**NOTE** *Clinical Pathology*

# Serological Evidence of Akabane Virus Infection in Northern Israel in 2001

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ABSTRACT. In February 2002 the first cases of a "blind newborn calves" syndrome with hydranencephaly appeared in Israel. Eighty-one serum samples, from 54 animals on farms where the syndrome was recorded and 27 others from unaffected farms were examined by neutralization of Akabane virus (AKAV, strain OBE-1) by the micro-titer method. Forty-seven of the 54 samples from the affected farms contained high serum neutralization titers against AKAV (mean SN titer 79.5 and ± 44.7, standard deviation), whereas only one of the 27 samples from the unaffected farms was positive (titer of 8). These results suggest that the vector(s) of AKAV was circulating in Israel in August through December, 2001.

KEY WORDS: Akabane virus, hydranencephaly, Israel.

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An outbreak of congenital malformations in dairy calves characterized by the appearance of the arthrogryposis and hydranencephaly syndrome (AHS) first appeared in Israel in 1969 and continued until 1970 [6, 8, 11]; similar malformations were seen in lambs and kids. On the basis of serological [6, 12, 13], epidemiological [6, 11], clinical and pathological [6, 8], histopathological [8], and serological data [3], the causal agent was diagnosed as Akabane virus (AKAV) [2, 4, 9]. Experimental inoculation of pregnant cows with AKAV in Japan elicited similar responses to those of the natural infection, proving the teratogenic potency of this virus [5]. AKAV and Aino virus (AINOV) belong to the Simbu sero-group of the genus Bunyavirus, family Bunyaviridae [2].

Since 1969/70 similar malformations have been seen sporadically in Israel. All these sporadic cases were attributed to an "unknown etiological agent", because a conclusive diagnosis could not be made and all attempts to isolate AKAV were unsuccessful. Moreover, the most recent serological survey by serum-neutralization (SN) was carried out by Kalmar *et al.*, in 1973/4 [3]. That survey included two populations from affected farms: one comprised older animals and the other, animals less than 3 years old. Only the former group was alive when the malformations were seen, during the 1969/70 epidemic. The conclusion of that study was unequivocal after the epidemics, no evidence for AKAV could be established [3].

In February 2002 the first cases of "blind newborn calves" (BNC) appeared in the Bet She'an Valley (northern Israel) in two large neighboring dairy herds. During the subsequent 4 months, dozens of farms from 88 settlements reported similar problems (Table 1). At necropsy of these BNC, the cerebellum appeared normal, while severe microhydranencephaly was seen. The cerebrum was only 5 to 10 g in weight and contained 200–300 m*l* of cerebrospinal fluid (CSF). The cortical tissue was completely replaced with CSF-filled sacs. A shell of edematous, thin brain tissue, replaced the cerebral cortex and the cystically dilated lateral ventricles were filled with CSF. The meninges, especially the pachymeninges, were much thickened. Arthrogryposis was rarely noted. No parallel findings were recorded in sheep or goats. All the cases were confined to the northern valleys of Israel.

In an attempt to identify the causative agent of the congenital abnormalities, 81 serum samples were taken from cattle raised in two geographical zones; 54 of the samples were from affected farms and 27 from unaffected farms. All the affected farms were located in the northern part of Israel while five unaffected farms were located in the coastal and the inner and central coastal plains. The 54 sera from the affected zone were from 4 pre-colostral and 22 of post-colostral BNC, 15 from cows 2 to 6 years old, which had given birth of BNC. The remaining 13 sera were from calves 8 to 10 months old, considered as sentinels, since they could reflect new infections only. The sera from the unaffected area included 2 sera from pre-colostral and from 8 post-colostral young calves, 10 cows between 2 to 6 years old and the remaining 7 were heifers aged 8 to 10 months. Sera were stored at –20°C pending shipment to Japan.

SNT was carried out by micro-titration with AKAV (strain OBE-1) and AINOV (strain JaNAr28). Fifty-microliter aliquots of serial two-fold dilution of serum, in duplicate rows, were mixed with an equal volume of 100 $TCID_{50}$

Table 1. Number of settlements in which at least one case of "blind calf" was recorded in Israel during the 2002 outbreak

| | Number of sites |
|---|---|
| Reported by farmer or practitioner | 72 (7)* |
| Confirmed by SNT | 16 (12)** |
| Total number of sites with birth of "blind calves" | 88 |

SNT- serum neutralizing test.
( )* Number of cases confirmed also by post mortem examination performed by local practitioner.
( )** Number of cases confirmed also by post mortem examination performed by Department of Pathology, Kimron Veterinary Institute.

Table 2. The results of serum activity to Akabane and Aino viruses according to the geographical origin

| Area of origin | N | sero-positive (%) AKAV | AINOV | AKAV titer* (mean ± SD) | AINOV titer* (mean ± SD) |
|---|---|---|---|---|---|
| Affected | 54 | 47 (87) | 26 (45.5) | 79.5 (± 44.7) | 17.6 (± 17.6) |
| Non-affected | 27 | 1 (3.7) | 8 (29.6) | 8** | 16.4 (± 16.4) |

*Only the positive cases were taken in account for the calculation of the mean antibody titers. ** No "mean" could be calculate for only one positive sample.

of virus in flat-bottomed microplates. After incubation for 1 hr at 37°C, 0.1 m*l* of HmLu-1 cell suspension in GIT medium (Wako Junyaku, Osaka, Japan) was added to the mixture, which was then incubated for 7 days at 37°C in 5% $CO_2$ incubator. The antibody titer was expressed as the reciprocal of the highest serum dilution at which CPE was inhibited. Serum exhibiting an antibody titer of 2 or more was regarded as positive.

The results of the SNT for the presence of anti-AKAV antibodies are shown in Table 2. The SNT antibody to AKAV was detected at a much higher rate (47/54, 87%) in the sera taken from cattle raised on affected farms than in those from unaffected farms (1/27, 3.7%). The mean and standard deviation (SD) values are presented in Table 2. The mean SNT titer of sera originating from the affected zone was much higher (79 ± 44.7) than that of sera taken in the other zone (8, only one sero-positive). The percentages of positive sera to AINOV were relatively low in affected farms. From the results of the SNT, AKAV was most probably the one involved in the epidemic of BNC in northern Israel in 2002.

The clear-cut findings of sero-reactivity to AKAV in the affected zone, in contrast to the negative findings in cattle from the unaffected farms (47/54 and 1/27, respectively) is indicative of AKAV's causative nature. The period during which the clinical signs were observed, namely from February to May 2002, coincides with AKAV vector-activity in August through December of the previous year (2001). The absence of arthrogryposis is not surprising, since similar findings were documented elsewhere [4]. This was not the only difference from the AHS epidemic of 1969/70 in Israel [6, 11], since besides to the different clinical presentations, sheep and goats were not affected this time. Geographically, the outbreak of 1969/70 occurred in the same area, namely northern Israel, and was recorded in the following year (1970) about 200 km further south. The same distribution was noted in the current outbreak in Israel which is now (2003) experiencing the same clinical feature in the central and inner coastal plain and in farms located near the Red Sea, 250 km and 400 km further south, respectively (personal communication).

It was surprising to find a relatively high sero-reactivity to AINOV in 29.6% (8/27) of the sera that came from the "control" geographical zone; of these only one sample was AKAV sero-positive (Table 2). This was probably because of a cross-reaction that exists within the Simbu sero-group [4,7] (SNT for AKAV was 1:8 while for AINOV it was >1:64; data not shown). A search of the literature failed to reveal evidence of another Simbu group virus circulating in the Middle East. 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 5–6 years ago.

It is not clear whether AKAV in Israel is endemic or occurs sporadically. Other open questions concern whether AKAV infection appears alone or in combination with other arbovirus(es), and other Simbu group viruses have invaded Israel. There is also uncertainly concerning AKAV's probable (major) vector in Israel and in the eastern Mediterranean Basin [7]; *Culicoides imicola* is present worldwide between latitudes 35°S and 40°N [7], and its presence in northern Israel is known, but the source of the AKAV (or of other Simbu group arboviruses) has not yet been identified. The distribution of *Culicoides* spp depends on climatic (macro-global and micro-regional) changes and reasons for its appearance are unresolved.

This probable reintroduction of AKAV into our region, the evidence of the presence of AINOV and the epidemics of bluetongue virus in the south of Europe and the east Mediterranean basin [7], together with the presence of West Nile virus in Israel [10] and North America [1], are all indications of the potential and actual spread of arboviruses into previously uninfected areas.

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