

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* species in 4 locations while polymerase chain reaction analysis identified *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); Jarash (Jarash 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

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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

Homogenizer (Omni International, GA, USA). The genomic DNA was isolated from insects' samples using Promega Wizard® Genomic DNA Purification Kit according to manufacturer instructions (Promega, WI, USA). Oligonucleotide specific-*Culicoides* spp. and *C. imicola* primers for the PCR assay were selected based on the published nucleotide sequence to amplify DNA fragment of the ITS-1 rDNA subunit in the *Culicoides* spp. genome and for amplification of 316 bp DNA fragment of the ITS-1 rDNA subunit in the *C. imicola* genome as described by Cetre-Sossa and colleagues.¹²

The morphologically identified insect pooled samples were tested for presence of epizootic hemorrhagic disease virus, blue tongue virus and bovine ephemeral fever virus. The genome of the described viruses was extracted using extraction kits (Intron, South Korea) according to the manufacturer's instructions. The samples were tested for the presence of those viruses using specific RT-PCR kits according to the manufacturer's instructions (Primer Design, UK).

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 $\geq 18 \pm 28^\circ\text{C}$, 75-85% and ≤ 2.5 m/s respectively.¹³⁻¹⁵ 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

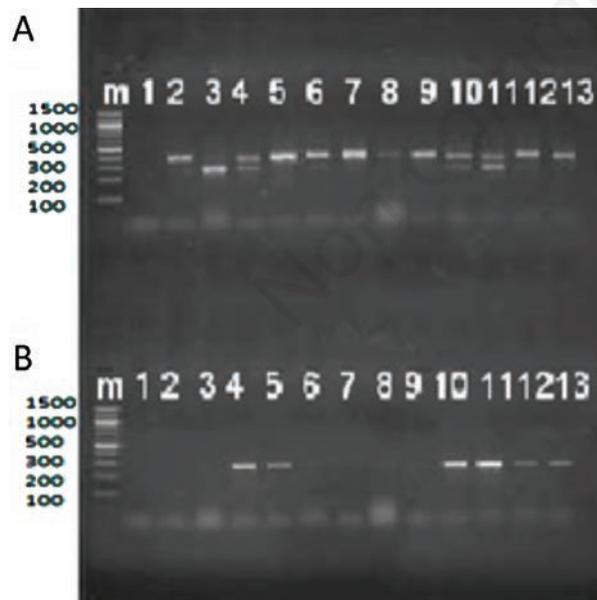


Figure 1. Representative gel electrophoresis for polymerase chain reaction products. (A) Indicate amplification of the *Culicoides* species common ITS1 sequence (PanCulF/PanCulR). (B) Indicates amplification of *C. imicola* ITS1 specific sequence. M indicates 100 bp DNA ladder, lane 1 indicates negative control, lanes 2, 6, 7, 8, 9, 11, 12, 13 indicate samples from Deir Alla, lane 3 indicates samples from Aq-Dulayl, lane 4 indicates samples from Bani-kenaneh, lanes 5 and 10 indicates samples from Al-Shouneh.

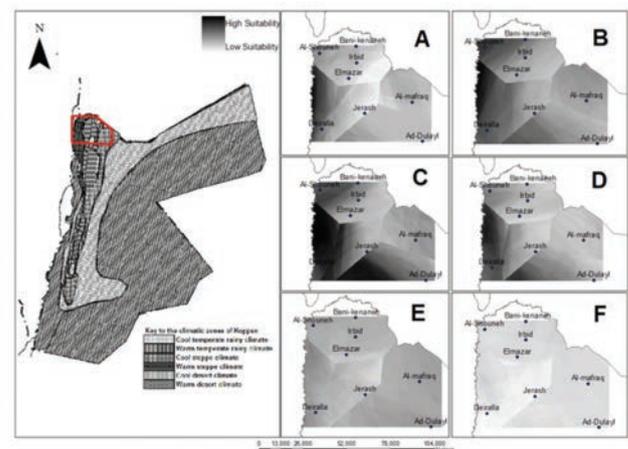


Figure 2. Categories of the climatic zones of the study locations and the monthly geographical information system suitability maps for *C. imicola* for the study regions from June (A) through November (F) during 2011. The darker the color means the more suitable conditions for occurrence of *C. imicola*.

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 obvious monthly variation in the occurrence rate and monthly distribution of *Culicoides* spp. and *C. imicola*. Our results

showed that *Culicoides* spp. are most abundant during September while *C. imicola* can occur during July, September, October and November. There are existing substantial research efforts that investigated the effect of several factors on the activity of *Culicoides* spp. including air temperature, wind speed, humidity, soil moisture, topography, land use, vegetation cover, presence of ruminant hosts.^{24,25} In the current work, suitability map for *C. imicola* was created by layering and compiling climate data from trapping site into the geographical information system.¹⁴ The suitability map identified geographical regions and time that match optimal conditions suitable for occurrence of *C. imicola*. There is a considerable variation in the climatic parameters and altitude of study areas. The altitude of the study regions ranged from 223 m BMSL to 808 m BMSL.²⁶ It is noticeable that *Culicoides* was found 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.

Collection area	June	July	Aug	Sep	Oct	Nov
Deir Alla	0	0	752 (344)	2585 (550)	981 (400)	628 (228)
Elmazar	265 (0)	437 (0)	309 (0)	209 (0)	322 (0)	NC
Aḍ-Ḍulayl	4356 (0)	845 (0)	276 (0)	756 (0)	403 (0)	423 (0)
Al-mafraq	0	0	635 (0)	817 (0)	400 (0)	185 (0)
Jerash	645 (0)	237 (0)	209 (0)	414 (0)	657 (0)	NC
Al-Shouneh	756 (0)	894 (130)	840 (120)	851 (143)	1040 (150)	760 (133)
Irbid	345 (0)	834 (0)	548 (0)	430 (0)	212 (0)	NC
Bani-kenaneh	888 (220)	708 (145)	745 (320)	973 (187)	547 (124)	NC

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.

Collection area	Elevation (m)	June	July	Aug	Sep	Oct	Nov
Deir Alla	-223	*	*	Cu/Cm*	Cu/Cm*	Cu/Cm*	Cu/Cm
Elmazar	808	Cu			Cu		
Aḍ-Ḍulayl	579	Cu			Cu		
Al-mafraq	705						
Jerash	620				Cu		
Al-Shouneh	-201	Cu*	Cu/Cm*	Cu*	Cu*	Cu/Cm	Cu
Irbid	568		Cu				
Bani-kenaneh	417	Cu	Cu/Cm*	Cu*	Cu	Cu	

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

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