Temporospatial distribution of Culicoides species and Culicoides imicola in northern Jordan

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Abstract

The aim of this study was to estimate geographical distribution of Culicoides species and Culicoides imicola in northern governorates of Jordan. The study was conducted by placing light traps in four climatically different geographical locations during 2011. Suitability maps were created by layering and compiling climatic parameters into the GIS data to highlight locations and time suitable for growth of C. imicola. Collected insect samples were assorted by morphology to identify Culicoides species. Molecular analysis was used to identify Culicoides spp. and C. imicola. In total, 25,196 insects were trapped of which 3491 (12.7%) were morphologically identified as Culicoides spp. The highest counts Culicoides spp. were recorded in Deir Alla (47%), Bani-Kenaneh (31%) and Al-Shouneh (21%) respectively. The peak activity was recorded during August through October. Morphological identification failed to identify Culicoides spp. in all locations except Al-mafraq. C. imicola could only be identified in Deir Alla, Bani-Kenaneh and Al-Shouneh. There was no evidence of viral genome of epizootic hemorrhagic disease virus, blue tongue virus and bovine ephemeral fever virus in the trapped midges.

Introduction

Culicoides are small biting midges belonging to the Ceratopogonidae family, which are of human and veterinary significance.1 There are more than 1300 identified Culicoides species worldwide. Taxonomic identification of Culicoides species is traditionally based on morphological characterization of the insect’s wing pattern.2 Culicoides spp. are characterized by the presence of two radial cells that are absent from other genera in the family.3 Morphological identification of Culicoides spp. might be time-consuming and difficult.4 Culicoides species have been reported to be a vector of many economically significant diseases in horses and ruminant including African horse sickness and blue tongue.5 The African horse sickness is considered a notable disease by the World Organization for Animal Health. In the Middle East, Culicoides imicola has been implicated to play a role in the occurrence of two economically significant diseases that affected the cattle industry including epizootic hemorrhagic disease (EHD) and bovine ephemeral fever.6 In 2006, serotypes 6 and 7 of EHD virus were detected in Jordan Valley in Israel.7 In Jordan, few suspected cases have been reported in the same year to be serologically positive for the EHD virus however the virus has never been isolated.8 Furthermore, serotype 7 of EHD virus was detected in Turkey in 2007.9 Till today, there has been no evidence for the described viruses, although Culicoides have been recorded in the region. Since then, no reported research efforts were undertaken to further investigate and characterize occurrence of Culicoides spp. in Jordan. Therefore, the broad goals of the research presented here were: to evaluate the spatiotemporal distribution of Culicoides spp. and C. imicola in northern governorates of Jordan and to establish vector suitability map to help highlight locations and time suitable for occurrence of C. imicola. Furthermore, to test the presence of epizootic hemorrhagic disease virus, blue tongue virus and bovine ephemeral fever virus in the morphologically identified pooled samples by real time polymerase chain reaction (RT-PCR). Our hypothesis is that C. imicola does occur in Jordan and will be identified in some environmentally suitable locations.

Materials and Methods

Collection sites

Insect samples were collected from eight geographical sites located within five governorates in northern Jordan during June through November 2011. Those locations are: Irbid (Irbid city, Bani-Kenaneh, Elmazar and Al-Shouneh); Al-mafraq (Al-mafraq city); Jerash (Jerash city); Zarqa (Ad-Dulayl) and Albalqa (Deir Alla). Study locations were classified based on climate into: Deir Alla and Al-Shouneh where the climate is characterized as warm steppe climate; Ad-Dulaul and Al-mafraq have a cool desert climate; Jerash, Irbid and Bani-Kenaneh have a warm temperate climate and Elmazar which considered as cool temperate rainy climate.10

Collection of insects

Insect samples were collected from farms were permitted by owner after signing a consent form. Insects were collected using CDC-light traps. The traps were placed inside and around the cattle farms one meter above ground level in the dark. 3-4 traps were placed overnight per each farm and the trapped insects were then transferred to the laboratory in a container containing sugar solution-soaked cotton.

Processing and analysis

Upon arrival to the parasitology laboratory, the collected insect samples were washed with mild soap detergent. Initial morphological identification of the Culicoides spp. was based on the wing pattern using dissecting and light microscope as described by Glukhova.11 The morphologically identified Culicoides spp. were assorted into groups of 10-100 insects and stored in a 2 mL microcentrifuge tube containing 0.5 mL PBS at −80°C for further molecular analysis. The identified samples were homogenized using Omni Bead Ruptor
Climate parameters

Climate parameters used to establish suitability maps for study locations for the occurrence of *C. imicola* were ambient daily temperature, wind speed and humidity. The climate parameters were monthly averaged and combined to create vector suitability maps using Arc Gis (Version 9.3) during the period June through November 2011. The reported optimal temperature, humidity and wind speed for *C. imicola* peak activity is $\pm 18 \pm 28^\circ C$, $75-85\%$ and $\pm 2.5 \text{ m/s}$ respectively. The study locations were classified based on potential risk value ranging from low to high.

Results

The number of collected insect samples during the study period is characterized in Table 1. The total numbers of collected insects were 25,196 samples of which 3194 samples (12.7%) were morphologically identified as a *Culicoides* spp. according to the wing pattern. There was a considerable variation in the occurrence of *Culicoides* spp. in the studied region. The lowest occurrence of *Culicoides* activity was in recorded in June while the highest activity was recorded in September. In the study regions, 47, 31 and 21% of the trapped *Culicoides* were recorded in Deir Alla, Bani-Kenaneh and Al-Shouneh respectively.

The analysis of the PCR data indicated presence of *Culicoides* spp. in all study locations except Al-Mafraq. *C. imicola* could be identified only in three locations: Al-Shouneh, Bani-Kenaneh and Deir Alla. Morphological characterization could not identify *Culicoides* spp. in four locations while PCR findings confirmed that some of the insect samples initially presumed to be morphologically non-*Culicoides* are actually identified as *Culicoides* (Figure 1, Table 2). The RT-PCR findings suggest absence of epizootic hemorrhagic disease virus, blue tongue virus and bovine ephemeral fever virus in the tested insect samples.

The generated suitability map highlighted locations and time suitable for occurrence of *C. imicola*. Analysis of the suitability maps suggests that *C. imicola* does occur in particular areas in Jordan. The data of the suitability maps are color-coded into Table 2 and Figure 2. It is noticeable that regions where *C. imicola* identified have an altitude of lower than approximately 400 m AMSL (Table 2).

Discussion

The current study was undertaken to estimate occurrence and geographical distribution of *Culicoides* spp. and *C. imicola* in four climatically different regions in northern Jordan during 2011. The suitability maps identified geographical locations and time suitable for the occurrence of *C. imicola*.

The abundance rate of insects morphologically characterized as *Culicoides* spp. was estimated to be approximately 12.7% of the total trapped insect samples. However, authors speculate that the actual abundance rate of *Culicoides* in the study locations might be higher. Findings of PCR analysis showed that pooled samples, initially characterized as non-*Culicoides* spp., actually identified as *Culicoides* spp. The authors concluded that some of the captured insect samples could not be easily classified morphologically. Previous
research efforts have suggested that identification of some Culicoides spp. based on morphological traits might be challenging.\(^{16,17}\) This might explain the mischaracterization of some insect samples based on morphology. The PCR analysis therefore could potentially be more practical to accurately identify mixed collections of insects especially when examining massive number of samples during outbreaks.\(^{18,19}\) In our study, the PCR analysis identified Culicoides spp. in all study locations except Al-mafraq area while morphological characterization could only identify Culicoides in four locations and C. imicola were identified in three locations. Previous research has characterized occurrence and distribution of different Culicoides spp. For instance, the prevalence rate of the identified Culicoides species varied depending on the study region. In Zimbabwe and Namibia, C. imicola was the most abundant Culicoides spp. with a prevalence rate of 80.4 and 99.4% respectively.\(^{20,21}\) In Kingdom of Saudi Arabia, abundance rate of C. imicola was estimated to be 19% of the morphologically identified Culicoides spp. and the peak activity was recorded during May of 2004-2006.\(^{22}\) While in Israel, the peak activity of C. imicola was recorded in September.\(^{23}\) In here, there was no molecular evidence of C. imicola occurrence in all location except Al-mafraq while C. imicola was found in three regions. In here, the effect of the availability of the surface water on the activity of C. imicola was not studied. However, it is worth mentioning that areas found that have more abundant C. imicola had relatively more abundant surface water sources such as open irrigation systems, springs and man-made ponds. The combination of climatic parameters in addition to the availability of water source may have provided suitable environment for occurrence of C. imicola. It is noticeable that regions where C. imicola identified have an altitude of lower than approximately 400 m AMSL.

In here, there was no molecular evidence of the viral genome in the trapped Culicoides midges. However, at the time of the insect’s collection there were no reports of clinical cases related to the described viruses in the study regions. Previous reports have suggested that detection of vector-borne viral diseases is usually low even during occurrence of outbreaks. Experimental study designs have only reported of less than 1% recovery rate of viruses from insects fed on infected blood.\(^{27}\)

In this work, there are only a few limitations. The study regions were limited to the northern parts of Jordan. Establishing effective preventive or control strategies require further research that covers more regions over multiple years span to overcome the climatic fluctuations and variation. The significance of study reported here is considered the first research efforts to document occurrence of C. imicola in Jordan. The results reported here highlighted areas suitable for optimal C. imicola infestation. Understanding the spatiotemporal distribution of C. imicola is of clinical veterinary significance when implementing diagnostic and surveillance measures particularly during occurrence of outbreaks.

### Table 1. Total monthly number of trapped insects in the study regions.

<table>
<thead>
<tr>
<th>Collection area</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deir Alla</td>
<td>0</td>
<td>0</td>
<td>752 (344)</td>
<td>2585 (550)</td>
<td>981 (400)</td>
<td>628 (228)</td>
</tr>
<tr>
<td>Elmazar</td>
<td>265 (0)</td>
<td>437 (0)</td>
<td>309 (0)</td>
<td>209 (0)</td>
<td>322 (0)</td>
<td>NC</td>
</tr>
<tr>
<td>Aq-Duayl</td>
<td>4556 (0)</td>
<td>845 (0)</td>
<td>276 (0)</td>
<td>756 (0)</td>
<td>403 (0)</td>
<td>423 (0)</td>
</tr>
<tr>
<td>Al-mafraq</td>
<td>0</td>
<td>0</td>
<td>635 (0)</td>
<td>817 (0)</td>
<td>400 (0)</td>
<td>185 (0)</td>
</tr>
<tr>
<td>Jerash</td>
<td>645 (0)</td>
<td>237 (0)</td>
<td>209 (0)</td>
<td>414 (0)</td>
<td>657 (0)</td>
<td>NC</td>
</tr>
<tr>
<td>Al-Shouneh</td>
<td>756 (0)</td>
<td>894 (130)</td>
<td>840 (120)</td>
<td>851 (143)</td>
<td>1040 (150)</td>
<td>760 (133)</td>
</tr>
<tr>
<td>Irbid</td>
<td>345 (0)</td>
<td>834 (0)</td>
<td>548 (0)</td>
<td>430 (0)</td>
<td>212 (0)</td>
<td>NC</td>
</tr>
<tr>
<td>Bani-kenahe</td>
<td>888 (220)</td>
<td>708 (145)</td>
<td>745 (320)</td>
<td>973 (187)</td>
<td>547 (124)</td>
<td>NC</td>
</tr>
</tbody>
</table>

Parenthesis indicates the number of Culicoides species identified morphologically; NC, no collection was performed.

### Table 2. Summary for detection of Culicoides species and C. imicola using polymerase chain reaction.

<table>
<thead>
<tr>
<th>Collection area</th>
<th>Elevation (m)</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deir Alla</td>
<td>-223</td>
<td>*</td>
<td>*</td>
<td>Cu/Cm*</td>
<td>Cu/Cm*</td>
<td>Cu/Cm*</td>
<td>Cu/Cm</td>
</tr>
<tr>
<td>Elmazar</td>
<td>688</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
</tr>
<tr>
<td>Aq-Duayl</td>
<td>579</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
</tr>
<tr>
<td>Al-mafraq</td>
<td>705</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
</tr>
<tr>
<td>Jerash</td>
<td>620</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
<td>Cu</td>
</tr>
<tr>
<td>Al-Shouneh</td>
<td>-201</td>
<td>Cu*</td>
<td>Cu/Cm*</td>
<td>Cu*</td>
<td>Cu*</td>
<td>Cu/Cm</td>
<td>Cu</td>
</tr>
<tr>
<td>Irbid</td>
<td>568</td>
<td>Cu</td>
<td>Cu/Cm*</td>
<td>Cu*</td>
<td>Cu*</td>
<td>Cu</td>
<td>Cu</td>
</tr>
<tr>
<td>Bani-kenahe</td>
<td>417</td>
<td>Cu</td>
<td>Cu/Cm*</td>
<td>Cu*</td>
<td>Cu*</td>
<td>Cu</td>
<td>Cu</td>
</tr>
</tbody>
</table>

Cu, detection of Culicoides species; Cu, detection of C. imicola; * high risk for occurrence of C. imicola according to the generated suitability maps.
References