Research Article

Spatial and Seasonal Distribution of Culicoides Species in Morocco in Relation to the Transmission of Bluetongue Viruses

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Abstract | The present study was undertaken to examine the data collected from a retrospective surveillance of Culicoides (Diptera: *Ceratopogonidae*) biting midge vectors from April 2009 to March 2010 in Morocco. In order to assess the implementation of integrated control measures and disease risk analysis, this entomological survey was performed on the risk of transmission of bluetongue virus (BTV) and identification of the main breeding sites of Culicoides. From April 2009 to March 2010, the study was carried out at 14 sites distributed across the country. Results indicated that the two most abundant Culicoides species were *Culicoides imicola* (94.2-95.85%) and *Culicoides newsteidi* (2.21-2.72%). The rate of prevalence of these species was bi-phasic and higher from April to June, and October to November 2009. Interestingly, the infectivity rate of BTV was also higher during these periods indicating that high risk of infection is directly proportional to that of transmission vectors. Moreover, results demonstrate that the life stage "bumper" of the female imicola is the indicator of the infecting activity of *C. imicola*. Collectively, the distribution patterns and emergence of BT can be predicted by the abundance of the Culicoides species in a specified locality so that control measures can be implemented well in time to contain the disease spread.

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Introduction

Bluetongue (BT) is a subacute to acute insect-borne disease of ruminants transmitted by blood-feeding Culicoides midges (Diptera: *Ceratopogonidae*) in temperate and tropical areas throughout the world, and its distribution lies roughly between latitudes 40°N and 35°S (Anthony et al., 2009). The disease is caused by the BT virus (BTV), which is an arthropod-borne orbivirus (family *Reoviridae*) with at least 26 distinct serotypes reported so far. In Morocco, BTV was reported for the first time in 1956 (Placidi, 1957). A vector survey study was conducted in the eighties to model the abundance of *Culicoidesimicola* (Baylis et al., 1997 and 1998). In 2003, a particular interest was focused on modeling the abundance of the vector in the Mediterranean basin including Morocco (Purse et al., 2007).

In the history of Morocco, at least three episodes of BTV can be identified in last two decades. First cascades of disease outbreaks occurred in the western regions of Morocco in 2004 involving BTV-4 serotype (OIE, 2004) and was extended to other parts of the country, and within two months, the balance of this episode has attained 230 outbreaks in 14 provinces. In September 2006, BT re-appeared in the eastern regions and laboratory investigations revealed that the disease is caused by a new BTV serotype, BTV-1. During this epidemic, a total of 500 outbreaks were reported in 19 provinces (OIE, 2006). Recently, in 2007, the disease appeared intense and new outbreaks spread to different area of Morocco. Moreover, it was speculated that the weather conditions favoured the multiplication and the proliferation of insect vectors during this episode of BT outbreaks (OIE, 2007). In 2007 year, 5222 cases were reported from 1076 outbreaks. Since these outbreaks of BTV, both BTV-1 and BTV-4 serotypes are endemic in the country.

The study was conducted as part of epidemiological surveillance of vector animal diseases at national level from April 2009 to March 2010 in Morocco. The entomological survey program aims to follow dynamics of *Culicoides* population vector in Morocco offending in the transmission of BT and the possible implementation of *Culicoides* populations on new geographic areas in Morocco. Efficient understanding of the factors that favour the abundance of the Culicoides in a given environmental conditions would be fundamental to develop seasonal control measures in the country that will help to restrict any future outbreak of the disease

The *Culicoides* surveys over a period of 12 months in 14 areas to define which *Culicoides* species could be implicated in BT transmission, to identify areas with high risk of transmission of bluetongue and also to better appreciate the environmental factors that predispose to the risk of emergence or re-emergence of such disease in Morocco

Materials and Methods

Light traps

Light traps "Onderstepoort" were used to collect *Culicoides* biting midges (*Diptera: Ceratopogonidae)* at fourteen sites in nine provinces through Morocco (Figure 1 and Table 1). Light traps are located in identified stations (presence of the disease and/or vector). They are operated at monthly intervals throughout the year to allow regular monitoring of the dynamics of *Culicoides* throughout April 2009 to March 2010. The choice of these sites was based on the presence of BT and/or the vector. Thus, in 14 provinces spread over different parts of the country, were designated 14 trapping stations, chosen for their location near wetland biotops where Culicoides midge abundance is known to be relatively high. As described in coming sections, the device responds to a schema of an embodiment that considers starting off traps mode, trapping procedures and collection methods, coding and routing the samples.

Collection procedures

Traps were hung 1.4 m above ground level and as close to the cattle as practically possible and to prevent interference traps were located at least 15 m apart. Insects were collected into water to which 0.5% of Antiseptic (contains Clorhexidinegluconate 0.3 g/100 ml and Cetrimide 3.0 g/100 ml) was added to break the surface tension of the water and allow midges to sink to the bottom of the collection container. After retrieval in the morning, the collected insects were transferred to 80% ethanol and stored in the dark at 4°C until analysed. Large collections were sub-sampled following the method of Van Ark and Meiswinkel (1992). Females of all species were, based on abdominal pigmentation (Dyce, 1969), age-graded into nulliparous, parous, gravid females or freshly blood-fed females. Males and all other insects captured were also counted.

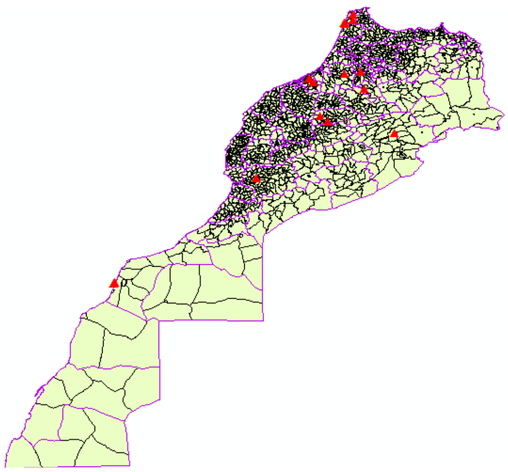

Figure 1: *Locations where the traps were used to collect Culicoides midges*

On all trial nights when no or very few Culicoides midges were collected, due to adverse weather conditions or trap failure, collections were repeated the following night.

Results

The outcome of the study provide a reliable Culicoides surveillance in 2009 (April-to December) and early 2010 (January to March). It should be noted that of the 14 initially designated trapping sites for this study, only 8 sites have provided samples on a regular basis. During three quarters of 2009, a total of 176 trapping samples (88.8% of the expected trappings) were collected and 73.4% were sorted and identified.

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Table 1: *Information of locations where the traps were used to collect Culicoides midges*

Provinces	Communes	Code Sites	X	Y	Code G O
Tanger	Sahel chamali	HOMA	-6,05	35,3	511.03.11.
	Sahel chamali	TINDE	-6,05	35,3	511.03.11.
Meknes	Meknes	MEK 1	-5,52	33,8	061.01.01.
Benimellal	Sidi hammadi	AGROPLUS	-6,58	32,2	091.05.13.
	Beni oukil	AET	-6,86	32,5	091.09.05.
Khemissat	Ait siberne	KHEM	-5,89	33,8	291.03.05.
Errachidia	Errachidia	ERRA	-4,43	31,9	201.01.05.
Ifrane	Ain leuh	IFRA 1	-5,31	33,2	271.03.01.
Laayoune	Foum el oued	LAAY1	-13,3	26,8	321.03.05.
	Foum el oued	LAAY2	-13,3	26,8	321.03.05.
Taroudant	Sidi dahmane	TARO1	-8,79	30,5	541.09.45.

Table 2: *Summary of Culicoides midges collected in different provinces during April 2009-March 2010, Morocco*

Location	Site	Code Site	T°Ann al Average max	T°Annual Average min	No.Non-Culicoides	No. C. IMIC	No. Culicoides	No.Collection made
Benimellal	Sidi Hammadi	AET	26.71	15.37	0	175	205	205
Benimellal	Benioukil	AGROPLUS	28.82	16.72	0	35	79	79
Errachidia	Errachidia	ERRA	26.69	14.96	54	15	16	70
Ifrane	Ain Leuh	IFRA1	19.75	9.00	29	655	705	734
Khemisset	Ait Siberne	KHEM	27.60	16.40	13	4	9	22
Laayoune	Foum El Oued	LAAY1	24.00	13.60	0	8	35	35
Laayoune	Foum El Oued	LAAY2	25.16	15.50	0	1	19	19
Meknes	Meknes	MEK1	28.22	17.66	130	461	773	903
Tanger	Sahel Chamali	HOMA1	28.40	18.40	0	24	77	77
Tanger	Sahel Chamali	TIND2	28.40	18.40	0	17	96	96
Taroudant	Sidi Dahmane	TARO	28.40	15.90	3	387	427	430
Total					229	1782	2441	2670

Spatial distribution of Culicoides

Geographical Distribution of Culicoides midges during April- December 2009

In 176 collections (88.8% of the expected trappings), 2441 Culicoides midges were collected and identified (Table 2). Among all the sites tested, traps at Ifrane, Meknes, Taroudant and to a less extend Benimellal collected the highest number of Culicoides (88.48% of the total catch) whereas traps at Khemisset and Errachidia collected the lowest (5.6% of the total catch). Significant differences were found in the average number of midges collected in Ifrane, Meknes, Taroudant as compared to Khemisset, Tanger, Laayoune and Errachidia. On average, midges collected at the central regions were 2.1 times more than those collected at the southeastern and 2.6 times more than northern regions.

Out of 2432 Culicoides identified were belonging to 10 different species namely, *C. imicola*, *C pulicaris*, *C. newsteadi*, *C. punctatus*, *C nubeculosus*, *C. circumscriptus*, *C newsteadi*, *C. oxystoma*, *C. obsoletus*and *C. helviticus* (Table 3).

Their distribution however was very different from site to site. All traps indicated that *C. imicola* is the most abundant Culicoides species and it was the only common species that was collected in all 14 collections. While *C. obsoletus* species is present in the North and Northwest with very low abundance (Figure 2), *C. imicola*s species are present abundantly in the northern center of the country and at higher density as compared to southern regions (detailed in Figure 2). Its abundance ranged from 76% in Tanger to 9.3% in Taroudant. Relatively small but statistically significant differences were found in relation of the numbers of *C. imicola* to that of other Culicoides species

Table 3: *Summary of other Culicoides midges collected in different provinces during April 2009-March 2010, Morocco*

Location	Site	Code Site	C_IMIC	C_OB-SOL	C_PULIC	C_NEW	C_PUNC	C_NUB	C_CIR	C_OXY	C_H.P	C_UV
Benimellal	Sidi Hammadi	AET	175	0	0	0	12	0	9	3	0	6
Benimellal	Benioukil	AGROPLUS	35	0	0	0	6	0	20	18	0	0
Errachidia	Errachidia	ERRA	15	0	0	0	0	0	1	0	0	0
Ifrane	Ain Leuh	IFRA1	655	0	6	39	5	0	0	0	0	0
Khemisset	Ait Siberne	KHEM	4	2	1	2	0	0	0	0	0	0
Laayoune	Foum El Oued	LAAY1	8	0	0	11	0	0	16	0	0	0
Laayoune	Foum El Oued	LAAY2	1	0	0	1	0	0	17	0	0	0
Meknes	Meknes	MEK1	461	0	20	214	17	0	61	0	0	0
Tanger	Sahel Chamali	HOMA1	24	10	3	2	14	0	14	0	0	10
Tanger	Sahel Chamali	TIND2	17	6	6	13	10	0	17	0	0	27
Taroudant	Sidi Dahmane	TARO	387	0	0	0	0	0	0	0	1	0
Total			1782	18	36	282	64	39	155	21	1	43

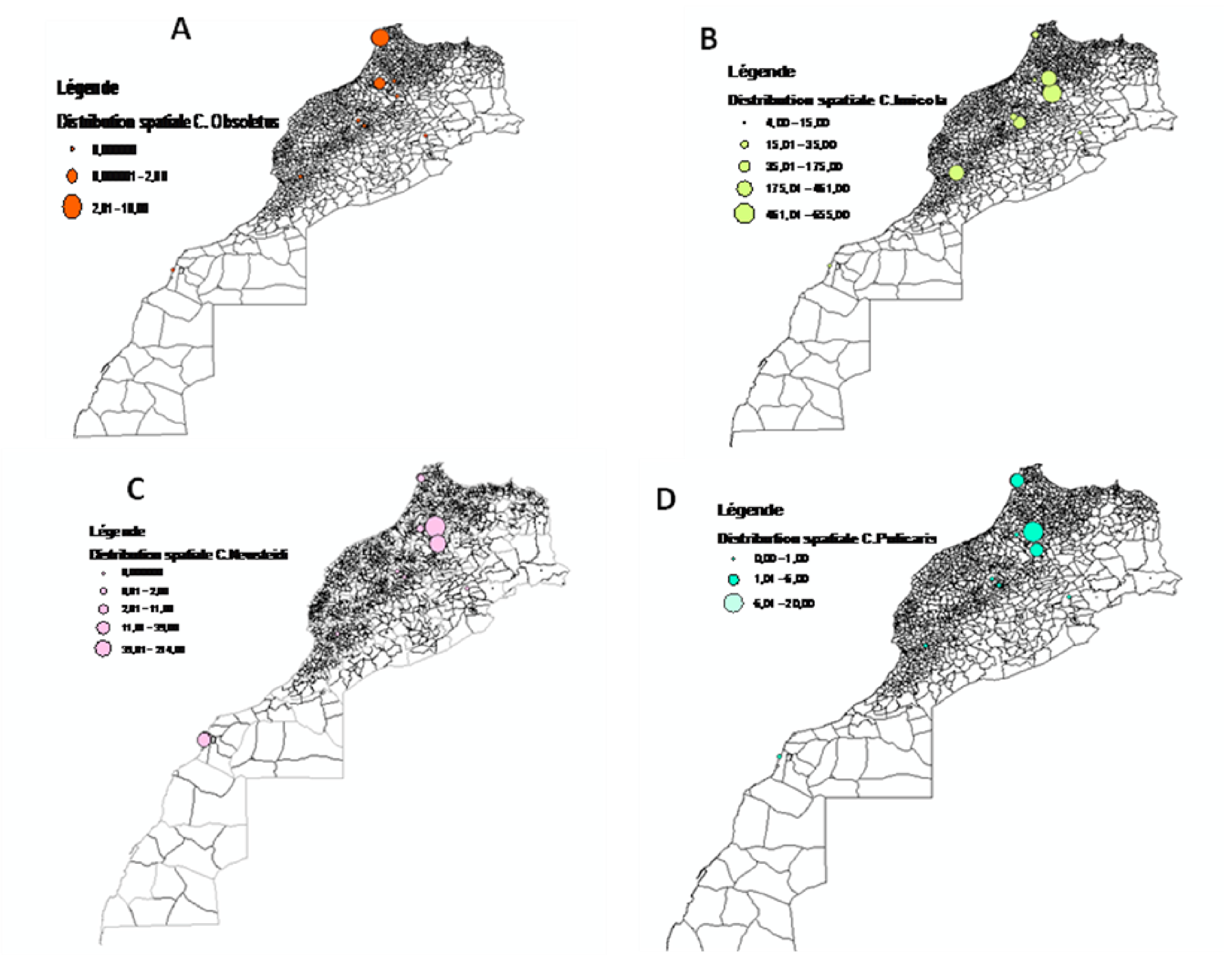

Figure 2: *Geographical Distribution of Culicoides midges April 2009-March 2010*

between the different regions. Interestingly, *C. newsteidi*species is also present in abundance at the same locations as the *C. imicola* species (Figure 2). Similar to *C. newsteidi*, relatively small differences were found in the proportion of *C. pulicaris*in the north central and northern regions (Figure 2).

Temporal distribution of Culicoides

In this period a total of 2441Culicoides belonging to 10 different species were captured on the second quarter of 2009 from April, with an increase in May and June marked by a slight increase in temperatures. The species of the C. *imicola* complex are largely dominant (67% of total catches), which is typically seen in the early season.

During the period from April to July 2009, the presence of Culicoides is significant because of the observed temperature at this time. A second peak of activity of Culicoides is observed from October through December when the Culicoides are still active throughout the north center and east of the country. However, declining enrollment is observed in August, linked to rising temperatures, but it is not maintained due to lower temperatures. A new progressive downsizing is observed from late November with a small boost in January although some provinces still exceeded the threshold of 5 pares females / trap / night (mostly in the Centre - North and East.

The sharp fall in temperatures from late November downsized. The monthly trapping from January 2010 confirms the drastic reduction of activity of populations (Figure 3), which indicates the inactivity of populations from this month. On average, less than 0.75 Culicoides / trap / night are captured in January 2010 mainly represented by C. *circonspectus*species.

Interactions between the vector and the disease

The analysis of vector's data collection and notifications of the disease in 2009, emphasizes on the relationship between the Culicoides and BT. This connection depends primarily on the density of the vector and the density of susceptible animals (Figure 3).

The *C. imicola*is characterized by its presence throughout the national territory with different dynamics in different regions. The *C. imicola* females are most abundant in the whole areas except sites of Laayoune where males predominate. This heterogeneous spatial distribution of males influences the distribution of the life stages of female imicola mainly two stages "parous" and "blood-fed", which are the infective in females (odds ratio: OR = 3.00 IC [1,1 - 19.7] P-value <0.05).

The spatial distribution of other species of likely Culicoides suggests a *Newsteidi*'s complex auxiliary role in the transmission and maintenance of the virus circulation in ruminant herds (OR = 1.76 CI [1.31 to 2.37] P value < 0.001). This species is present in the South but also the North of the country that has previously undergone several episodes of Bluetongue (Figure 4).

The species *C. imicola* remain the proved species of the disease in Morocco. The notifications of the disease in

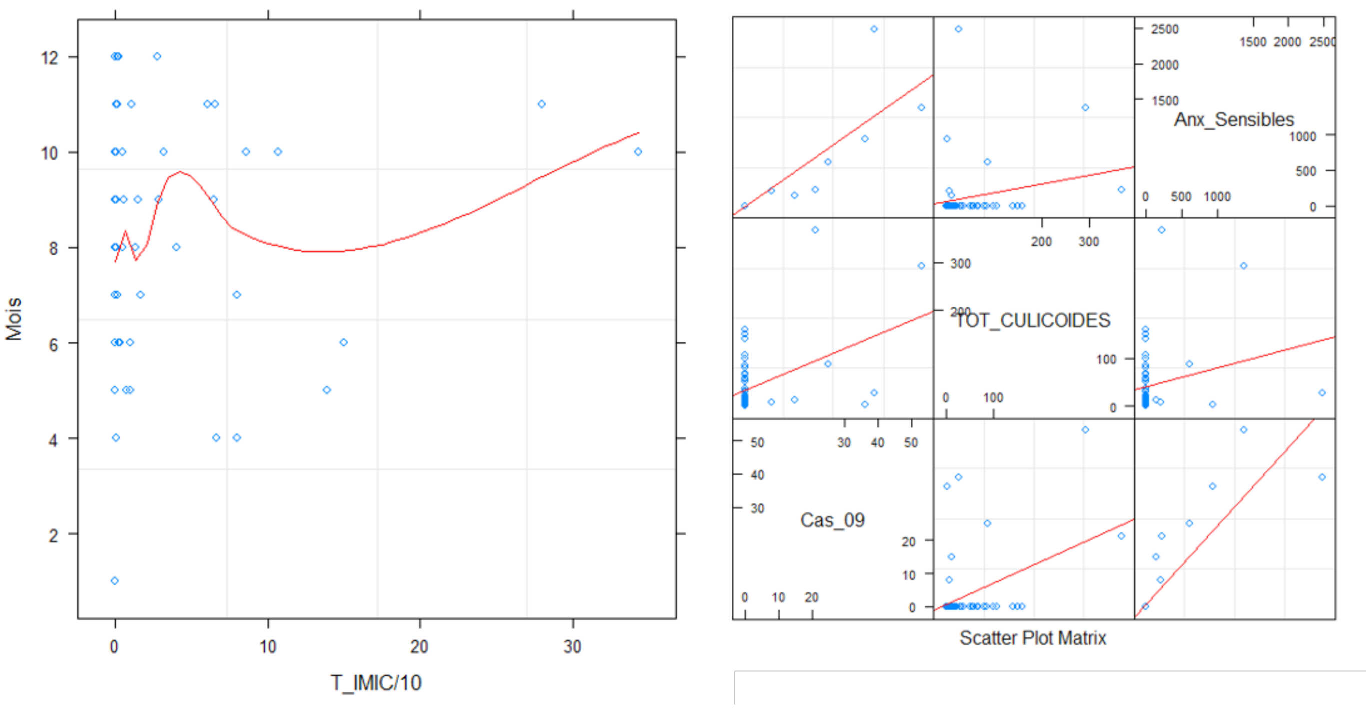

Figure 3: *Characteristics of disease-vector interaction in 2009*

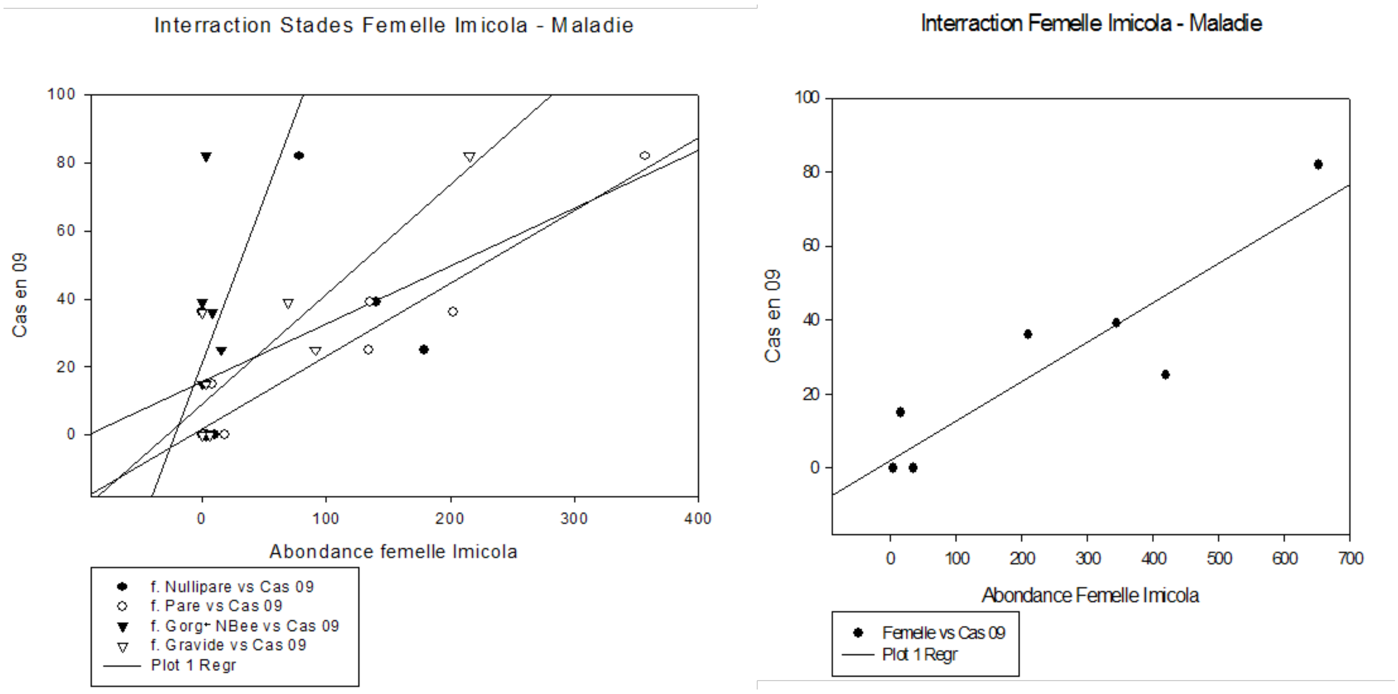

Figure 4: *Interactions of C. imicola life stages and Bluetongue in 2009*

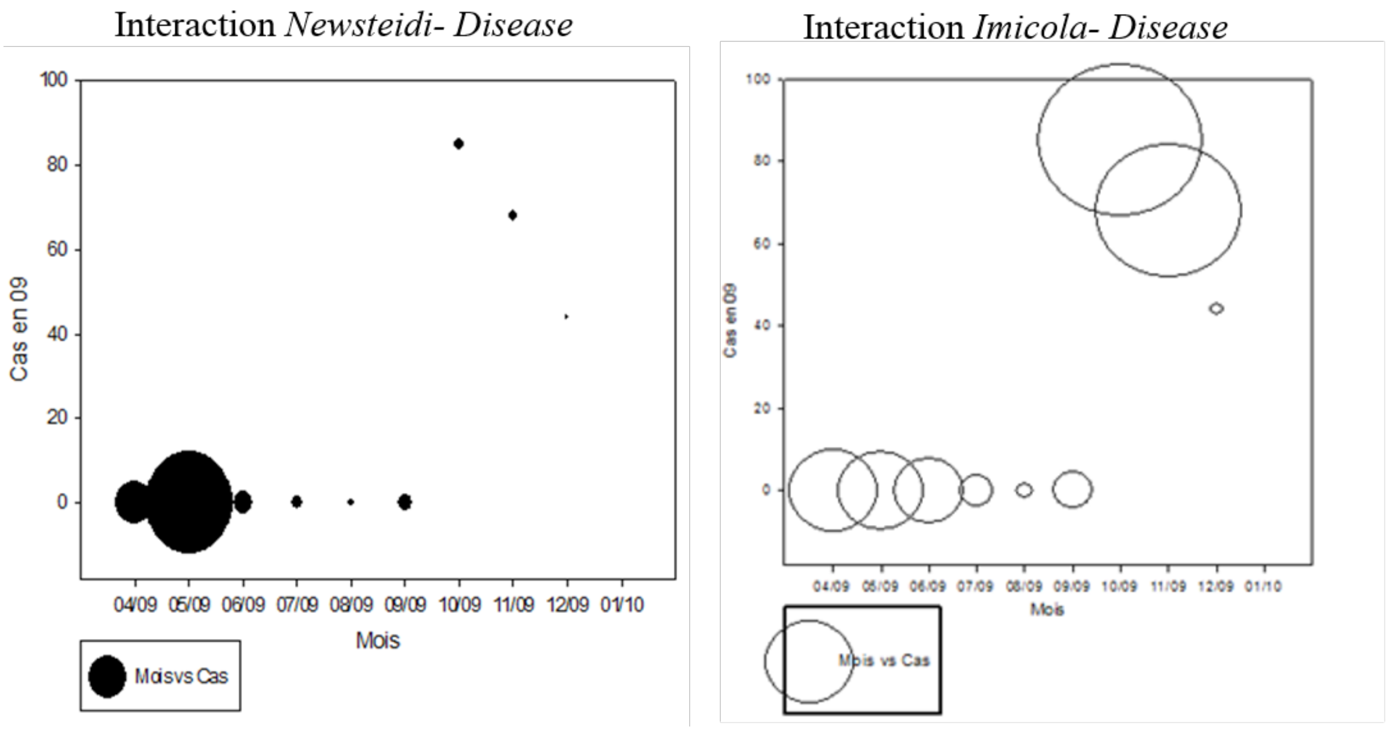

Figure 5: *Interactions of the Newsteidi-imicola Complex and the Disease.*

2009 were positively correlated with significant abundance of this specie particularly in temperate time of the year. Indeed, the trend line shows the average number of *C. imicola* observed since the second quarter of the year with high abundance in months 10 and 11of 2009 (Figure 5).

The crossing of the factors "time", "disease" and "abundance of the vector" for the year 2009, shows that *imicola* and *newsteidi* species have a very similar dynamics for the second and third semester of the year with average abundances around the month of May. The appearance of cases of the disease towards October and November of the year seems much more linked to the strong presence of *C.imicola* than *C.newsteidi* (Figure 5).

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Discussion

The investigations have shown huge Culicoides population dynamics, including *C. imicola* and *C. newsteidi*, especially for two periods: spring (April to June) and fall (October-November).

The life stage "parous" of the female imicola is the indicator of infective activity of *C. imicola*. The notifications of the disease in 2009 coincide with periods of peak activity of this stage. Two periods at risk of the disease were observed: late spring-early summer and autumn coinciding with periods of activity of the infective stage of *C. imicola*.

Retrospective analysis of data on outbreaks of the disease reported in national territory shows that the degree of risk varies from one region to another. Some areas at risk for the disease are identified taking into account the dynamics of the vector and disease occurrence at the level of these areas mainly North Central and Southeast of the country.

Considering that several uncertain risk factors such as climate change influence on the geographic distribution of vectors, vector density, frequency and intensity of animal diseases and animal movements, it seems sensible to introduce into our analysis of the environmental and climatic data (rainfall pattern and / or the vegetation index, herd immunity, temperature, wind direction and speed) to enable the building of occurrence risk maps and installation of the disease patterns throughout the national territory.

Trapping the end of the summer and early winter are very informative of the decrease in activity of Culicoides populations. It is therefore essential to continue to trap and send the samples regardless of weather conditions and quantity of insects (trapping performance). The study of the determinants of Culicoides activities will have answers on weather conditions that induce the end and the resumption of the activity and see if it is a one-off occasions of activity at very temperate periods. These additional weekly trappings will determine if it is a total cessation of activity of Culicoides during winter. This is fundamental to whether the persistence of viral circulation from one year to another is assured only by hosts (long viraemia in cattle host, goat or sheep) or if the vectors also have a role in this persistence.

Conclusion

Our study highlights the dynamism of the BTV–*Culicoides* system in Morocco. It provides a strategy for a fuller examination of the relationship between BTV transmission and the abundance and distribution of different vector species. Future surveillance and research effort for bluetongue should aim to record and explain the relationship between the vector distribution and environmental factors.

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Conflict of Interest

There exists no conflict of interest.

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