Final Scientific Report

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Project Title: Exploration of the Epidemiology of a Newly Emerging Cattle-Epizootic Hemorrhagic Disease Virus in Israel

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Abbreviations commonly used in the report, in alphabetical order:

BTV – bluetongue virus, EHDV – epizootic hemorrhagic disease virus, WTD – white-tailed deer

Budget: IS: $ 150,000 US: $ 157,000 Total: $ 307,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Appendix G6a
Final Scientific Report

Publication Summary (numbers)

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<tr>
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Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

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-2-
Abstract

In September 2006 an outbreak of ‘Bluetongue like’ disease struck the cattle herds in Israel. Over 100 dairy and beef cattle herds were affected. Epizootic hemorrhagic disease virus (EHDV) (an Orbivirus closely related to bluetongue virus (BTV)), was isolated from samples collected from several herds during the outbreaks. Following are the aims of the study and summary of the results: which up until now were published in 6 articles in peer reviewed journals. Three more articles are still under preparation:

1. To identify the origin of the virus: The virus identified was fully sequenced and compared with the sequences available in the GenBank. It appeared that while gene segment L2 was clustered with EHDV-7 isolated in Australia, most of the other segments were clustered with EHDV-6 isolates from South-Africa and Bahrain. This may suggest that the strain which affected Israel on 2006 may have been related to similar outbreaks which occurred in north-Africa at the same year and could also be a result of reassortment with an Australian strain (Wilson et al. article in preparation). Analysis of the serological results from Israel demonstrated that cows and calves were similarly positive as opposed to BTV for which seropositivity in cows was significantly higher than in calves. This finding also supports the hypothesis that the 2006 EHD outbreak in Israel was an incursive event and the virus was not present in Israel before this outbreak (Kedmi et al. Veterinary Journal, 2011)

2. To identify the vectors of this virus: In the US, Culicoides sonorensis was found as an efficient vector of EHDV as the virus was transmitted by midges fed on infected white tailed deer (WTD; Odocoileus virginianus) to susceptible WTD (Ruder et al. Parasites and Vectors, 2012). We also examined the effect of temperature on replication of EHDV-7 in C. sonorensis and demonstrated that the time to detection of potentially competent midges decreased with increasing temperature (Ruder et al. in preparation). Although multiple attempts were made, we failed to evaluate wild-caught Culicoides insignis as a potential vector for EHDV-7; however, our finding that C. sonorensis is a competent vector is far more significant because this species is widespread in the U.S. As for Israeli Culicoides spp, the main species caught near farms affected during the outbreaks were C. imicola and C. oxystoma. The vector competence studies performed in Israel were in a smaller scale than in the US due to lack of a laboratory colony of these species and due to lack of facilities to infect animals with vector borne diseases. However, we found both species to be susceptible for infection by EHDV. For C. oxystoma, 1/3 of the Culicoides infected were positive 11 days post feeding.

3. To identify the host and environmental factors influencing the level of exposure to EHDV, its spread and its associated morbidity: Analysis of the cattle morbidity in Israel showed that the disease resulted in an average loss of over 200 kg milk per cow in herds affected during September 2006 and 1.42% excess mortality in heavily infected herds (Kedmi et al. Journal of Dairy Science, 2010). Outbreak investigation showed that winds played a significant role in virus spread during the 2006 outbreak (Kedmi et al. Preventive Veterinary Medicine, 2010). Further studies showed that both sheep (Kedmi et al. Veterinary Microbiology, 2011) and wild ruminants did not play a significant role in virus spread in Israel (Kedmi et al. article in preparation). Clinical studies in WTD showed that this species is highly susceptible to EHDV-7 infection and disease (Ruder et al. Journal of Wildlife Diseases, 2012). Experimental infection of Holstein cattle (cows and calves) yielded subclinical viremia (Ruder et al. in preparation).

The findings of this study, which resulted in 6 articles, published in peer reviewed journals and 4 more articles which are in preparation, contributed to the dairy industry in Israel by defining the main factors associated with disease spread and assessment of disease impact. In the US, we demonstrated that sufficient conditions exist for potential virus establishment if EHDV-7 were introduced. The significant knowledge gained through this study will enable better decision making regarding prevention and control measures for EHDV and similar viruses, such as BTV.
Achievements:

1. Serological evidence demonstrates that the outbreak of EHDV was probably due to a new invasion of the virus. However, genetic evidence shows that the virus which caused the outbreak may have evolved from reassortment of virus from Australia and a virus that circulated in the region prior to the outbreak.

The virus identified was fully sequenced and compared with the sequences available in the GenBank. It appeared that while gene segment L2 was clustered with EHDV-7 isolated in Australia, most of the other segments were clustered with EHDV-6 isolates from South-Africa and Bahrain. This may suggest that the strain which affected Israel on 2006 may have been related to similar outbreaks which occurred in north-Africa at the same year and could also be a result of reassortment with an Australian strain.

Analysis of the serological results from Israel demonstrated that cows and calves were similarly positive as opposed to BTV for which seropositivity in cows was significantly higher than in calves. This finding also supports the hypothesis that EHDV invaded Israel during 2006 and was not present there before this outbreak.

2. Analytical assessment of morbidity, mortality and losses associated with EHDV, which will enable evidence based decision making regarding resource allocation for disease control.

Herds affected during the first, second, and third month of the outbreak (September-November) experienced an average loss of 207 (95% CI=154-261), 137 (63-211), and 52 (27-76) kg of milk/milking cow, respectively, during the outbreak period. An average excess mortality and involuntary culling of 1.47/100 cows was documented in herds affected in September. High correlation was observed between EHDV seroprevalence and milk loss; average milk loss for herds with seropositivity of 26 to 50, 51 to 75, and 76 to 100% was 84, 133, and 204 kg of milk/milking cow, respectively. A 1.42% (0.91-1.93%) increase in mortality was observed in herds with seroprevalence above 50%. Losses for the dairy cattle industry interpolated from these data were estimated at US$2,491,000 (US$1,591,000-3,391,000), an average loss of US$26.5/cow in the Israeli dairy cattle. This equals 0.55% of the average total value production of a dairy cow in Israel. This is the first study to estimate the production losses caused by EHDV or any bluetongue-like disease.

3. Determination of the main environmental factors associated with disease spread which enables better understanding of spread of vector borne diseases and helps prediction of spread of the next
A significant association between exposure to EHDV and BTV was demonstrated in both univariate and multivariate analyses. Recent exposure to BTV and EHDV (demonstrated by seroprevalence in calves) was clustered in different geographical locations, indicating that the two viruses had different patterns of spread, that of EHDV being influenced by winds and terrain barriers and that of BTV by herd immunity. The analysis of EHDV spread revealed that both the hazard and the rate of outbreak spread to the south and to the north of Israel 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 animals to feedlots or slaughter facilities 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.

4. No involvement of sheep and wild ruminants (except buffalo) was observed in the EHDV outbreak in Israel. In the US, experimentally infected WTD demonstrated severe disease and strong viremia while infected cattle showed no clinical signs and only mild viremia. Together, these findings demonstrate that the host population structure and not just the environment, determines outbreak outcome. This has implication on the method for disease control in both Israel and the US.

Sixty-six sheep and lambs scattered in seven herds were compared to 114 cows and calves scattered in 13 dairy cattle herds, matched to the sheep herds by location. While antibody prevalence to EHDV was high in cattle (35.2% within the outbreak zone) no evidence of exposure to EHDV was found in sheep (p<0.0001). Antibodies to BTV were apparent in both cattle and sheep though in the former it was significantly higher (63.2%, 16.7% respectively, p<0.0001), suggesting higher exposure of cattle to biting Culicoides midges. In addition, a serological investigation of wild ruminants in Israel suggests that wildlife did not play a role in the outbreak. Only 7 out of 619 examined sera were serologically positive for EHDV. Five of these were domesticated buffalo (Bubalus bubalis) sera collected at two locations in Israel. The only other EHDV positive wild animal was an Iranian sheep (Ovis aries) of
which two serum samples were collected before the 2006 outbreak. The results of this study indicate that in the EHDV outbreak that occurred in Israel on 2006, wild ruminants and sheep did not play a major role in the transmission of EHDV.

In the US, six, 8-mo-old WTD were experimentally infected with EHDV-7, and all became infected and exhibited varying degrees of clinical disease. Clinical signs, clinicopathologic abnormalities, and postmortem findings were consistent with previous reports of orbiviral hemorrhagic disease (HD) in this species. Four of six animals died or were euthanized because of the severity of disease, one on post-inoculation day (PID) 5 and the remaining WTD on PID 7. All deer had detectable viremia on PID 3, which peaked on PID 5 or 6 and persisted for as long as PID 46 in one animal. Deer surviving the acute phase of the disease seroconverted by PID 10. Based on the 67% mortality rate we observed, this strain of EHDV-7 is virulent in WTD, reaffirming their role as a sentinel species for the detection of endemic and nonendemic EHDV. Further, the observed disease was indistinguishable from previous reports of disease caused by North American EHDV and bluetongue virus serotypes, highlighting the importance of serotype-specific diagnostics during suspected HD outbreaks.

Following the WTD study, we performed two experimental trials in Holstein cattle, first in mature cows then in calves, both yielding subclinical infections (Ruder et al. in preparation). For the cow trial, a blood inoculum prepared in WTD was administered by SC and ID injection to each cow. Three of four mature cows developed a transient, low-titer viremia (<$10^{2.3}$ median tissue culture infective doses (TCID$_{50}$/ml) and all four seroconverted. For the calf trial, different inoculation methods were attempted: SC and ID injection; SC, ID, and IV injection; or midge bite. These alterations were made in an attempt to replicate disease observed in the field. Other than a febrile response by the calves that were inoculated with an IV component, no clinical abnormalities were observed. All six calves developed viremia ($10^{2.63}$-$10^{3.5}$ TCID$_{50}$/ml) beginning 3 days post-infection and persisting for the duration of the study (18 dpi). Although disease was not replicated, we showed cattle are susceptible to infection experimentally.

5. *Culicoides sonorensis*, the main vector of EHDV in the US was infected after feeding on infected WTD and transmitted the virus to susceptible WTD. In Israel *C. oxystoma* and *C. imicola* were positive 12 days after infection.

To evaluate the susceptibility of *C. sonorensis*, midges were fed on EHDV-7 infected WTD, held at 22 ± 1°C, and processed individually for virus isolation and titration on 4-16 days post feeding (dpf).
Midges with a virus titer of $\geq 10^{2.7}$ TCID$_{50}$/midge were considered potentially competent. To determine if infected C. sonorensis were capable of transmitting EHDV-7 to a host, a susceptible WTD was then fed on by a group of 14-16 dpf midges. From 4-16 dpf, 45% (156/350) of midges that fed on WTD with high titer viremia ($>10^7$ TCID$_{50}$/ml) were virus isolation-positive, and starting from 10-16 dpf, 32% (35/109) of these virus isolation-positive midges were potentially competent ($\geq 10^{2.7}$ TCID$_{50}$/midge). Midges that fed on infected deer transmitted the virus to a susceptible WTD at 14-16 dpf. The WTD developed viremia and severe clinical disease. We also examined the effect of temperature on replication of EHDV-7 in C. sonorensis and demonstrated that the time to detection of potentially competent midges decreased with increasing temperature: 12 days post-feeding (dpf) at 20 °C, 6 dpf at 25 °C, and 3 dpf at 30 °C. The findings are consistent with previous studies of related orbiviruses, showing that increasing temperature can shorten the apparent extrinsic incubation period (Ruder et al. in preparation). In addition, wild-caught, adult Culicoides insignis were trapped in Florida, USA on multiple occasions during September 2011. The midges were brought back to the University of Georgia and experimental infection attempts were made by offering midges a blood meal spiked with EHDV-7. Despite multiple attempts, none of the midges (n=185) took a bloodmeal using these artificial techniques. Although we failed to evaluate C. insignis as a potential vector for EHDV-7, our finding that C. sonorensis is a competent vector is far more significant because this species is widespread in the U.S.

As for Israeli Culicoides spp., the main species caught near farms affected during the outbreaks were C. imicola and C. oxystoma. The Culicoides EHDV-7 susceptibility studies performed in Israel were on a smaller scale than in the US due to lack of a laboratory colony of these species and due to lack of facilities to infect animals with vector borne diseases. However, we found both species to be susceptible for infection by EHDV. For C. oxystoma, 1/3 of the Culicoides infected were positive 11 days post feeding.
Details of cooperation:

The BARD project resulted in a very fruitful cooperation between the American and Israeli partners. The scientific cooperation resulted in two joint published articles and three articles that are still under preparation. In addition, there were mutual visits of PhD students supported by this grant and a visit by the Israeli PI to UGA. During November 2010, Dr. Maor Kedmi traveled to UGA, where he performed together with Dr. Mark Ruder the cattle infection study with the Israeli EHDV (US aim 4). While staying in UGA, Dr. Kedmi actively participated in the clinical trial as well as the laboratory work, including post-mortem evaluation, virus isolation, handling of Culicoides and PCR testing. Dr. Kedmi also gave a seminar at UGA, reporting the results of the studies performed in Israel as part of the BARD project. During September 2012, Dr. Mark Ruder traveled to Israel. He spent time with faculty, staff, and students at the Koret SVM and shared his experience with virus isolation techniques from both animal and insect samples. While staying in Rehovot, Dr. Ruder also traveled to northern Israel to visit dairy and beef herds and was able to assist with a field investigation of an active lumpy skin disease outbreak. Dr. Ruder gave two seminars while in Israel (one at the Kimron Veterinary Institute and one at the Koret SVM), reporting the results for the BARD project.
List of publications:


