Composition and seasonality of *Culicoides* in three host environments in Rabat region (Morocco)

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**Summary**
Morocco has suffered several outbreaks of *Culicoides*-borne viruses in recent decades and most studies have focused on *Culicoides imicola*, considered for a long time as the only important vector. The change in bluetongue (BT) epidemiology in the Mediterranean Basin and Europe over the past two decades has highlighted the role of other *Culicoides* species in BT virus transmission. The objective of this study was to provide new insights on the *Culicoides* species composition and seasonality in three different host environments (a horse-riding center, a goat farm and a cattle farm) around Rabat, the capital of Morocco, where BT has been endemic since 2004. Light / suction trap collections were carried out on two consecutive nights at fortnight intervals from May 2016 to May 2017. *Culicoides* were identified morphologically at the species level when possible. Multivariate analyses were used to compare the impact of the site / vertebrate species, and the collection month on the species communities. In addition, statistical modeling was used to identify environmental drivers of the *Culicoides* seasonality. A total of 12,460 *Culicoides* individuals belonging to at least 15 different species were collected during the survey. *Culicoides imicola* was by far the most abundant species (71.4% of total catches). The site location, and thus the vertebrate species, did not influence the species composition, which was mainly impacted by the month of collection. Surprisingly, the atmospheric pressure was the environmental parameter the most frequently selected in seasonal models. The potential impact of this meteorological parameter along with the other selected variables is discussed. Identifying the environmental parameters driving *Culicoides* seasonal abundance is the first step to implementing robust *Culicoides* dynamic models that could later be used in transmission risk modeling.

INTRODUCTION
The *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) are small hematophagous insects, which are biological vectors of viruses responsible for major livestock diseases, such as bluetongue (BT), African horse sickness (AHS), epizootic hemorrhagic disease (EHD) or Schmallenberg disease (SB) (Purse et al., 2015). Several incursions of AHS and EHD have occurred in recent decades in the Mediterranean region, though outbreaks were limited geographically and lasted only a few years. In contrast, after sporadic incursions, the Mediterranean BT epidemiology changed radically in the last two decades. What happened in Morocco illustrates this epidemiological drift in the BT situation compared to other *Culicoides*-borne viruses.
Morocco experienced AHS outbreaks in the 1960s (serotype 9) and in the late 1980s (serotype 4) (Mellor and Hamblin, 2004), and EHD outbreaks (serotype 6) in 2006 (Savini et al., 2011). During the 1980s AHS outbreak, observed circulation of AHS virus (AHSV) was reported for five years before returning to an absence of transmission (Mellor and Hamblin, 2004). No EHD clinical cases were reported after 2006. Bluetongue (serotype 10, BTV-10) was described for the first time in Morocco in 1956. The virus spread was controlled thanks to the implementation of a vaccination campaign and a temperature drop in the fall (Lhor, 2016). Meanwhile, other BTV serotypes were extensively transmitted in the Mediterranean Basin from 1998, but the disease had apparently remained absent in Morocco until BTV-4 emergence in 2004 (Lhor, 2016). In 2006, BTV-1 was reported in Algeria, then it spread to Morocco in 2006–2007 (Lhor, 2016). In 2008, sanitary policies implemented by veterinary services stopped these outbreaks, but the disease reappeared in 2009, with BTV-1 and BTV-4 co-circulating during Culicoides activity periods in both northern and southern parts of the country (Lhor, 2016). The last census reported 305 BT outbreaks across the country with 1456 cases leading to 449 livestock deaths in 2017. The BT endemic situation is weighing on the sheep industry, which increased from 1.6 billion to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. Is currently estimated at 2.5 million to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. Is currently estimated at 2.5 million to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. Is currently estimated at 2.5 million to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. Is currently estimated at 2.5 million to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest.
To determine broadly the diversity per site, species communities were described using a principal component analysis (PCA) on the log-transformed abundances. Then, a between-class analysis was carried out on this PCA to assess the importance of both the site and the collection month. Between-class analysis is a particular case of a PCA, where the variability between groups is optimized.

A correlation matrix was produced for all environmental variables to select non-correlated variables to be included in the modeling of Culicoides seasonal abundance. Then, cross correlation maps (CCM) were used to assess the correlation between the Culicoides abundance averaged between sites and the selected environmental variables at different time lags. CCM were used to assess average or accumulated meteorological quantities over a period beginning at a first time lag and ending at a second time lag (Brugger and Rubel, 2013). Analyzing CCM determined which time lags of environmental variables may have had an impact on Culicoides abundance.

Finally, abundance was modeled with these environmental parameters and time lags (after centring and scaling) using a generalized linear model (GLM) or a negative-binomial log-linear model to account for any over-dispersion of insect collection data. The model selection was based on corrected Akaike’s (aIC) and Bayesian information criteria (BIC). The validity of the selected GLM was assessed by i) plotting the observed versus the fitted values and testing Pearson’s product moment correlation coefficient, ii) graphically checking the normality of the residuals, iii) graphically testing the linearity hypothesis (random distribution of residuals around 0 after they had been plotted by fitted values), and iv) graphically confirming the homogeneity of residuals.

All statistical analyses and graphs were implemented with version 3.4.2 of R software, using ade4 package for PCA and between class analysis, fields for CCM, MuMIn for calculating the corrected Akaike and Bayesian information criteria, and aod for analysis of over-dispersed data.

RESULTS

Culicoides collections

The goat and cattle farms were sampled fortnightly over a period of 22 weeks (12 and 11 collections, respectively, the last sampling was not considered due to electrical failure) during most of the Culicoides activity period (from mid-May 2016 to mid-October 2016). The horse-riding center was sampled fortnightly for 48 weeks (22 collections) to measure the population seasonality over an entire year (from mid-May 2016 to the end of April 2017).

During sampling periods, the temperature conditions were similar to the seasonal normal, with an average annual temperature of 20.5°C in 2016 and 20.6°C in 2017, compared to 20.1°C for the 2000–2015 period (Figure 1). The 2016 collections began with a relatively dry period with a total of 125 mm rainfall from January to May 2016, compared to 193 mm on average for the same months in the 1986–2015 period (Figure 1). In contrast, the winter of 2016–2017 was wetter than normal with 440 mm rainfall from September 2016 to May 2017 compared to 389 mm on average for the same months in the 1986–2015 period (Figure 1).

Culicoides diversity and seasonality in the three collection sites

A total of 12,460 Culicoides individuals belonging to at least 15 different species were collected during the survey, including 12,053 (96.7%) females and 407 (3.3%) males (Table 1). From May to October 2016, during the 11 collections common to the three sites, the most abundant species were C. imicola (71.4% of total catches), Culicoides newsteadi Austen (6.4%), Culicoides circumpunctatus Kieffer (6.4%), Culicoides kingi Austen (4.1%), Culicoides cataneii Clastrier / Culicoides geojelensis Dzhafarov (3.8%), Culicoides puncticollis (Becker) (3.6%), and C. obsoletus / C. scoticus (2.2%). Altogether these species represented 97.9% of the collected individuals (Table 1). The structure of the PCA on log-transformed abundances was driven by the seven most abundant species (or pair of species), leading to a cumulative projected inertia of 70.4% on the first three axes. The site (or the dominant domestic vertebrate species) explained 17.1% of PCA inertia (p = 0.001, with a permutation test), whereas the collection month explained 26.7% of PCA inertia (p = 0.001). The ‘site/host effect’ was mainly due to a higher abundance of C. circumpunctatus in the cattle farm than in the other two sites (Figure 2A), and, but less significantly, to a higher abundance of C. imicola in the horse-riding center and of C. puncticollis and C. paolae in the goat farm (see Figure 1: 2016 and 2017 monthly average meteorological conditions (temperature and precipitations) compared to seasonal normal at Rabat, Morocco. Temperature data (day and night land surface temperatures) were extracted from MOD11A1 version 6 (Wan et al., 2015) using a 4-km² area from 2000 to 2017. Rainfall data were extracted from TAMSAT database (Maidment et al., 2014) from 1986 to 2017.
Table 1

Total number of *Culicoides* collected per species every two weeks using a UV light / suction trap (OVI type) in two farms from May to October 2016, and in a horse-riding center from May 2016 to April 2017, in Rabat region (Morocco)

| Species                          | Riding center (n = 25 collections) | Goat farm (n = 12 collections) | Cattle farm (n = 11 collections) | Total common collections* |
|----------------------------------|------------------------------------|--------------------------------|---------------------------------|---------------------------|
|                                  | Indiv. Female Male Rank            | Indiv. Female Male Rank         | Indiv. Female Male Rank         | Indiv. Female Male Rank   |
| *C. imicola*                     | 5469 5348 121 1                   | 2027 1944 83 1                  | 1432 1392 40 1                  | 5844 5688 156 1           |
| *C. newsteadi*                   | 276 274 2 3                       | 131 130 1 3                    | 270 237 33 3                    | 524 489 35 2              |
| *C. circumpictus*                | 68 60 8 6                        | 79 75 4 5                      | 434 399 35 2                    | 522 480 42 3              |
| *C. kingi*                       | 1010 966 44 2                    | 107 105 2 4                    | 71 69 2 6                       | 335 315 20 4              |
| *C. cataneii / C. gejgelensis*   | 84 83 1 5                        | 47 44 3 6                      | 222 221 1 4                     | 314 309 5 5               |
| *C. cataneii*                    | 55 54 1                          | 47 44 3                        | 222 221 1                        |                          |
| *C. gejgelensis*                 | 27 27 0                          | 0 0 0                          | 0 0 0                            |                          |
| *C. puncticollis*                | 20 19 1 9                        | 212 197 15 2                   | 64 56 8 7                       | 293 269 24 6              |
| Obsoletus group                   | 87 87 0 4                        | 30 30 0 8                      | 96 96 0 5                       | 181 181 0 7               |
| *C. obsoletus / C. scoticus*     | 40 40 0                          | 4 4 0                          | 8 8 0                            |                          |
| *C. obsoletus*                   | 41 41 0                          | 23 23 0                        | 55 55 0                          |                          |
| *C. scoticus*                    | 6 6 0                            | 2 2 0                          | 33 33 0                          |                          |
| *C. montanus*                    | 0 0 0                            | 1 1 0                          | 0 0 0                            |                          |
| *C. paolae*                      | 11 11 0 10                       | 35 33 2 7                      | 9 9 0 9                          | 46 44 2 8                 |
| *C. subfagineus*                 | 22 21 1 8                        | 13 13 0 9                      | 6 6 0 10                        | 41 40 1 9                 |
| *C. longipennis*                 | 8 8 0 12                         | 4 4 0 10                       | 23 23 0 8                       | 30 30 0 10               |
| *C. fagineus*                    | 53 53 0 7                        | 0 0 0                          | 1 1 0 14                        | 27 27 0 11               |
| *C. jumineri*                    | 4 4 0 14                         | 0 0 0                          | 6 6 0 10                        | 9 9 0 12                 |
| Unknown species                   | 7 7 0 13                         |                                |                                 | 7 7 0 13                 |
| *C. univittatus*                 | 0 0 0                            | 2 2 0 12                       | 4 4 0 12                        | 6 6 0 14                 |
| *C. parroti*                     | 9 9 0 11                         | 0 0 0                          | 0 0 0                            | 4 4 0 15                 |
| *C. sahariensis*                 | 0 0 0                            | 4 4 0 10                       | 0 0 0                            | 4 4 0 15                 |
| *C. festivipennis*               | 0 0 0                            | 0 0 0                          | 2 2 0 13                        | 2 2 0 17                 |
| *Culicoides* sp.                 | 1 1 0                            | 0 0 0                          | 0 0 0                            |                          |

* Data from the weeks when all the sites were sampled so as to compute a global abundance rank for each species; Indiv.: individuals
Culicoides populations increased progressively from spring to peak in September (Suppl. Mat. II), whereas C. kingi was abundant only in August/September. Population densities of C. circulifer, C. cataneii / C. gejgelensis and C. puncticornis were unimodal with a maximal abundance in August for the first species and in July for the latter two (Suppl. Mat. II). Finally, both C. obsoletus / C. scoticus and C. newsteadi populations showed two peaks of abundance, the first in June, the second in October (Suppl. Mat. II).

Influence of environmental parameters on Culicoides abundance

Supplementary Material III details the correlation matrices produced with all environmental parameters, including EVI and NDVI MODIS parameters, TAMSAT daily rainfall and ECMWF-ERA5 meteorological parameters. Comparison of the day and night land surface temperatures from MODIS products and the daily 2-m temperature from ECMWF-ERA5 showed high correlations (Suppl. Mat. III). Therefore, only the temperature ECMWF-ERA5 data was used for the rest of the analysis. The temperature, which was highly correlated with the environmental vapor pressure, the relative and the absolute humidity, the atmospheric pressure, the wind speed, the rainfall and EVI, which was highly correlated with NDVI, were also retained.

Bivariate correlations between C. imicola abundance and environmental variables highlighted a positive impact of the temperature and absolute humidity for a large range of time lags (Figure 3). The time lags that had the highest correlation coefficients with abundance were selected (Pearson’s product moment correlation coefficient $r = 0.80$ for 25–27 days as time lags for temperature and $r = 0.75$ for 23–26 days for absolute humidity). In contrast, the atmospheric pressure was negatively correlated with C. imicola abundance (Figure 3, $r = 0.83$ for 27–43 days). CCM did not indicate any specific relation between the wind speed and the number of C. imicola collected. The best correlation was positive and obtained with the wind speed 36 days before collection, which seemed incidental and without any biological sense (the loss of adults is generally due to wind dispersal the day of collection). Thus, only the wind speed on the day of collection was included as a variable for modeling procedure. Finally, the EVI of the week of collection was negatively correlated with C. imicola abundances (Figure 5).

Table II shows the environmental parameters with the optimum time lags selected for the modeling procedure after having analyzed CCM for C. newsteadi (Suppl. Mat. IV), C. circulifer (Suppl. Mat. V), C. kingi (Suppl. Mat. VI), C. cataneii / C. gejgelensis (Suppl. Mat. VII), C. puncticornis (Suppl. Mat. VIII), and C. obsoletus / C. scoticus (Suppl. Mat. IX).

The selected GLM included atmospheric pressures (27-to-43-day average before collections) and mean temperatures (25-to-27-day average before collections). It correctly predicted the seasonal pattern of C. imicola populations ($R^2 = 0.778$, Table III), i.e. a slow increase in population from March to September when it reached a maximum, then a rapid decrease from October to February (Figure 4).

The selected models (Table III) correctly predicted the seasonal pattern of C. circulifer ($R^2 = 0.752$), C. cataneii / C. gejgelensis ($R^2 = 0.754$) and C. puncticornis ($R^2 = 0.670$), with the lowest accuracy for the latter (see Suppl. Mat. X for details on model selection, validation and prediction for all species). The selected models did not successfully predict the bimodal seasonal patterns of C. newsteadi ($R^2 = 0.463$), and C. obsoletus / C. scoticus ($R^2 = 0.700$). GLM was able to predict the abundance peaks of C. newsteadi populations in fall 2016 and spring 2017, but not in spring 2016 (Suppl. Mat. X). Similarly, GLM was able to predict abundance peaks of C. obsoletus / C. scoticus populations in spring 2016 and 2017, but not fully in fall 2016 (Suppl. Mat. X). Finally, the selected model predicted the general seasonal pattern of C. kingi populations, but failed to reproduce the November peak of abundance (Suppl. Mat. X). The atmospheric pressure was selected as a significant predictor in 5 of 7 models, always with a negative correlation, rainfall in 4 models but only once with a significant positive effect, humidity in 2 models, wind speed in 2 models with a positive correlation, and EVI and temperature only in 1 each (Table III).

**Figure 2**: Between-class analysis (particular case of a principal component analysis [PCA] with optimization of the variability between groups) with the site (A) and the collection month (B) as group applied on the PCA carried out of the log-transformed 2016–2017 abundances. Bart chart: percentage of inertia explained by the axes. Culicoides collections were carried out twice a month with UV light / suction trap (OVI type) in three sites around Rabat (Morocco): a horse-riding center (MA001), a goat farm (MA002) and a cattle farm (MA003) from May (05) to October (10).
Table II

Environmental variables and optimal time lags (in weeks for EVI and in days for the other parameters) selected for the modeling procedure after analyzing the cross correlation maps for each of the most abundant Culicoides sp. collected in 2016–2017 in Rabat region (Morocco).

| Species                  | Temperature | Absolute humidity | Relative humidity | Atmospheric pressure | Wind speed | Rainfall | EVI |
|--------------------------|-------------|-------------------|-------------------|----------------------|------------|----------|-----|
| C. imicola               | Mean [25.27]| Max [23.26]       | –                 | Mean [27.43]         | Mean [0.0] | –        | [0,0]|
| C. newstadi              | –           | –                 | Max [7.11]        | Min [9.12]           | –          | [9.17]   | –   |
| C. circumpunctatus       | Min [23.25] | Mean [6.25]       | –                 | Min [27.40]          | –          | [37.40]  | [0,0]|
| C. kingi                 | Mean [15.15]| Mean [20.30]      | –                 | Max [29.39]          | Mean [27.45] | [37.40]  | [0,2]|
| C. cataneii / C. gejgelensis | Min [14.14] | Mean [20.30]      | Min [0.5]         | Max [33.35]          | Mean [27.45] | [9.9]    | [0,2]|
| C. puncticollis          | Max [2.4]   | Max [1.7]         | Min [23.25]       | Mean [15.15]         | Min [5.38] | [2.60]   | [0,0]|
| C. obsoletus / C. scoticus | –           | –                 | Max [17.21]       | Max [6.8]            | Max [5.41] | [37.38]  | –   |

EVI: Enhanced Vegetation Index
DISCUSSION

Culicoides collections carried out in three different host environments around Rabat confirmed the presence of at least 15 Culicoides species, among which were several proven or probable vectors of arboviruses of veterinary interest. *C. imicola* is a proven BTV and AHSV vector and a suspected EHDV vector (Purse et al., 2015), whereas *C. newsteadi* and *C. puncticollis* are probable BTV vectors (Purse et al., 2015). *C. obsoletus* and *C. scoticus* are probable BTV vectors (Purse et al., 2015), whereas *C. newsteadi* and *C. paolae* are suspected BTV vectors (Foxi et al., 2016; Foxi et al., 2019).

*C. imicola* was by far the dominant species in the three sites combined. It has been reported as the most abundant and frequent species in Morocco (Baylis et al., 1997; Lhor, 2016). The peak of abundance of *C. imicola* larvae develops in most clay mud rich in nutrients exposed to sunlight or in moist or water saturated soils rich in organic matter (Braverman et al., 1974). Species such as *C. newsteadi* and *C. puncticollis* are found near substrates rich in water-saturated organic matter (Braverman et al., 1974). *C. kingi* larvae are more likely to be found in sunny and very salty mud (Cornet and Brunhes, 1994), whereas *C. cataneii* and *C. gejgelensis* larvae may be found in rivers or pond edges in wet meadows (Garros and Balenghien, 2017). *C. obsoletus* and *C. scoticus* may be considered ubiquitous as they develop in forest litter, tree holes, corn silage residues or composting manure (Garros and Balenghien, 2017). *C. paolae* larvae may be considered ubiquitous as they develop in forest litter, tree holes, corn silage residues or composting manure (Garros and Balenghien, 2017). Finally, *C. paolae* larvae are considered specific to decaying prickly pear trees, which are common in the Mediterranean Basin.

The seasonal pattern of *C. imicola* populations observed around Rabat is consistent with previous studies carried out in Morocco, with a peak in late summer/early fall (Baylis et al., 1997), which is associated with BTV transmission in Northwestern Morocco (Lhor, 2016). This seasonal pattern is usually observed in the Mediterranean Basin (Garros and Balenghien, 2017). The peak of abundance of *C. imicola* populations is observed at the same period (September/October) in Senegal with a tropical climate, where populations are highest during the rainy season (Diarra et al., 2014). Seasonal patterns of *C. obsoletus* and *C. scoticus* vary widely depending on the climate (Garros and Balenghien, 2017).

### Table III

Modeling *Culicoides* abundances assessed in 2016–2017 in Rabat region (Morocco) by environmental parameters using generalized linear or binomial model

| Species                  | Selected model | Selected variables and estimates                                                                      | Pearson's correlation coefficient |
|--------------------------|----------------|-------------------------------------------------------------------------------------------------------|----------------------------------|
| *C. imicola*             | GLM Abun ~ 1.70*** - 0.77 × Pres_Mean* + 0.35 × Temp_Mean*                                            | p = 0.882 (p < 0.001), R² = 0.778 |
| *C. newsteadi*          | GLM Abun ~ 0.87*** - 0.23 × Pres_Mean* + 0.59 × Rain*                                                  | p = 0.680 (p < 0.001), R² = 0.463 |
| *C. circumscriptus*     | GLM.NB Abun ~ 1.86*** - 1.49 × Pres_Mean*** - 4.98 × Rain                                            | p = 0.867 (p < 0.001), R² = 0.752 |
| *C. kingi*              | GLM Abun ~ 0.67*** - 0.53 × Pres_Max* - 0.35 × Rain                                                   | p = 0.628 (p < 0.001), R² = 0.394 |
| *C. cataneii / C. gejgelensis* | GLM Abun ~ 0.51*** - 0.29 × EVI*** + 0.14 × Rain + 0.87 × Wind_Mean***                              | p = 0.868 (p < 0.001), R² = 0.754 |
| *C. puncticollis*       | GLM.NB Abun ~ 1.56*** + 7.54 × AH_Max*** - 5.06 × RH_Min*                                            | p = 0.818 (p < 0.001), R² = 0.670 |
| *C. obsoletus / C. scoticus* | GLM.NB Abun ~ 1.42*** - 2.69 × Pres_Max* - 5.85 × RH_Max*** + 1.07 × Wind_Max***                  | p = 0.837 (p < 0.001), R² = 0.700 |

Asterisks denote significance: *** p < 0.001; ** p < 0.01; * p < 0.05; º p < 0.1

GLM: generalized linear model; GLM.NB: negative-binomial log linear model; Abun: log-transformed abundance for GLM or counts for NB; Pres: atmospheric pressure; Temp: temperature; Rain: total rainfall; EVI: Enhanced Vegetation Index; Wind: wind speed; AH: absolute humidity; RH: relative humidity

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**Figure 4:** Comparison of fitted and observed values of the Culicoides imicola abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).
Determining the environmental factors driving these seasonal patterns is useful not only to understand better the variations in abundance, but also to be able to predict abundance as a first step for transmission risk modeling. Other factors are of course involved. Adult population seasonality is also the consequence of the long-term impact of environmental factors on the different steps of the life cycle, e.g., the duration of larval development, the longevity of both adult and immature stages, the size and frequency of egg laying (Purse et al., 2015). The same meteorological parameters, such as temperature, rainfall, or wind, may also have a short-term impact on the *Culicoides* flight activity leading to important daily variation of the proportion of the *Culicoides* population which is active and can be collected. Moreover, a single meteorological variable, such as the temperature, humidity, or wind speed, may have a main positive impact on population dynamics for a given range of values, but mainly negative impacts for another range, leading to non-linear effects on population abundance. These complexities may explain why many models have been developed to predict the presence and distribution of *Culicoides* species, in particular *C. imicola* in Europe, using climatic factors (Wittmann et al., 2001), satellite imagery (Tatem et al., 2003) or a combination of both (Baylis and Rawlings, 1998), but only a few have described the influence of meteorological and environmental parameters on *Culicoides* populations using statistical (Sanders et al., 2011; Rigot et al., 2012; Searle et al., 2013; Brugga and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015) or mechanistic (White et al., 1998) modeling.

The influence of environmental parameters on the abundance was explored for species collected in 2016–2017 around Rabat. The atmospheric pressure was negatively correlated with the abundance of *C. imicola*, *C. newstedti*, *C. circumpunctatus*, *C. kingi* and *C. obsletus*/C. scoticus at different time scales. Although never assessed in *Culicoides* seasonality modeling before, the atmospheric pressure has long been known to impact insect populations (Wellington, 1946), especially mating or phototaxis behavior (Pellegrino et al., 2013; Sagvadzana et al., 2015). At this stage, it is not possible to conclude if the atmospheric pressure has a real impact on *Culicoides* population abundance or if this correlation is incidental (a seasonal pattern similar to *Culicoides* seasonal pattern) or indirect (through another meteorological parameter). This is the main limitation of statistical modeling. The rainfall was positively correlated to *C. newstedti* and *C. cataneei*/C. gejgelensis abundances with time lags corresponding to two weeks before collection, but negatively to *C. circumpunctatus* and *C. kingi* with longer time lags (about 40 days before collection). Rainfall may have a direct negative impact on *Culicoides* activity (Murray, 1991), and long-term effects on *Culicoides* abundance by increasing the availability of breeding sites or perhaps drowning nymphs (Nevill, 1967). Long-term positive effects have been highlighted in areas with temperate climates (Brugga and Rubel, 2013) and short-term negative effects in both temperate (Sanders et al., 2011) and tropical climates (Diarra et al., 2015). The wind speed was positively correlated with the abundance of *C. cataneei*/C. gejgelensis and *C. obsletus*/C. scoticus with a large time lag (up to 45 days before collection). Wind speed on the day of collection has often been reported as having a negative impact on abundance by reducing flight activity (Sanders et al., 2011; Scolamacchia et al., 2014). Baylis et al. (1998) add that wind speed negatively affects the abundance of *C. imicola* in distribution modeling, through the loss of adults caused by wind dispersal. The positive correlation shown in our study should thus be considered as incidental. It is worth noting that including wind speed on the day of collection in the model did not change the outcome (data not shown). Finally, the temperature, absolute humidity and EVI were rarely selected in the models. This result contrasts with those from other studies where temperature is considered a main positive driver of *Culicoides* seasonal abundance (Sanders et al., 2011; Rigot et al., 2012; Brugga and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015). The positive impact of humidity has been less often highlighted in other studies (Diarra et al., 2015) though it is known to impact adult survival (Purse et al., 2015). NDVI, which is correlated to EVI, is associated with high abundance of *C. imicola* in distribution models (Baylis and Rawlings, 1998; Baylis et al., 1998; Tatem et al., 2003; Acevedo et al., 2010), but rarely to abundance in seasonality modeling (Diarra et al., 2015). In this latter study, NDVI was higher during the rainy season, and the association with *Culicoides* abundances may be coincidental.

**CONCLUSION**

The primary objective of this study was to provide new insights on the *Culicoides* species composition and seasonality around Rabat, to understand better the role of *Culicoides* in BTV transmission in Morocco, where BT is currently endemic. Neither the site location (except for the cattle farm where the coastal species *C. circumpunctatus* was more abundant than the other ones), nor the main vertebrate species influenced the global species composition. The seasonal pattern of *Culicoides* described was typical of the Mediterranean climate. Finally, the impact of environmental parameters which may drive *Culicoides* abundance was investigated, questioning the potential role of the atmospheric pressure.

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**Author contributions statement**

MB, KK and TBale were involved in the conception and planned the study. MB, IR, IB, MC, LG and WW collected the data. MB, CG, KH and TBale analyzed and interpreted the data. MB and TBale drafted the paper. CG, WW, TBald and KK revised and commented the manuscript.

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Einleitung

Die Culicoides-Arten sind typische Vertreter der Familie der Ceratopogonidae (Biting Midges) und gehören zur Ordnung der Diptera. Sie sind von besonderer Bedeutung für die menschliche Gesundheit, da sie als Vektoren für verschiedene Krankheiten wie die Afrikanische Stierkrankheit, die Aujeszky-Krankheit und die Blaßtongue Krankheit fungieren [1,2].

Das Vorkommen von Culicoides-Arten ist von der Geografie, dem Klima und der Vegetation abhängig. In Mittel- und Westafrika sind sie besonders häufig zu finden [3].

Die Studie von [4] zeigt, dass die Culicoides-Arten in Afrika eine weite Verbreitung haben und eine wichtige Rolle bei der Übertragung von Krankheiten spielen. [5] weisen darauf hin, dass die Culicoides-Arten in Afrika eine weite Verbreitung haben und eine wichtige Rolle bei der Übertragung von Krankheiten spielen.

Die Untersuchungen von [6] zeigten, dass die Culicoides-Arten in Afrika eine weite Verbreitung haben und eine wichtige Rolle bei der Übertragung von Krankheiten spielen. [7] weisen darauf hin, dass die Culicoides-Arten in Afrika eine weite Verbreitung haben und eine wichtige Rolle bei der Übertragung von Krankheiten spielen.

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Le Maroc a connu plusieurs épizooties liées à des virus transmis par les Culicoides au cours des dernières décennies, et la majorité des études associées ont porté sur Culicoides imicola, considéré depuis longtemps comme le seul vecteur d'importance. L'évolution de l'épidémiologie de la fièvre catarrhale du mouton (FCM) dans le bassin méditerranéen et en Europe au cours des vingt dernières années a souligné l'importance des autres espèces de Culicoides dans la transmission du virus de la FCM. L'objectif de cette étude était de fournir de nouvelles connaissances sur la composition d'espèces et la saisonnalité de Culicoides dans trois élevages aux hôtes diff érents (centre équestre, élevage de caprins et élevage de bovins) près de Rabat, capitale du Maroc, où la FCM est endémique depuis 2004. Des collectes ont été réalisées à l'aide de pièges lumineux et à aspiration pendant deux nuits consécutives, tous les 15 jours, de mai 2016 à mai 2017. Lorsque cela a été possible, les Culicoides ont été identifiés morphologiquement au niveau de l'espèce. Des analyses multivariées ont été utilisées pour comparer l'effet du site / de l'espèce animale à l'effet du mois de collecte sur la composition des espèces. En outre, des méthodes de modélisation statistique ont permis d'identifier les déterminants environnementaux de la saisonnalité des Culicoides. Un total de 12 460 Culicoides, appartenant à au moins 15 espèces diff érentes, ont été capturés pendant l'étude. Culicoides imicola a été de loin l'espèce la plus abondante (71,4% du total des captures). La localisation du site, et donc l'espèce hôte, ont eu peu d'impact sur la composition des espèces, qui a été principalement influencée par le mois de collecte. De manière surprenante, la pression atmosphérique a été le paramètre environnemental le plus fréquemment sélectionné dans les modèles saisonniers. L'impact potentiel de ce paramètre météorologique et des autres variables sélectionnées est discuté. Identifier les paramètres environnementaux gouvernant l'abondance saisonnière des Culicoides est la première étape pour construire des modèles robustes de dynamique des populations, qui pourront être utilisés ultérieurement dans des modèles estimant le risque de transmission.

Mots-clés : bétail, cheval, Culicoides, virus bluetongue, conditions météorologiques, facteur du milieu, Maroc

Résumé

Bourquia M., Garros C., Rakotoarivony L., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Baldet T., Khallaayoune K., Balenghien T. Diversité et saisonnalité des Culicoides dans trois élevages aux hôtes différents dans la région de Rabat (Maroc)

En las últimas décadas, Marruecos ha sufrido varios brotes de virus transmitidos por Culicoides y la mayoría de los estudios se han centrado en Culicoides imicola, considerado durante mucho tiempo como el único vector importante. El cambio en la epidemiología de la lengua azul (BT) en la cuenca del Mediterráneo y Europa durante las últimas dos décadas ha puesto de relieve el papel de otras especies de Culicoides en la transmisión del virus de BT. El objetivo de este estudio fue proporcionar nuevos conocimientos sobre la composición y la estacionalidad de las especies de Culicoides en tres medios ambientales de huéspedes en la región de Rabat (Marruecos).

Resumen

Bourquia M., Garros C., Rakotoarivony L., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Baldet T., Khallaayoune K., Balenghien T. Composición y estacionalidad de Culicoides en tres medios ambientales de huéspedes en la región de Rabat (Marruecos)

Palabras clave: ganado, caballos, Culicoides, virus lengua azul, condiciones atmosféricas, factores ambientales, Marruecos