Serological and clinical evidence of a teratogenic Simbu serogroup virus infection of cattle in Israel, 2001-2003

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Summary

During the last 35 years, two major outbreaks of Akabane virus (AKAV) infection were recorded in cattle in Israel in 1969/1970 and 2002/2003. Congenital malformations of calves characterised by the appearance of an arthrogryposis and hydranencephaly syndrome first appeared in Israel in 1969. Based on epidemiological, clinical, pathological, histopathological and serological data, this syndrome was strongly correlated with seroreactivity to AKAV, a member of the *Bunyaviridae*, Simbu serogroup.

In February 2002, the first cases of 'blind newborn calves' (BNC) were observed on farms located in the northern valleys of Israel. Microtitre serum neutralisation (SN) tests of serum from malformed calves and their dams were conducted using Akabane and Aino viruses (AINOV). The first SN test was performed at the reference laboratory of the Clinical Virology Section, Kyushu Research Station, National Institute of Animal Health, Kagoshima, Japan. The clear-cut findings of seroreactivity to AKAV by cattle located in the affected zone, in contrast to negative findings in cattle from unaffected farms (87% and 3.7%, respectively) was indicative of AKAV infection. In contrast, seroreactivity to Aino virus was relatively low in both affected and non-affected areas during the 2002 outbreak. In order to establish Israeli laboratory standards for Simbu serogroup diagnosis, 57 serum samples tested by the Japanese laboratory were retested by SN in Israel. An almost complete homology (96.5%) was found between the two SN panels of sera (kappa = 0.92). SN and ELISA kits enabled the surveillance of this arbovirus epidemic in the second consecutive year (2003). Moreover, AKAV was identified in trapped midges by hemi-nested PCR and real-time PCR. With these techniques, the geographical limits of the BNC epidemic that appeared in some areas of Israel was identified for the first time and was recorded in the Arava Rift Valley, 400 km south of the epicentre of the 2002 outbreak. The reintroduction of AKAV into this region, together with some evidence of AINOV activity and epidemics of bluetongue (BT) in the southern parts of Europe and the eastern Mediterranean, and renewed outbreaks of West Nile virus infection in Israel, Italy and southern France, are all evidence of the potential spread of arbovirus activity into southern Europe from the Mediterranean Basin.

Keywords

Aino virus – Akabane virus – Arthrogryposis – Congenital malformations – Hydranencephaly – Israel – Neonatal ruminants – Simbu serogroup.

Introduction

Akabane virus (AKAV) and Aino virus (AINOV) are known to cause epidemics of abnormal parturitions in cattle, including abortion, stillbirths and calf deformities. This is known as the congenital arthrogryposis-hydranencephaly syndrome (CAHS) affecting the musculo-skeletal and nervous systems, respectively (5, 8, 9). AKAV was originally isolated from mosquitoes and from the midge, *Culicoides imicola*, which is now considered to be the major vector (1, 5, 7). Serological studies have since classified AKAV and AINOV in the Simbu

serogroup of the family *Bunyaviridae* (7). Congenital disease associated with AKAV is found principally at the extremities of its normal geographical distribution.

Outbreaks of congenital malformations in dairy calves characterised by the CAHS were first recorded in Israel in 1969 and persisted until 1970 (6, 10, 12, 14). Similar malformations were seen in lambs and kids (14). Based on epidemiological (10), clinico-pathological (10, 12), histopathological (12) and serological data (6), CAHS was diagnosed as being caused by AKAV (17). Thirty-two years later, in February 2002, the first cases of 'blind newborn calves' (BNC) (Fig. 1) appeared in two neighbouring large dairy herds in northern Israel. During the four months that followed, dozens of farms had reported similar problems (Figs 1 and 2). All the BNC cases presented hydro- or micro-hydranencephalic signs, or both, on post mortem. In these cases, the cranium of the necropsied BNC contained an apparently normal cerebellum, but instead of the cerebrum there remained only 5-10 g of tissue encasing 200-300 ml of liquid (Figs 1 and 2) (3).

Figure 1
A blind neonatal calf with micropthalmia

Micro-SN for AKAV and AINOV was performed in the Laboratory of the Clinical Virology Section of the Kyushu Research Station, National Institute of Animal Health, Kagoshima; AKAV was identified as responsible for the newly emerging epidemic. This conclusion was based on the clear serological findings of AKAV in the affected zone in contrast to the negative findings in cattle from unaffected zones during the 2003 outbreak (87% and 3.7%, respectively) (3). This 2002 outbreak persisted until the end of April 2002, suggesting that the activity of AKAV began in August 2001 and ended prior to November the same year (3).

In February 2003, BNC was recorded again and this time the syndrome was observed throughout Israel (Fig. 3). Moreover, BNC appeared in 2003 beyond the southernmost point recorded in the 1969/1970 outbreak (10) (Fig. 4). While the 1969/1970 outbreak was contained above the latitude of 31°00´ (10), in 2003, BNC was noted on dairy farms located very close to the Red Sea 29°30´ (Fig. 3) about 400 km from the 2002 epicentre.

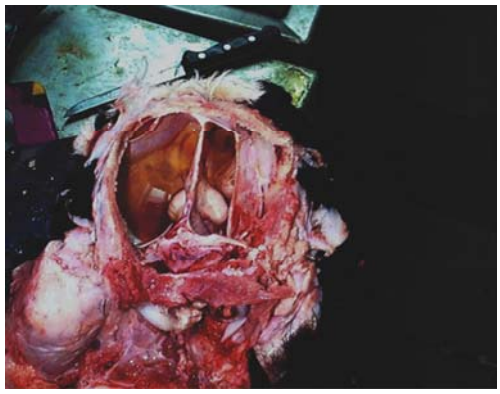

Figure 2
A brainless hydranencephalic blind calf

This paper describes the diagnosis of the AKAV infection in 2003 and defines the geographic limits of its spread.

Materials and methods

Defining the geographical boundaries of the epidemic

Between February and May 2003 the 25 settlements that reported the birth of blind calves were located along the inner coastal plain, the Negev and the Arava Rift Valley. All these areas were below 31°00´ latitude and had not reported neonatal malformations during the 2002 epidemic (Figs 3 and 4).

The blocking ELISA for Simbu serogroup antibodies was used to test serum samples from twelve of these settlements.

Serology

Serum neutralisation test

A panel of 37 positive and 20 negative serum samples was chosen on which the diagnostic test used was established (Table I). These samples were tested previously by the Japanese laboratory to identify the causative agent responsible for the outbreak of congenital malformations seen in 2002 (3). Four of the positive sera included two pre-colostral sera of affected newborn calves and two from their dams. To compare results with those of the Japanese Reference Laboratory, an agreement test was performed and the kappa value was

calculated. SN tests for AKAV and AINOV are described elsewhere (3).

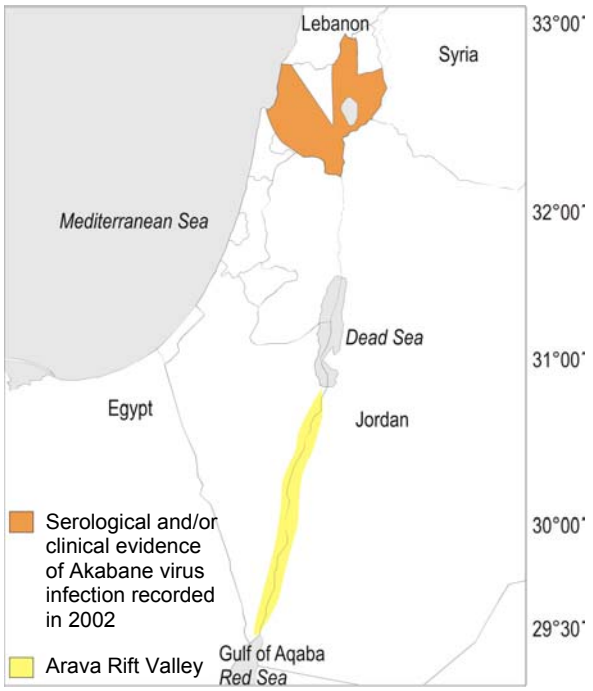

Figure 3
The epicentre of blind neonatal calves in the 2002 outbreak

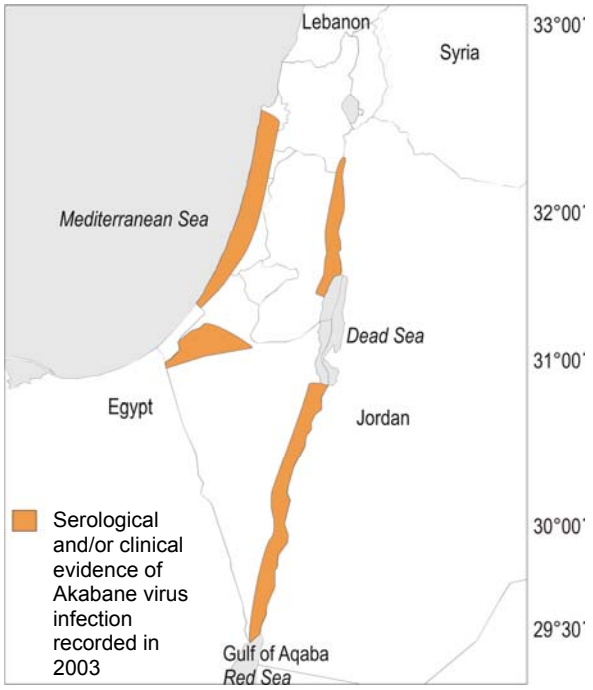

Figure 4
The epicentre of blind neonatal calves in the 2003 outbreak

Enzyme-linked immunosorbent assay

The extent of the Simbu serogroup infection in Israel from February until May 2003 was determined by employing a blocking ELISA for Simbu serogroup antibodies (Elizabeth Macarthur Agricultural Institute, Australia). The procedure was conducted in accordance with the instructions of the manufacturer (5).

Table I
Agreement between the serum neutralisation test results on a panel of serum samples taken during the outbreak of congenital malformations, February-May 2002

Location	National Institute of Animal Health, Japan		Total
	Seropositive	Seronegative	
Kimron Veterinary Institute, Israel	Seropositive	36	1	37
Kimron Veterinary Institute, Israel	Seronegative	1	19	20
Total		37	20	57

Polymerase chain reaction and real-time PCR

PCR and real-time PCR for AKAV, AINOV and Simbu group viruses were developed by Stram *et al.* (15, 16) and were used to identify and sequence AKAV from *Culicoides* (Fig. 5).

Results

Defining the geographical boundaries of the epidemic

The latitude of 32°00´ was the southernmost line of the 2002 epidemic in Israel. From May 2002 to February 2003, no additional cases were reported in Israel. In February 2003, BNC reappeared south of this latitude (Fig. 4) reaching the Arava Rift Valley (Fig. 4), 400 km south of the 2002 epicentre. Moreover, the 2003 BNC outbreak appeared beyond the southernmost point recorded in the 1969/1970 epidemic (10) in areas where it had never previously been recorded. The 1969/1970 outbreak was contained above 31°00´ (10). In 2002 and 2003, BNC was noted on dairy farms located very close to the Red Sea 29°30´ (Fig. 4). In 2003, BNCs were also recorded from a very limited number of settlements in the northern part of Israel, and in some of them it appeared twice in two consecutive years (data not shown).

Serology

Serum neutralisation test

Table I summarises the results of the comparison made between the SN tests that were conducted in Israel and in Japan. An almost complete homology (96.5%) was found between the two SN test locations with a kappa value of 0.92.

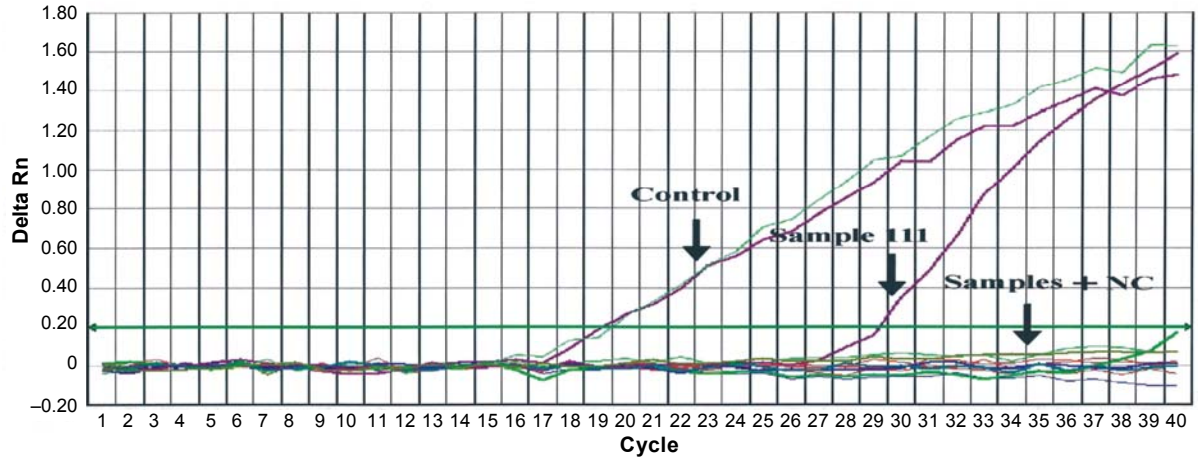

Figure 5
The real-time polymerase chain reaction of Akabane virus isolated from *Culicoides* biting midges

Enzyme-linked immunosorbent assay

Simbu serogroup antibodies were demonstrated in all 12 settlements, using the blocking ELISA.

Polymerase chain reaction and real-time PCR

Figure 5 depicts the real-time PCR results and the sequences (Fig. 6) of the Israeli AKAV recovered from trapped *Culicoides* (15, 16).

Conclusion

This paper describes the methods that were used to diagnose the causative agent of neonatal ruminant malformations in the northern part of Israel in 2002 and to trace its progression to other zones in the second year of the epidemic. The rationale behind the methodology employed to investigate the 2002-2003 epidemic is described elsewhere (2). The clear findings of seroreactivity to AKAV in the affected zones are very suggestive of the causative effect of AKAV during the 2002 epidemic, and are consistent with the main period of activity of *C. imicola* between August and November in this region (3). The geographical distribution of the 2002 outbreak that was first noted in northern Israel was similar to the 1969 outbreak (10). During the second year of these two epidemics, the disease expanded southwards. During the 2003 epidemic, BNC appeared beyond the southernmost point recorded in the 1969/1970 outbreak (10) (Fig. 3). The 1969/1970 outbreak was contained above the latitude of 31°00´ (10), while in 2003, BNC was detected in some dairy farms located very close to the Red Sea at 29°30´ (Fig. 4) about 400 km south of the 2002 epicentre.

The demonstration for the first time of AKAV in trapped *C. imicola* (15, 16) strengthens the assumption that teratogenic arboviruses were involved in 2002-2003 epidemics and were probably involved in some of the sporadic births of defective neonatal ruminants in our region.

1	ATGGCAAATc	aATTTATTTT	caACGATGGT	ccaCAACGGA	ATGCAGCTAC
51	ATTTAACCCG	GATGCAGGGT	ATGTGGCATT	TATCAGTAAG	TATGGGCAGG
101	AGCTCAACTT	TACTGTTGCT	AGAGTCTTCT	TCCTCAAGGA	GAAGAAGGCC
151	AAGATGGTCT	TACATAAGAC	GCCACAACCA	AGTGTCGATC	TTACTTTTGC
201	AGGGGTCAAA	TTTACAGTGG	TTAATAACCA	TTTTCCCCAG	TACACTGCAA
251	ATCCAGTGTC	AGACACTGCC	TTTACGCTCC	ATCGCATCTC	GGGCTACTTA
301	GCTCGCTGGG	TTGCTGGAGCA	GTGCAAGGCT	AATCAGATCA	AATTCGCAGA
351	GGCAGCTGCC	ACAATTGTGA	TGCCGCTGGC	TGAGGTGAAG	GGTTGCACCT
401	GGAGTGATGG	GTATGCAATG	TACCTAGGAT	TTGCCCCTGG	TGCTGAGATG
451	TTTTTGGAAA	CCTTTGAGTT	TTACCCACTG	GTTATCGACA	TGCACCGTGT
501	GCTAAAGGAT	GGGATGGATG	TCAACTTCAT	GAGAAAGGTC	TTGCGCCAGA
551	GGTACGGGCA	GCTGACTGCA	GAGGAGTGGA	TGACATCTAA	GTTGGATGCA
601	GTCAAGGCTG	CATTTAGCTC	AGTTGCCCAG	ATATCCTGGG	CCAAATCTGG
651	TTTCTCACCT	GCAGCAAGAG	CTTTCCTGGC	TCAATTTGGT	ATCCAGATCT
701	AAT				

Figure 6
The sequence of the S segment of the Israeli Akabane virus lineage ISR-01

Stram *et al.* (16) proposed the presence of a new AKAV lineage in Israel, different from those published in Japan and Australia. It would be beneficial to define the new lineage or to confirm its similarities with the African lineage, but unfortunately, the African lineage genome segment S has not yet been published.

It was surprising to find relatively high seroreactivity towards AINOV in 29.6% of the sera that originated from the zone that served as 'controls' during the 2002 epidemic. These sera were also seronegative to AKAV (3). In light of the maximal life span of the average Israeli dairy cow, it appears that the initial infection with AINOV must have occurred some five to six years ago.

It is not known whether AKAV is endemic or sporadic in Israel. Other unanswered questions are whether AKAV infection appears alone or in combination with other arbovirus(es), and whether different viruses in the Simbu group have invaded Israel. In this study, the authors have confirmed that the probable (major) vector of AKAV in Israel and in the eastern part of the Mediterranean Basin (11) is *Culicoides imicola*; this vector is present in the Old World in a belt between 35°S and 40°N (11). On the other hand, the source of AKAV (or of any other arbovirus included in the Simbu group) has not yet been identified. The distribution of *Culicoides* spp. depends on climatic (macro-global and micro-regional) changes and reasons for its presence or absence in certain defined zones are as yet unsolved.

This probable reintroduction of AKAV into this region, the evidence of the presence of AINOV and the epidemics of BT virus in southern Europe and the eastern Mediterranean Basin (11), together with the potential alert to the presence of West Nile virus in Israel (13) and Italy (4), all provide evidence of the potential and actual spread of arboviruses into previously uninfected areas.

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