Neutralising antibodies to Akabane virus in ruminants in Cyprus

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Summary

Neutralising antibodies to Akabane virus, a cause of arthrogryposis and hydranencephaly, were demonstrated in serum samples from 33 sheep, 3 goats and 1 bovine among 285 serum samples collected in south-eastern Cyprus from December 1970 onwards. Twenty-four of the 29 sheep having positive antibodies came from one farm in Liopetri. No positive sera came from animals born after 1969, no association with abortions or stillbirths was noted and no arthrogryposis or hydranencephaly was observed in Cypriot animals in 1969 or before. It is suggested that Akabane virus was carried to Cyprus from the eastern Mediterranean mainland by infected midges on the wind in 1969 and possibly also in 1968, but that no disease was observed since infection took place after 50 days of gestation when damage to the foetus was unlikely.

Introduction

Epidemics of abortion, stillbirths, arthrogryposis and hydranencephaly in cattle, sheep and goats have been reported in Israel (Markusfeld and Mayer, 1971), Japan (Inaba, Kurogi and Omori, 1975) and Australia (Hartley, Wanner, Della-Porta and Snowdon, 1975). The cause has been shown to be Akabane virus (a member of the Simbu group of the Bunyaviridae), which after infection of the adult multiplies in the placenta and the foetus giving rise to foetal abnormalities (Kurogi, Inaba, Takahashi, Sato, Satoda, Goto, Omori and Matumoto, 1977). No clinical signs are seen in the animal. The disease has been called Akabane disease and the virus is transmitted by Culicoides midges.

From 1970 onwards a team from the Animal Research Institute, Pirbright has been investigating the ecology of bluetongue in Cyprus (Sellers, 1975; Sellers, Gibbs, Herniman, Pedgley and Tucker, 1979). During this period collections of serum samples were made from cattle, sheep and goats especially in the south-eastern region of the island. Abortions and stillbirths have been associated, among other causes, with infection by bluetongue virus and it was decided as part of the differential diagnosis to examine the serum samples for the presence of antibodies to Akabane virus.

Materials and Methods

Virus

Akabane virus and specific antiserum were kindly supplied by Dr Y. Inaba of the National Institute of Animal Health, Tokyo. The virus, JaGAr39, was received as the 19th passage in suckling mouse brain. The second passage in BHK21 cells was used in the serum neutralisation tests.

Serum samples

The serum samples had been collected during twice-yearly visits to Cyprus from December 1970 and were stored at -20°C. The samples originated from animals on farms in the Famagusta and Larnaca districts. Sera from sheep in quarantine were also tested. The sera were heated at 56°C for 30 min before testing.
Serum neutralisation test

The tests were carried out in microplates with flat-bottomed wells. For screening tests 0.1 ml of serum diluted 1/10 in PBS supplemented with 0.2% w/v bovine albumin was placed in the wells; to this was added 0.1 ml of virus containing an estimated 100 TCID₅₀. The mixture was incubated at 37°C for 60 min and at 4°C overnight. On the following day 0.05 ml of BHK21 cell suspension containing 5 × 10⁵ cells/ml of maintenance medium was added to each well. After sealing the microplates were incubated at 37°C. Monolayers (70% cover) were established within 24 h. The wells were observed microscopically and the extent of cytopathic effect recorded. Full cytopathic effect was observed on the 4th and 5th days. Sera with antibody at the 1/20 final dilution were titrated using a 2-fold dilution series. The 50% end point was based on the presence or absence of cytopathic effect. Antibody levels of 1/20 or greater were considered positive.

RESULTS

Two hundred and fifty sheep sera, 30 goat sera and 5 cattle sera from 190 sheep, 19 goats and 5 cattle were tested for antibodies to Akabane virus. Two or more bleedings were tested from some of the sheep and goats. Thirty-three sheep sera, 3 goat sera and 1 cattle serum had antibody levels of 1/20 or higher to Akabane virus and they represented 29 sheep, 3 goats and 1 cow. Twenty-four of the 29 positive sheep sera came from 1 farm at Liopetri and represented 24 of the 67 sheep sampled over the years. The antibody titres on that farm (1/20–1/640) were the highest found and the titre of the 1 positive sheep serially bled did not diminish over 2 years (1/178–1/355).

No antibodies were found in sheep born after 1969 or in goats born in 1970 and afterwards. Serum samples from 10 Awassi sheep in quarantine in 1971 and 181 in quarantine in 1972 (both lots from Israel) were tested. No antibody to Akabane virus was found.

DISCUSSION

The results indicate that sheep, goats and possibly cattle in the south-eastern region of Cyprus experienced infection with Akabane virus. The year or years that infection occurred cannot be estimated accurately since serum samples were not collected until December 1970. However, the finding that positive serum samples came from animals born in years up to and including 1969 suggested that infection possibly occurred during 1969 although it could also have occurred in earlier years especially in 1968. Negative results from animals born in subsequent years suggest that the virus did not persist in Cyprus.

Abortions and stillbirths are reported in most years in Cyprus. Serum samples from ewes that had aborted in 1971 and 1972 did not show evidence of infection with Akabane virus or indeed with bluetongue virus. There are, moreover, no reports of arthrogryposis or hydranencephaly in the Annual Reports of the Cyprus Veterinary Services. The owner of the flock at Liopetri did not recall anything unusual in 1968 to 1970 nor did other farms in the area.

Where Akabane disease is endemic, young animals are infected with virus before they become pregnant for the first time and develop antibodies which persist. Thus during pregnancy a bite from infected midges does not lead to infection of the foetus and subsequently to arthrogryposis and hydranencephaly. However, the finding that Akabane infection did not persist in Cyprus beyond 1970 and the likelihood that