evidence of Akabane virus activity.

A small number of Jordanian horse sera was collected in 1979 without determining the ages of the donors; 3 out of the 45 samples tested were found to be positive with titres ranging from 80 to 240.

#### *Cyprus*

The results of tests undertaken with samples collected in Cyprus in 1980 and 1981 are given in Table 7. They suggest that Akabane virus could have been present on the island in 1978 and was certainly present in 1980 (Table 7). Titres were in a range from 80 to 1280 with 81% of the samples having antibody levels equal to, or greater than 240.

#### *Distribution*

Figures 1 and 2 attempt to map the distribution of Akabane virus in the Middle East in 1979 and 1980. This has been done on the basis of the evidence available from the Turkish provinces sampled in each of those years together with the evidence available from Syria and Cyprus.

### DISCUSSION

The serological evidence obtained in 1980 clearly indicates that Akabane virus infected domestic livestock in a number of provinces along the south coast of Turkey in 1979 (Fig. 1). As far as Aydın is concerned, it is to be supposed that the virus gave rise to subclinical infections in a number of pregnant susceptible cattle in the autumn of 1979 and that during the course of these infections the virus

crossed the placenta and infected the developing foetuses. This would account for the epidemic of congenitally deformed calves born in the spring of 1980 and the presence of Akabane neutralizing antibodies in the pre-colostral serum from calf 244 (Table 1).

Both the case records and the opinion of resident clinicians in the Aydin veterinary hospital suggested that this was the first time an outbreak of arthrogryposis and hydranencephaly had occurred in that province. Sellers and Pedgley [14] described the meteorological conditions associated with the wind-assisted transfer of insects infected with Akabane virus to Aydin Province in 1979. They suggested an origin of infection in eastern Turkey and northern Syria. However, the present results show that Antalya Province could have been the source, and that the virus could have spread along the south coast from Adana. As Akabane disease appeared to be confined to Aydin, it could be supposed that Adana and Antalya provinces were infected earlier in the summer and that cattle became immune either before they were bred or that they were infected either sufficiently early or late in pregnancy for the foetus to remain unharmed by the virus.

It also seems clear that, in contrast to bluetongue infection in Turkey [13], Akabane virus did not spread to the provinces of the northeastern coast or to the upland provinces of central Anatolia. This suggests that the two viruses are transmitted by different vectors or, if by a single vector species, then one which transmits bluetongue virus with greater efficiency than Akabane virus.

The occurrence of neutralizing antibodies in animals born in 1980 from Aydin, Denizli and Mugla provinces suggests that the virus made a second incursion from an external source, that it over-wintered in one or all of these western Provinces or that the animals in question, although born in 1980, had in fact been infected late in pregnancy in 1979 and had been born with an active immunity to Akabane virus. However, in the absence of samples from suitable sentinel animals and in the absence of evidence of infection in Antalya province in 1980, it seems more likely that limited over-wintering occurred in these western Provinces. On the other hand, as the virus did not apparently recur in Aydin, Denizli or Antalya in 1981, it appears that over-wintering is not a regular event even in the most favourable areas of the Turkish coast, probably due to the limited winter activity of the vector [15].

The results from Syria and Jordan suggest that Akabane virus can establish itself from time to time in the Jordan and Orontes river valleys, although it is not suggested that the virus is endemic in either locality. During our surveys it appeared that Akabane virus had replicated in cattle in the Orontes valley in 1979 in northern Syria but had not persisted into 1980. In Jordan the antibodies found in young calves in 1979 had disappeared by the time they became yearlings indicating that they were due to maternal immunity from adults infected at an earlier date and on this basis we would suggest that Akabane virus did not reach the Jordan valley in 1979. At the same time there was considerable evidence to show that the virus does occasionally infect livestock living in or near the Euphrates river valley. Within the region, horses appear to be among the species infected with Akabane virus but the extent to which they are involved in the epidemiology of the disease is unknown.

While the size of the Akabane virus endemic area has yet to be defined exactly, it is likely to involve Pakistan, the Indian subcontinent and Indochina. Al-Busaidy and colleagues [11] were of the opinion that countries in the Arabian peninsula were not part of this area but rather, that they were constantly at risk of infection from an extraneous source. Nevertheless once established, Akabane infection could persist on the Arabian peninsula for a number of consecutive years. A somewhat similar though less tenacious situation appears to exist in Turkey and in some neighbouring countries.

The route whereby Akabane gains entry to the Turkish coast remains uncertain but it is most unlikely that it invades through Jordan or Syria. On the other hand the fact that the Turkish Provinces of Diyarbakir and Gaziantep were also infected in 1979 leads us to suggest that Iran and Iraq are at the periphery of the endemic area and that there is a pathway around the so-called fertile crescent that allows the spread of Akabane virus to eastern Turkey. From here it might, under favourable conditions, spread westwards to and along the Turkish coast, or move southwards down the rift valley system into Syria, Jordan or Israel. The existence of such a pathway through eastern Turkey would account for the very low incidence rate of infections in northern Syria. Taking this argument to its conclusion, it should be possible to develop an early warning system to predict the reappearance of the virus throughout the region.

Cyprus was probably infected in 1978 and 1980 through infected vectors transported by the wind from southern Turkey [16]. Even though it is possible that over-wintering occurs the virus is unlikely to persist on the island indefinitely, and Cyprus should be regarded as a sentinel station for events taking place on the mainland.

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