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Sero-epidemiology of bluetongue in Algerian ruminants

Moustafa KARDJADJ[1]*, Pam Dachung LUKA[2] and Meriem Hind BENMAHDI[1]

[1]Ecole Supérieure en Science de l'Aliments, Algiers, Algeria.
[2]Biotechnology Division, National Veterinary Research Institute, Plateau State, Nigeria.

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A total of 2871 blood samples were collected (450 cattle, 1932 sheep and 489 goats) from 225 randomly sampled herds between January and June 2014. Competitive enzyme-linked immunosorbent assay (C-ELISA) was used to detect antibodies from sera as an indicator of exposure to bluetongue virus (BTV). The results show an overall herd seroprevalence of 16.44% (95% CI 9.42-23.46); 21.3% (95% CI 17.1-25.6) for cattle and 13.33% (95% CI 9.86-16.8) for small ruminants, respectively. At the individual level; our results revealed an overall seroprevalence of 6.96% (200/2871); 13.7% (62/450) for cattle and 5.70% (138/2421) for small ruminants. The risk factor analysis performed by univariate analysis followed by multiple logistic regressions indicated that transhumance, mixed herds, presence of wetlands nearby the herds and lack of *Culicoides* controls strategies were the major risk factors for bluetongue sero-positivity in Algerian ruminant herds.

Key words: Algeria, bluetongue, seroprevalence, risk factors, ruminants.

INTRODUCTION

In Algeria, ruminants are one of the main sources of meat production (with an approximate population of 33 million heads; 2 million cattle, 27 million sheep and 4 million goats) and a significant financial income of an important part of the Algerian households. By virtue of its geographical location and its borders with the North African and Sahel countries, Algeria is vulnerable to several trans-boundary diseases, including bluetongue (MADR, 2014). Bluetongue (BT) is an infectious, non-contagious disease of ruminants transmitted by *Culicoides* biting midges. It is caused by the bluetongue virus (BTV) and it is classified as a reportable disease by the World Organization for Animal Health (OIE). Clinical disease is often observed in sheep, occasionally in goats, and rarely in cattle (Maclachlan, 2010). There are at least 26 distinct BTV serotypes including serotypes 25 and 26, which were recently identified from Switzerland and Kuwait (Hofmann et al., 2008; Maan et al., 2011). Due to the large number of circulating BTV serotypes in the Mediterranean Basin, it is generally very difficult to predict the serotype for a specific region (Saegerman et al., 2008). Maclachlan (2010) reported that several serotypes tend to circulate simultaneously in the same region; this is showed to be the case in Algeria where two serotypes were co-circulating; BTV-1 and BTV-4 (OIE, 2014). Saegerman et al. (2008) reported that the vast majority of BTV strains occurring in Europe have a direct northern African origin. However, few data are available

*Corresponding author. Email: drkardjadj@live.fr. Tel: +213796915906.

on BTV epidemiology in North Africa including Algeria. Therefore, the objectives of the present study were to estimate the bluetongue seroprevalence in Algerian ruminants, to describe its distribution and classify the risk factors associated with bluetongue herd seropositivity.

There is no well-defined control strategy for bluetongue in Algeria and vaccination against bluetongue is forbidden. The diagnosis of bluetongue in Algeria includes early recognition and notification of a suspect clinical situation by the field veterinarians and laboratory tests on blood to detect the specific antibodies (MADR, 2014). Furthermore; entomological monitoring for *Culicoides* research was recently set in the country in order to improve the efficiency of the *Culicoides* control operations by the appropriate use of insecticide (MADR, 2014).

MATERIALS AND METHODS

Study area and design

Algeria is located between latitudes 19°N and 37°N and longitudes 9°W and 12°E. It is the largest country in Africa. It has a long coastline at the Mediterranean Sea; most of the coastal area (northern region) is hilly, sometimes even mountainous. South of the northern region is a steppe; farther south, there is the Sahara desert. Administratively, Algeria is divided into 48 districts but for the present study purposes and according to geographical and farming management specificity, five regions were delimited and each region contained 7 to 12 districts; north-central (35.3°-36.8°N and 1°E-4.7°E), north-western (35°-36.3°N and 2°W-1°E), north-eastern (35.3°-37°N and 4.7°E-8.5°E), steppe (33°-35.3°N and 2°W-8.5°E) and Sahara region (19°-33°N and 8.8°W-12°E).

A cross sectional study with a two-stage selection design as described by Toma et al. (2009) was carried out across the country between January and June 2014 to investigate BTV sero-epidemiology in Algerian ruminants. The flock size (n = 225 herds; 150 small ruminants herds +75 cattle herds) was determined at a 95% confidence level using a 15% expected prevalence (Madani et al., 2011) and an absolute precision of 4%. Herds were selected from each region using random numbers generated by an electronic calculator; within each flock, animals were selected randomly by lottery. Ruminant owners participating in the study were informed about the purpose of the study and their verbal agreement was obtained. A questionnaire including individual and herd management risk factor attributes was administrated at all selected herds.

The questionnaire took account of species, age, sex, herd size, type of herd, contact with other flocks (yes or no), *Culicoides* control (use of insecticide or not) and the presence of nearby wetlands (yes or no).

Laboratory analysis and statistical analysis

A total of 2871 blood samples were collected (450 cattle, 1932 sheep and 489 goats) by jugular vein puncture in 5 ml sterile vaccutainer tubes using venoject needles (Venoject, UK). Blood samples were transported on ice for analysis. A competitive enzyme-linked immune-sorbent assay (c-ELISA) was performed using a commercially available BTV antibody c-ELISA Kit (Veterinary Medical Research and Development Laboratory, USA). The sera were screened for IgG anti-VP7-BTV antibodies according to the manufacturer recommendations.

For the risk factor analysis, an initial exploratory analysis of the data (univariable) was conducted for the selection of variables with $P \leq 0.2$ by chi-square test or Fisher's exact test. Subsequently, the variables that passed this cut-off were subjected to multivariable logistic regression (Hosmer and Lemeshow, 2000). The fit of the final model was verified using Hosmer and Lemeshow test, and collinearity between independent variables was verified by a correlation analysis. For those variables with a strong collinearity (correlation coefficient > 0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (Dohoo et al., 1996). Confounding was assessed by monitoring the changes in the model parameters when adding new variables. The calculations were performed using SPSS software version 20.0.

Confidence Interval (CI) at 95% = P +/- Pa, where P is the obtained Prevalence and Pa is the absolute precision was calculated for a two-stage random sampling, taking into account the variability that is likely to exist between and within flocks, using the following formula:

$$Pa = 2^* \sqrt{(1 + p\mathrm{m}) * \frac{\mathrm{P}(1-\mathrm{P})}{\mathrm{nm}}}$$

Where, p is a within-class coefficient, n is the flock size and m is the mean of sampled animals within each flock (Toma et al., 2009).

RESULTS AND DISCUSSION

Bluetongue is a great veterinary concern to small ruminant producers, wildlife managers and veterinary diagnosticians because of the frequent occurrence of outbreaks among domestic and wild ruminants in geographical regions previously known to be BT-free (Saegerman et al., 2008). According to recent studies (Mellor et al., 2008; Maclachlan, 2010), there was an evidence of occurrence of BT disease in tropical and subtropical countries. In these areas the disease appears sub-clinically and did not attract attention, in such circumstances, the presence of the virus is often confirmed via serological evidence.

The serological evidence of BTV exposure was observed in 37 (17 cattle and 20 small ruminants herds) out of 225 herds accounting for 16.44% (95% CI 9.42-23.46) herd seroprevalence; 21.3% (95% CI 17.1-25.6) for cattle and 13.33% (95% CI 9.86-16.8) for small ruminants. The difference observed between the herd seroprevalence within the studied regions (Table 1) were not significant (P>0.05). At the individual level our results revealed an individual seroprevalence of 6.96% (200/2871); 13.7% (62/450) for cattle and 5.70% (138/2421) for small ruminants. This means a significant reduction in the seroprevalence compared to that stated by Madani et al. (2011) that report 15.58%, suggesting an improvement of the sanitary status after some years of vector control strategies.

The findings of this study also suggested that antibodies to bluetongue virus in Algeria are widely distributed nationally among sheep, goats and cattle. This is in agreement with previous studies by Madani et al. (2011). Seropositivity in ruminants re-established the

Table 1. Univariable analysis for risk factors associated with bluetongue herd seropositivity.

Variable	Categories	Small ruminants			Cattle		
		No. of herds sampled	No. of positive herds (%)	*P*	No. of herds sampled	No. of positive herds (%)	*P*
Region	North-Cenral	20	3 (15%)	0.09*	20	4 (20%)	0.59
	North-Western	17	3 (17.64%)		20	4 (20%)	
	North-Eastern	23	6 (26.08%)		20	6 (30%)	
	Steppe	80	7 (8.75%)		15	3 (15%)	
	Sahara	10	1 (10%)		There was no cattle in sahara		
Herd size	Small herds	69	9 (13.04%)	0.34	57	12 (21.06%)	0.34
	Large herds	81	11 (13.6%)		18	5(27.7%)	
Type of herds	Sheep or cattle herds	99	14 (14.14%)	0.12*	44	6 (13.6%)	0.02*
	Mixed herds	51	6 (11.76%)		31	11 (35.5%)	
Grazing system	Sedentary herds	118	13 (11%)	0.03*	There was no transhumnt in cattle in Algeria		
	Transhumant herds	32	7 (21.87%)				
Contact with other herds	No	36	2 (5.55%)	0.13*	19	3 (15.8%)	0.11*
	Yes	114	18 (15.78%)		56	14 (25%)	
Culicoides control	No	114	1 (0.9%)	0.00*	52	15 (28.8%)	0.01*
	Yes	36	19 (52.77%)		23	2 (8.7%)	
Wetlands nearby	No	94	8 (8.51%)	0.01*	48	8 (16.7%)	0.01*
	Tes	56	12 (21.42%)		27	9 (33.3%)	

*Variables selected and used in the multiple analysis ($P \leq 0.2$).

circulation of BTV in Algeria (Wilson and Mellor, 2009; Madani et al., 2011). However, to avoid the menace of BT outbreaks in the future, more surveillance of the disease incidence and entomological data should be encouraged and general preparedness to counter outbreaks be advocated (Wilson and Mellor, 2009).

At the end of the univariate analysis (Table 1); region, type of herds, grazing system, contact with other herds, *Culicoides* control and the presence of wetlands nearby were subjected to logistic regression for the risk factor study as potential risk factors. However, the risk factor analysis using multivariable logistic regression (Table 2) did recognize the transhumance, mixed herds, *Culicoides* control and the presence of wetlands nearby as risk factors for bluetongue seropositivity in Algerian ruminants herds.

In the present study the risk of bluetongue seropositivity in small ruminants herds was 2.44 (95% CI 1.30-3.58; P = 0.041) times higher in transhumant herds than in sedentary herds. This could be attributed to the fact that the transhumant movement of herds from dry area to wetlands increased the risk of vector exposure and BTV transmission. Arguably, transhumant herds experience transportation stress, underlying parasitic infection and other predisposing factors which might lower individual or herd immunity and thereby increased the susceptibility of animals to the infection (Mellor et al., 2008).

Likewise the odd ratio for cattle and small ruminants was 2. 19 (95% CI 1.02-3.36; P = 0.03) and 3. 39 (95% CI 1.20-5.48; P = 0.023) times higher in regions nearby wetlands than in dry areas. Climatic factors play an important role in the occurrence of BTV infection in animals and influence the size of vector populations (Mellor et al., 2008). Although more than 1000 species of *Culicoides* are known worldwide, relatively few of these species have been incriminated as BTV vectors; species of vector insects that transmit BTV differ amongst regions. *Culicoides imicola* is the traditional African-Asian vector of BTV (Tabachnick, 2004). Our study shows that the preventive measures, such as routine application of anti-*Culicoides* insecticide such as *deltamethrin* in the selected herds by spraying or dipping decreased the odds for bluetongue seropositivity, respectively for cattle

Table 2. Risk factor (logistic regression) associated with bluetongue herd seropositivity.

Risk factor	*B*	SE	Odds ratio	95% CI	*P*
Small ruminants					
Transhumant herds	0.658	0.363	2.44	1.30 - 3.58	0.041
Culicoides control	0.875	0.524	7.87	3.99 - 11.75	0.018
Presence of wetlands nearby	0.827	0.419	3.39	1.20 - 5.48	0.023
Cattle					
Mixed herds	0.689	0.427	2.10	1.20 - 3	0.03
Culicoides control	0.714	0.469	2.21	1.17 - 3.25	0.02
Presence of wetlands nearby	0.784	0.523	2.19	1.02 - 3.36	0.03

Hosmer and Lemeshow test: Chi-square = 4.145; df = 2; P = 0.126

and small ruminants, by 2. 21 (95% CI 1.17-3.25; P = 0.02) and 7.87 (95% CI 3.93-11.75; P = 0.023) times compared to non-sprayed herds.

The present study shows also that the risk of bluetongue seropositivity in herds was 2.10 (95% CI 1.20-3.; P = 0.03) times higher in mixed herds than in cattle's only herds. This could be attributed to the fact that sheep are more sensitive to BTV. Therefore, the presence of sheep in cattle farms increases the risk of BTV transmission.

In conclusion, our results indicate a serological evidence of exposure to bluetongue virus that was widely distributed in Algeria. Lack of adequate *Culicoides* control measures the practice of mixed herd's, transhumance and the presence of nearby wetlands, were identified as risk factors for bluetongue seropositivity in ruminants in Algeria. Targeting the vector factors especially; the *Culicoides* control measures in disease prevention strategy may play a key role in controlling and preventing the transmission of BTV in Algeria. Consequently, entomological monitoring for *Culicoides* was recently launched in the country in order to improve strategies on the appropriate insecticides to be used for the control of *Culicoides* in the country.

Conflict of Interests

The authors have not declared any conflict of interests.

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