



The association of winds with the spread of EHDV in dairy cattle in Israel during an outbreak in 2006

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ABSTRACT

Winds may play a major role in spread of arthropod-borne viruses (arboviruses). Arboviruses like epizootic hemorrhagic disease virus (EHDV), bluetongue virus and bovine ephemeral fever virus (BEFV) frequently cause major outbreaks in Israel with a unique pattern of spread. Most of these outbreaks begin in the Jordan valley, near the Sea of Galilee and then spread to the north, south and west through the major valleys of Israel. The aim of this study was to describe the spread pattern in such an outbreak and to find if this pattern can be explained by winds. Herein, we compared the spread rate to each direction and used Cox proportional hazards model to test factors associated with the spread of EHDV, which emerged in dairy cattle in Israel during the summer of 2006. Documented, clinical and serological data on spread of the outbreak were then compared with wind data collected by meteorological stations along the trail of virus spread and with modeled winds at high altitude (>500 m). The analysis revealed that both the hazard and the rate of outbreak spread to the south and to the north were significantly higher than to the west. Average rate of outbreak spread during periods in which at least 3 h of winds to spread direction were recorded was 20,880 m/week (SD = 13,230) vs. 7486 m/week (SD = 4936) in periods during which no such winds were recorded. Serological evidence demonstrated exposure to the virus up to 166 km away from the location of the initial outbreak center. Modeled wind data showed that this spread may be explained by winds at high altitudes. Animal movements due to shipments of feedlot calves and slaughters could not explain the spread pattern observed during the outbreak. This study therefore shows that winds are probably a major contributory factor for long and medium distance spread of *Culicoides* borne viruses in this region.

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1. Introduction

It was previously suggested that winds may be associated with spread of arthropod-borne (arboviruses) through active or passive dispersal of their vectors (Reynolds et al., 2006). Two of the most important arboviruses of livestock are epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). Both are double stranded RNA

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viruses which belong to the genus *Orbivirus* and the family *Reoviridae* and are transmitted by several midge species of the genus *Culicoides* (Du Toit, 1944; Mellor et al., 2000; Paweska et al., 2005). As these are very small insects (only a few mm in size) they may be carried easily by winds. Winds, therefore, may have an important role in the rate of virus spread. Indeed, previous findings have suggested that winds may played an important role in the spread of BTV-8 in Europe (Hendrickx et al., 2008), the dissemination of bluetongue among herds in Turkey, Greece and Bulgaria (Ducheyne et al., 2007) its introduction into the Balearic Islands in 2000 (Alba et al., 2004) and into Israel (Braverman and Chechik, 1996). Spread of BTV-8 into England was predicted by analysis of wind patterns (Gloster et al., 2007).

The climate in the Jordan valley which is located in the north of the Israeli part of the Rift valley, allows the maintenance of many vector species from different biogeographical areas. Therefore, livestock diseases caused by arboviruses are frequent in this region (Brenner et al., 2004; Purse et al., 2004; Yeruham et al., 2005). The small size of Israel as well as its unique topography, with the Rift valley at the east connected by western valleys to the western coastal plain enables strict analysis of the association of winds and animal movements with arbovirus dispersion. During 2006, a cattle virulent epizootic hemorrhagic disease virus appeared in Israel (Yadin et al., 2008) and caused production losses of 2.5 millions USD to the dairy cattle industry in Israel (Kedmi et al., 2010). This outbreak allowed us to examine the effect of winds on spread of a *Culicoides* borne viruses in this unique region. Epidemiological investigation which included analysis of outbreak spread as well as winds and animal movement in this period enabled us to perform an assessment of the major factors that may influence its spread in Israel.

2. Materials and methods

2.1. Outbreak dataset

We analyzed the data collected during a large outbreak of EHDV, which took place in Israel, during the summer and autumn of 2006. Surveillance of the outbreak was made by a mutual effort of the 'Hahaklait', the veterinary school and the veterinary institute. 'Hahaklait' is a farmers' cooperative that provides veterinary services to about 80% of the cattle population in Israel. During the outbreak, a directive was sent by the chief veterinarian of the 'Hahaklait' to all veterinarians to report any clinical symptoms suggestive of EHD, according to the following case definition of an affected herd: conjunctivitis and/or salivation and/or stiff gate and/or congested mucous membranes in two or more cows, accompanied by an abrupt reduction in milk production in several cows in the herd. These data were recorded on a daily basis.

The first affected herd in this outbreak was reported on August 28, 2006. The virus was spread from this herd southward towards the Dead Sea and northward along the trail of the Rift valley and up to the northern border of Israel with Lebanon. Morbidity was then expanded from the primary outbreak region westward through the valleys and into the

coastal plain. Overall, the disease was reported in 83 dairy herds located in 78 rural localities and from 22 beef herds. Most of the herds were affected during the first 8 weeks of the outbreak. However, a few more newly affected herds continued to be reported for 5 weeks more, until the third week of November.

2.2. Sample collection

Serum samples were collected from 71 dairy herds at least 1 month after first appearance of clinical signs in the herd. In order to detect clinically unapparent infection, sera were collected from a representative sample of 73 dairy herds which did not report of EHDV-suggestive clinical signs. All were dairy herds of high-producing Israeli Holstein cows (average national annual production 11,281 kg/cow), held under zero grazing in free-stall sheds. All were closed herds, i.e. breed their own replacement heifers. Veterinarians were instructed to collect the following serum samples from each case herd: five samples from calves aged 6–12 months, five from apparently healthy cows, five from cows that had begun showing clinical signs (including reduction in milk production) at least 2 weeks before blood sample collection and five samples from cows that were sick during collection. In the control herds, serum samples were collected only from calves and healthy cows. The sample size rationale was to favor sampling of wide geographical distribution over collection of high number of samples from smaller number of herds. According to this sampling strategy the probability to miss infection in a herd in which 20% of the cows were affected was calculated to be 10%. For a 30% affected herd this probability was less than 3%. This was calculated according to the formula $u = (1 - P)^n$ where u is the probability to miss infection, P is the seroprevalence and n is the number of sampled cows.

2.3. Serological assays

A competitive enzyme-linked immunosorbent assay (c-ELISA) similar to an assay previously presented by Thevasagayam et al. (1996) was used to identify the presence of antibodies against EHDV in the serum samples. This ELISA was previously shown to have no cross-reaction with bluetongue virus and to detect exposure to all serotypes of EHDV. Specificity and sensitivity of this assay were shown to be 100% (as compared to agar immuno-diffusion test) (Thevasagayam et al., 1996). Briefly, the EHDV c-ELISA was performed as follows: EHDV-1, purified as previously described (Thevasagayam et al., 1995), was incubated at a dilution of 1:250 at 37°C for one hour on an orbital shaker. The plates were washed three times prior to loading of the test serum samples, control sera and competing monoclonal antibody (EHDV VP7-specific, at a dilution of 1:100). Four wells were loaded with all reagents but no sera and were considered zero-competition controls (CO). After incubation (1 h, 37°C), the conjugate (Dako rabbit anti-mouse horseradish peroxidase 1:1000) was added and the plate was incubated for 1 h at 37°C. The substrate (Zymed TMB single solution) was added and after incubation (15 min), the reaction was stopped by the addition of 1 M sulfuric acid. The plates were read at

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