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Distribution of bluetongue virus in Turkey, 1978–81

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SUMMARY

Information about the distribution of bluetongue in Turkey during 1978–81 has been obtained by serological surveys in cattle, sheep and goats. The group-specific immunodiffusion test was used to identify the presence of bluetongue virus (BTV) in a given province and the type-specific microneutralization test to decide which virus types had been in circulation. By drawing sera from accurately aged donor animals in May and August 1980, it was possible to draw up a general outline of the distribution of BTV in Turkey between early 1978 and mid 1980. By combining the same technique with spring and autumn field visits it became possible to make detailed inferences about the distribution of BTV serotypes in 1980 and 1981. The results support the conclusion that BTV was widespread in central and western Turkey for a number of years and suggests that overwintering can be a regular occurrence in that country. When compared with contemporaneous results from Syria and Jordan, a unifying and well-defined bluetongue virus ecosystem becomes apparent.

INTRODUCTION

Clinical bluetongue was virtually unknown in Turkey until an epidemic due to bluetongue virus type 4 (BTV4) occurred in late 1977 in the Aegean Province of Aydin. BTV4 persisted in western Turkey for the next 2 years, and spread to involve a number of coastal and contiguous inland provinces [1]. The outbreaks were eventually controlled by the widespread use of live attenuated BTV4 vaccine in sheep. It was suggested [1] that the reappearance of a pathogenic variant of the same type of virus in each of 3 successive years implied an ability to overwinter in western Turkey.

In 1980 the United Nations Food and Agriculture Organisation initiated a joint research programme on the epidemiology of bluetongue in Turkey involving the Institute for Animal Health, Pirbright, England and the Etlik Veterinary Control and Research Institute, Ankara, Turkey. In 1981 this programme continued with the support of the Overseas Development Administration of the British Government. After 1979 no clinical cases of bluetongue were reported from any part of Turkey and therefore further information on the distribution of the virus

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could only be obtained using seroepidemiological techniques. Consequently, the serum collections made in 1980 consisted of samples drawn from animals in as many provinces as could be visited in the time available. In 1981 attempts were made to obtain more detailed information on the distribution of BTV in selected central and western provinces.

Taylor and colleagues [2] made an earlier attempt to interpret the results of these surveys. Based on neutralizing antibody results, these authors suggested that Turkish livestock could have been infected with BTV types 2, 4, 9, and a hitherto unrecognized virus type. More recently it was suggested [3] that in some mediterranean sheep breeds the type specific antibody response to BTV may fall rapidly to below the 1/20 threshold of the microneutralization test employed and that this, rather the presence of a new virus type, might account for the large number of sera with group specific antibodies which failed to neutralize any of BTV types 1–20. Nevertheless, by searching for neutralizing antibody clusters among such weak responses as did occur, and by matching these with the occasional high-titre monospecific antibody valuable epidemiological insights were possible [4].

In the light of these observations the earlier results from Turkey have now been re-evaluated and used to show the widespread nature of BTV in that country between 1978 and 1981.

MATERIALS AND METHODS

Serum samples

Sera were collected in evacuated 10 ml bleeding tubes during a series of field trips to various parts of the Republic. Generally, blood samples were obtained from unvaccinated cattle, sheep and goats belonging to outlying farmers and shepherds, or by visiting households within a village. On some occasions samples were taken from stocks held on Government farms, and in 1980 a small number of samples were collected from sheep that had been vaccinated against BTV4.

The collecting pattern was structured only to the extent that no more than 2–3 days' work was undertaken in any given province during any individual field visit, that samples were generally gathered close to major trunk roads, and that samples were not collected from nomadic animals. The ages of the animals sampled were determined by consultation with the owner, together with an examination of the individual eruption patterns of permanent incisor teeth.

As far as practicable, serum was removed from the clotted blood samples within 24–48 h of collection using the facilities available in the Provincial Veterinary Investigation Laboratories or in the Etlik Laboratory.

In May 1980 the eastern and western Provinces of Adana, Afyonkara-Hisar, Aydin, Balekesir, Bursa, Cannakale, Denizli, Diyarbakir, Gaziantep, Istanbul and Izmir were visited whilst the central Provinces of Ankara, Konya and Kayseri and the northern Provinces of Amasya and Samsun were visited in August. These trips were intended to provide background information on the distribution of BTV in Turkey. In the spring of 1981 the Provinces of Afyonkara-Hisar, Ankara, Antalya, Aydin, Balekesir, Bursa, Denizli, Isparta, Izmir, Konya and Mugla were visited in order to collect sera that could provide specific information on the distribution of

Table 1. *The incidence of precipitating antibodies to bluetongue virus in serum samples collected in Turkey in May 1980*

Province	Species	No. locations sampled	Year of birth		
			1979	1978	⩽1977
Adana	Ox	1	0/6	0/5	1/6
	Sheep	3	0/10	0/6	2/13
	Goat	2	3/7	2/2	7/10
Afyonkara-Hisar	Sheep	1	2/8	—	4/10
Aydin	Ox	2	0/1	—	4/5
	Sheep	1	0/1	2/2	2/2
Balekesir	Ox	1	0/2	0/1	0/2
	Sheep	2	0/7	—	—
	Goat	1	1/3	—	4/4
Bursa	Ox	1	0/7	—	0/7
	Sheep	1	—	2/5	0/2
Canakkale	Ox	1	1/2	0/1	2/8
	Goat	1	3/5	1/1	2/3
Denizli	Ox	1	0/1	2/2	—
	Sheep	3	8/23	1/12	6/11
	Goat	1	0/3	—	2/2
Diyarbakir	Ox	2	0/12	0/5	2/5
	Sheep	3	8/18	3/3	7/7
	Goat	3	3/4	1/2	2/4
Gaziantep	Ox	2	0/7	1/4	3/4
	Sheep	2	9/17	2/6	3/5
	Goat	2	5/10	0/1	4/6
Istanbul	Ox	1	0/3	—	0/5
	Sheep	1	—	2/2	5/8
Izmir	Ox	1	—	1/1	1/1
	Goat	1	0/2	—	1/5
	% Positive		27·0	32·8	47·4

BTV in 1980. In October 1981, a final visit was made to look for evidence of virus activity during 1981 itself.

Serological tests

Group-specific antibodies to BTV were identified using the agar-gel immunodiffusion (AGID) test of Lefevre and Taylor [5]; samples were regarded as positive only if a line of complete identity was established with control positive serum. Thereafter all positive sera were titrated against BTV international serotypes 1–20 using the microneutralization test as described by Herniman and colleagues [6].

Statistical methods

Fisher's exact test [7] was used to determine possible significant differences in annual antibody prevalence rates.

RESULTS

Although BTV may overwinter in some coastal provinces of Turkey, the results of Yonguc and colleagues [1] and the review of Sellers and Mellor [8] support the general concept of a winter lull in virus transmission, particularly in upland areas of the country. Allied to a pattern of spring lambing it follows that yearling or 2-

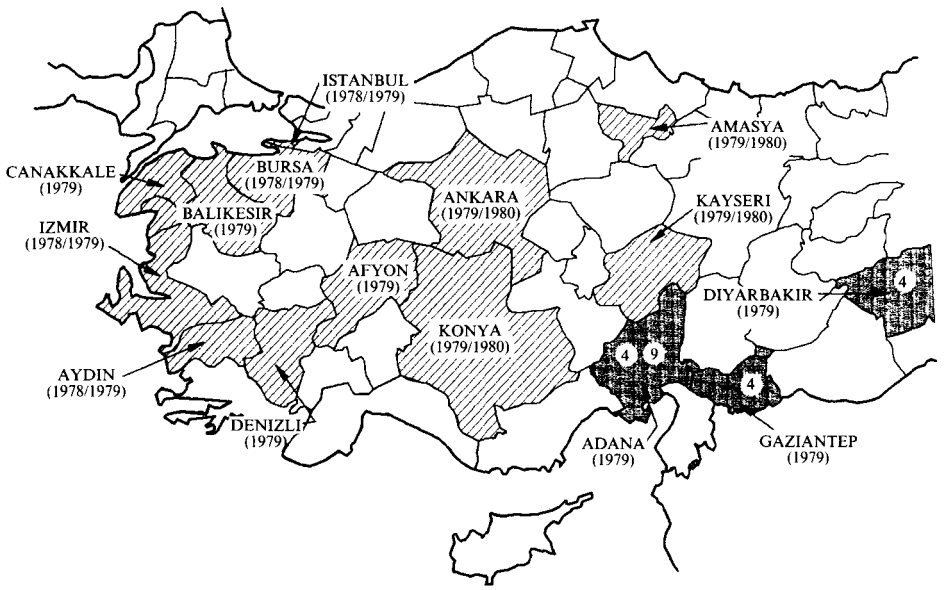

Fig. 1. Distribution of BTV and BTV serotypes in Turkey in 1978, 1979, or early 1980. ▨, BTV group specific antibodies detected; ■, microneutralising antibodies detected to a specific BTV type. The name of the province followed by the year(s) in parentheses indicates the name of affected province and probable year of most recent BTV infection.

Table 2. *The incidence of precipitating antibodies to bluetongue virus in samples collected in August 1980*

Province	Species	No. locations sampled	Year 1979	Year 1978
Amasya	Ox	1	—	0/4
	Sheep	2	0/5	2/9
Ankara	Ox	3	—	1/15
	Sheep	3	0/15	4/15
Kayseri	Ox	3	—	1/15
	Sheep	3	7/15	11/15
Konya	Ox	3	—	0/15
	Sheep	3	4/15	6/15
Samsun	Ox	2	—	0/11
	Sheep	2	0/10	0/11
	% positive		18·3	20·0

year-old animals sampled in the spring of 1980 could provide evidence relating to infections occurring in 1978 or 1979 but that samples obtained in mid-1980 could be affected by infections occurring in 1980 itself.

From the presence of group specific precipitating antibodies in the sera of yearling and 2-year-old animals collected in May 1980, it appears that BTV was active in the Provinces of Adana, Afyonkara-Hisar, Balekesir, Canakkale, Denizli, Diyarbakir and Gaziantep during 1979 and in Aydin, Bursa, Istanbal and Izmir in either 1978 or 1979 (Table 1, Fig. 1).

When the gross incidence rates for yearlings, 2-year-old, and older animals were

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Table 3. *The incidence of precipitating antibodies to bluetongue virus in samples collected in April and May 1981*

Province	Species	No. locations sampled	Year of birth		
			1980	1979	≤1978
Afyonkara-Hisar	Ox	2	1/7	0/2	0/8
	Sheep	2	1/37	1/1	—
Ankara	Ox	2	0/15	—	—
	Sheep	2	1/35	—	—
	Goat	1	0/10	—	—
Antalya	Ox	3	0/2	2/2	17/24
	Sheep	2	9/10	3/4	7/11
	Goat	2	5/8	3/3	5/7
Aydin	Ox	4	7/49	2/10	12/22
Balekesir	Ox	3	0/31	—	—
	Sheep	3	15/35	—	—
	Goat	2	15/22	—	—
Bursa	Ox	2	0/10	—	0/3
	Sheep	2	5/20	—	—
	Goat	1	10/11	—	—
Denizli	Ox	5	0/13	2/6	9/24
	Sheep	3	0/11	—	0/3
	Goat	3	10/23	9/11	10/18
Isparta	Ox	2	0/6	—	1/22
	Sheep	1	8/11	—	3/4
	Goat	1	6/7	6/6	5/5
Izmir	Ox	1	0/4	0/5	0/8
Konya	Ox	3	0/29	—	—
	Sheep	3	5/31	—	—
	Goat	1	—	10/10	—
Mugla	Ox	2	0/9	1/1	4/10
	Sheep	2	2/23	0/6	1/11
	Goat	2	11/22	4/5	6/7
	% positive		22·4	59·7	42·8

compared there was no statistical difference ($P = 0{\cdot}42$) between the infection rates in yearlings and 2-year-old but a significant increase in infection rate was observed between these two age groups and the older animals ($P = 0{\cdot}01$). This suggests that in some of these provinces BTV infection has occurred at least once prior to 1978.

Of the provinces sampled in August 1980 (Table 2), it appeared that Amasya and Ankara had been infected at some time between 1978 and mid-1980 while for Kayseri and Konya the timing could be narrowed to the period between the spring of 1979 and the summer of 1980. However, as there is no statistical difference ($P = 0{\cdot}55$) in the overall antibody incidence rates among the two cohorts of infected animals it can be suggested that they were only exposed to BTV infection once. Accordingly, in Fig. 1, each of these provinces is shown as having undergone infection during 1979–80.

The samples collected in the spring of 1981 showed that BTV had been active in the Provinces of Afyonkara-Hisar, Ankara, Antalya, Aydin, Balekesir, Bursa, Denizli, Isparta, Konya and Mugla during 1980 (Table 3, Fig. 2). Comparing the significantly increased antibody incidence between the 1 and 2 year olds ($P = 0{\cdot}004$) it is clear that a number of these provinces were infected with BTV in each

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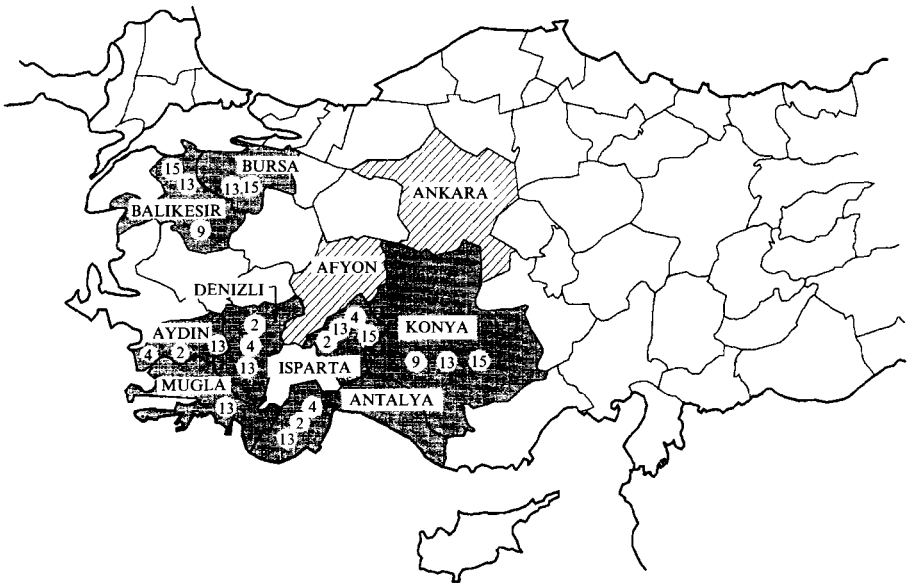

Fig. 2. Distribution of BTV and BTV serotypes in Turkey in 1980. ▨, BTV group specific antibodies detected; ▩, microneutralising antibodies detected to a specific BTV type.

Table 4. *The incidence of precipitating antibodies of bluetongue virus in samples collected in October 1981*

Province	Species	No. locations sampled	Year of birth		
			1981	1980	⩽1979
Antalya	Ox	3	7/29	—	—
	Sheep	1	0/6	1/4	5/10
	Goat	2	9/21	5/9	35/38
Aydin	Ox	2	3/48	7/21	2/7
Denizli	Ox	1	1/8	—	—
	Goat	2	0/22	3/11	2/10
Istanbul	Ox	1	0/8	—	—
	Sheep	1	0/26	0/6	0/1
	Goat	1	—	—	2/2
Konya	Ox	3	0/24	—	—
	Sheep	3	16/50	—	—
	Goat	1	1/5	—	—
	% positive		13·8	31·4	67·6

of 1979 and 1980. BTV was also active up to October 1981 in the Provinces of Antalya, Aydin, Denizli and Konya (Table 4, Fig. 3).

In 1980, 56 sera were collected from Merino or Dalig sheep that had been vaccinated with attenuated BTV4 vaccine. All had developed group-specific precipitating antibodies but low-level homologous neutralizing antibody titres (20–40) could be detected in only 10 samples.

Microneutralization tests on the extensive collections obtained in 1980 showed monospecific BTV4 responses in cattle from the eastern Provinces of Diyarbakir and Gaziantep which indicated that this type had occurred there as recently as

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Fig. 3. Distribution of BTV and BTV serotypes in Turkey in 1981. ▨, BTV group specific antibodies detected; ■, microneutralising antibodies detected to a specific BTV type.

Table 5. *Occurrence of monospecific BTV neutralizing antibody responses in Turkish livestock in 1980 and 1981*

Month and year of sampling	Province of origin	Host species	BTV type	No. of responses	Age(s) of hosts (in years)
May 1980	Diyarbakir	Ox	4	1	4
	Gaziantep	Ox	4	2	2 & 5
	Adana	Goat	9	1	1
May 1981	Antalya	Ox	2	4	2–8
	Aydin	Ox	2	1	1
		Ox	4	6	1–10
		Ox	13	1	1
	Denizli	Ox	2	1	3
	Isparta	Ox	2	1	12
		Goat	13	1	2
	Mugla	Goat	13	1	1
October 1981	Antalya	Ox	2	9	1–3
		Goat	2	8	1–5
	Aydin	Ox	2	2	1–7

1978 or 1979. In Adana there was good evidence in the form of clusters and a single monospecific response to suggest that both types 4 and 9 had been active in the province during 1979 (Tables 5 and 6).

From the results of microneutralization tests undertaken with sera collected in the spring of 1981 it would appear that BTV types 2, 4, 9, 13 and 15 had been active in 1980. Type 13 in particular appeared to have achieved a widespread distribution being recorded in 8 of the 9 provinces where BTV was shown to have occurred (Fig. 2). On the other hand, types 2 and 4 appeared to have been restricted to the south-western Provinces of Aydin, Denizli, Isparta and Antalya.

Table 6. *Occurrence of BTV neutralizing antibody clusters in Turkish livestock in 1980 and 1981*

Month and year of sampling	Province of origin	Host species	BTV type(s)	Age(s) of hosts (in years)	Remarks
May 1980	Adana	Goat	4 & 9	1–4	Same flock
April 1981	Balekesir	Goat	9, 13 & 15	1	Same flock
	Bursa	Goat	13 & 15	1	Same flock
	Konya	Sheep	9 & 13	1	Same flock
		Goat	9, 13 & 15	2–3	Same flock
May 1981	Antalya	Ox	2 & 13	2–10	Same herd
		Sheep	4	1	
		Goat	2, 4 & 13	1–2	Same flock
	Aydin	Ox	4	1–10	
	Denizli	Goat	2, 4 & 13	1–3	Same flock
	Isparta	Goat	2, 4, 13 & 15	1–3	Same flock
	Mugla	Goat	4 & 13	1–3	Same flock
October 1981	Antalya	Ox	2	1–2	
		Goat	2	1–4	
	Aydin	Ox	2	1	

In 1981 there was evidence for the presence of BTV in Antalya, Aydin, Denizli and Konya Provinces. Microneutralization tests failed to provide typing antibody results from Denizli or Konya, but in the other two provinces it was clear that type 2 had been active (Fig. 3).

DISCUSSION

The observation that a number of sheep vaccinated with BTV4 failed to develop measurable neutralizing antibodies within a few months of vaccination points to an inherent weakness associated with the use of this test in seroepidemiology with Mediterranean sheep. Nevertheless, we believe our data demonstrate that if sufficient samples are examined, and if the samples are collected within 12 months of infection, then antibody clusters will provide a means of determining which BTV serotypes have been in circulation. Occasional high-titre monospecific responses, although less convincing in themselves, provide useful corroborating evidence when they are directed at the same types as the clusters. This viewpoint is supported by studies undertaken in the Caribbean basin which demonstrated a high level of correlation between serological evidence for the presence of specific BTV types and their actual isolation [9–11].

It is clear from the surveys undertaken in 1980, which contributed a substantial amount of information related to events taking place between 1978 and mid-1980 (Fig. 1), that in that particular time period BTV achieved a wide distribution within Turkey. Taking into account the distribution of BTV based on clinical reports [1], the Provinces of Antalya and Kocaeli can be included on this map. In 1979 in particular, which was the last recorded year of the BTV4 outbreak in western Turkey, BTV was nonetheless active in western, central and eastern provinces of the country. Clearly, its total distribution was much wider than that suggested by this single 3-year long clinical outbreak. This observation is further

supported by evidence that, in the absence of clinical reports, BTV had probably infected livestock in some or all of the provinces listed in Table 1 in or before 1977.

Using both the BTV group and type specific antibody tests to analyse the serum samples collected in the spring of 1981, it was possible to assemble a fairly detailed picture of the distribution of BTV in west and west-central Turkey in 1980 (Fig. 2). Type 13 appears to have been widespread in this region while types 2 and 4 were restricted to the south west of the area sampled. Type 9 appeared to have been present only in Balekesir and Konya Provinces.

Although antibody clusters were detected to BTV 15 in a number of provinces, these were always found in groups of animals that showed clusters to other BTV types, particularly BTV13. Further, no BTV15 monospecific response was found in any positive sample. From this it is suggested that the BTV15 antibodies detected are cross-reactive and similar to those produced in animals that have been infected with two different BTV types within a few weeks of each other [12].

In the final phase of these surveys in the autumn of 1981, it was possible to visit five of the south-western provinces of Turkey. From this mission BTV activity was detected in four of the five provinces sampled and it was clear that in two of these BTV2 had been active in 1981.

In the earlier study of Yonguc and colleagues [1], an account was given of an outbreak of acute clinical bluetongue which commenced in October 1977 in the Province of Aydin. After a lapse of some 8 months, disease, due to the same bluetongue type (BTV4), reappeared in the autumn of 1978 in a group of western provinces contiguous both with each other and with Aydin. Following a similar winter–spring–summer break, further outbreaks occurred in the autumn of 1979. Although there were no further reported outbreaks of bluetongue in either 1980 or 1981, the present report makes it clear that BTV4 was still present in western Turkey in 1980, that a number of other serotypes were also present in the same area at the same time and that BTV persisted into 1981. Clearly, therefore, a large number of BTV infections in Turkish livestock escape detection, presumably because they are subclinical in nature.

Based on the present results it is entirely possible that BTV4 existed in eastern and southern Turkey prior to the 1977 outbreak, and that in that year it did no more than extend its overall distribution to western Turkey where it was able to overwinter. What is harder to understand is why, if BTV is circulating throughout the year, clinical disease should only appear in the autumn months and be associated with a particular virus type. Perhaps most BTV serotypes consist of various subpopulations each with a different level of innate virulence but existing within a situation where the relatively avirulent subpopulation(s) usually remain dominant. However, when these mixed subpopulations enter a totally susceptible livestock population, virus strains with high virulence levels may be selected and may dominate. This might be through a mechanism involving rapid alternating passages between mammalian hosts and the insect vectors, a mechanism which could be favoured by the build-up of insect numbers in the autumn months. Following the appearance of clinical disease in the autumn it is suggested that the virulent subpopulation disappears with the onset of winter and the coincident reduction in insect numbers. Then, according to this theory, a virus of similar

virulence would have to be re-selected the following year. If vaccine were widely used, as was the case with BTV4 in western Turkey, this could eventually slow down transmission rates enough to prevent the emergence of a virulent subpopulation but not enough to eradicate the virus completely. Elsewhere, the more endemic nature of BTV infection might give rise to a partly immune livestock population within which rapid serial virus passage would be an uncommon event. This explanation could account for the relatively rare emergence of clinical outbreaks of bluetongue in endemic areas.

The results of the present Turkish survey should be compared with the results of similar surveys undertaken as much the same time in Syria and Jordan [3]. In these it was concluded that BTV types 2, 4 and 9 were present in northern Syria in 1978, BTV9 and BTV13 in 1980, and BTV2 and BTV66 in 1981. Exactly the same BTV types were thought to have been present in Jordan in the period 1978–81. The similarity in BTV types prevailing in Turkey, Syria and Jordan between 1978 and 1981 is quite striking and clearly indicates that to a large extent these three countries form part of a common bluetongue ecosystem. In Israel, the only other Mediterranean country for which detailed information is available, BTV types 2, 4, 6, 10 and 16 are endemic [13]. It therefore appears that Israel and Turkey have both common and distinct serotypes and it is probably safe to conclude that the BTV ecosystems occurring in the two countries are somewhat different.

There is insufficient detail available from the present surveys to build a comprehensive picture of the natural history of bluetongue in Turkey. However, it is probably wise to assume that BTV is endemic in Turkey and is not, as was previously supposed, a chance intruder. This concept is borne out by the more recent survey work of Burgu and colleagues [14]. However, in view of the harsh winters in central Anatolia and the mountainous provinces of the east, it is likely that the virus overwinters on the western and southern coast and spreads inland each summer. This view is amply supported by the analytical report of Sellers and Mellor [8].

In order to comprehend fully the mechanics of this endemic situation all of the contributory factors including overwintering, the annual invasion of inland provinces, the insect vectors and the possible occurrence of virus hot spots require additional study. However, enough is now known of the epidemiology of the virus to make these studies profitable both in terms of understanding how the virus persists and in devising practical methods for reducing the possibility of infection occurring. Taking into account the widespread presence of Akabane virus in the same geographical area (Taylor and Mellor, 1993; unpublished results), the need for a broadly based Middle East collaborative research programme on insect transmitted diseases of livestock is plain to see.

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