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Abortion and various associated risk factors in small ruminants in Algeria

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ABSTRACT

Identification of the causes of abortion among the huge population of small ruminants in Algeria (≈31 millions heads), is an important task for the control of livestock productivity and viability scourges to the small ruminants industry. Optimal production and utilization is constrained by a number of factors: disease, poor feeding and low management skills. Therefore, in the present study the prevalence of abortion in Algerian small ruminant's flocks was estimated and its possible association was correlated with infectious (PPR, BT and Brucellosis seropositivity) and managerial (flock size, grazing system, type of farming, and contact with other flocks) risk factors. The present study showed an overall flock prevalence of small ruminant's abortion as 75.33% (113/150) [95% CI 71.72–78.94%]. The risk factor analysis using multivariable logistic regression recognized the north-western and the steppe region as well as PPR positivity as a risk factor for abortion in Algerian small ruminant's flocks. The odds of flock abortion was 11.47 [95% CI 2.39–54.88; $P = 0.002$] and 10.31 [95% CI 1.28–82.88; $P = 0.028$] times higher in north-western and steppe regions respectively compared to other region. Also the presence of PPRV infection in small ruminant flocks amplified the odds by 6 times [95% CI 2.221–17.427; $P = 0.001$].Surprisingly, the univariate analysis for the other risk factors associated with abortions in Algerian small ruminant flocks indicated no statistically significant links with bluetongue ($P = 1.000$) and brucellosis seropositivity ($P = 0.334$). Flock size ($P = 0.574$), type of farming ($P = 0.443$), grazing system ($P = 0.117$) and contact with other flocks ($P = 0.245$) was also not statistically significant. Our results revealed that abortion in small ruminants is a challenge to farmers and PPR was chiefly linked to it. Therefore an effective vaccination and control programme is advocated for small ruminants in Algeria.

1. Introduction

Small ruminant production is one of the main sources of meat production (≈31 millions heads) in Algeria that plays a vital role in food security. The small ruminants industry has the ability to improve the living standards of farmers and households, as well as increase animal protein for Algerians and consequently alleviating poverty. The small ruminant population in Algeria stands as 27 millions sheep and 4 millions goats respectively, where 65% of the total populations are females and 35% males. However, both sheep and goats are reared under traditional extensive system in Algeria, intensive husbandry systems has recently been introduced in the country (MADR, 2014).

In spite of the population advantage of small ruminants, diseases and poor herd-health management practices poses a significant challenge to optimal and efficient management and profitable small ruminants' production in developing world such as Algeria. However, the viability of sheep and goat farming depends largely on their reproductive performance which is invariably regulated by genetic and environmental factors (Mellado et al., 2006). Additionally, abortion represents the most dangerous livestock productivity and viability scourges and public health concerns posed by some of the zoonotic microorganism (Van Engelen et al., 2014; Benkirane et al., 2015).

Abortion of food producing animals have a negative impact on livestock production, animal health and ultimately rural economies since most small ruminants are kept by the rural poor as a means of

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alleviating poverty (Diallo, 2006). However, the farming system and communal grazing practiced enables infectious organism to spread very fast and therefore, there is the need for an improved diagnostic capacity, appropriate and adequate control strategies and periodic monitoring for healthy livestock and public health safety (Van Engelen et al., 2014). There are several potential factors underlying the causes of abortion which can be broadly categorized into infectious and non infectious (Entrican, 2009).

Infectious agents are the most plausible causes of abortion in sheep and goats as compared to non-infectious agents and are mostly zoonotic. The main infectious causes of abortion in sheep and goats are *Chlamydia abortus*, *Campylobacter* spp., *Toxoplasma gondii*, *Listeria* spp., *Yersinia pseudotuberculosis*, *Coxiella burnetii*, *Brucella miletensis*, and *Bluetongue virus* (BTV) (Entrican, 2009; Van den Brom et al., 2012; Lafi et al., 2013; Ababneh et al., 2014).

North Africa and the Middle East has been described as endemic areas for Brucellosis with characterized high incidences of human cases (Rubach et al., 2013). In North Africa, this scenario is supported by environmental factors, communal grazing mostly by the poor farmers (Spickler and Roth, 2008). Exposure to these pathogens can predispose an animal to abortion and severe public health implications (Holler, 2012; Benkirane et al., 2015).

Infection from early embryonic term to the end of the gestation period plays a critical role in the viability of the fetus. These diseases cause abortion, fetal loss and congenital abnormalities in lambs and kids (Radostits et al., 2007; Van Engelen et al., 2014). Recently, Peste des Petites Ruminants Virus (PPRV) infection of pregnant sheep and goats has been implicated in abortion in Turkey (Güler et al., 2014). Subsequently, Kardjadj et al. (2015a) described the first occurrence of the disease in both sheep and goats in Ghardaïa district, the isolated strain belongs to lineage IV of PPRV and was genetically closely related to the strain identified in neighboring Morocco and Tunisia. This infectious and highly contagious agent spreads easily among small ruminants in a system with low management practices. Furthermore, the fact that both sheep and goats are not vaccinated against PPRV makes this disease a serious threat to the livelihood of Algerian farmers.

In 2012, Hamza and Bouyoucef (2013) reported that 90% of Algerian farmers had observed abortions in their heads. However, the causes of these abortions remained undiagnosed. In order to protect and sustain the Algerian small ruminant industry and equally boost productivity, it is therefore critical to have adequate knowledge about the possible abortion causes. The present study attempted to investigate and estimate the prevalence of abortion in Algerian small ruminant's flocks during a national survey and correlate its possible association with infectious agents (PPR, BT and Brucellosis seropositivity) and managerial (flock size, grazing system, type of farming, and contact with other flocks) risk factors.

2. Materials and methods

2.1. Study area

Algeria is located between latitudes 19° 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 (1600 km); 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 (wilayas) but for the sake of this study and according to the geographical and farm management specificity, the country is delineated into five regions, with each region containing 7 to 12 districts; north-central (35.3°–36.8°N and 1°E–4.7°E), northwestern (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).

2.2. Study design and sample collection

Small ruminant owners participating in the study were informed about the purpose of the study and their verbal consent was obtained. The number of flocks to be sampled from each region was proportional to the percentage of small ruminants in that region. Flocks were selected from each region using random numbers generated by an electronic calculator.

A cross-sectional study with a two-stage selection design as described by Toma et al. (2009) was carried out for 6 months across the country between January and June 2014. The simple size (n = 150 flocks) and the number of animals to be sampled within each flock (m = 15 animals) was determined at a 95% confidence level using; an expected prevalence for bluetongue at 15% and brucellosis at 5%, respectively (Madani et al., 2011; Kardjadj, personal communication), with an absolute precision of 4% and 2.5%, an estimated within-class coefficient of P = 0.4 and 0.6 and an inflation coefficient of 7 and 10.

From the 150 flock, a total of 2421 blood samples were collected (1,932 sheep and 489 goats) by jugular venous puncture in 5 ml sterile vacutainer tubes using venoject needles (Venoject, UK). Blood samples were transported on ice to Institut National de Medecine Vétérinaire (INMV), Algiers, Algeria for analysis. The samples were centrifuged at 3000 rpm for 5 min and separated into a sterile tube and stored at −20 °C until tested.

2.3. Laboratory analysis

The 150 selected flocks were screen for antibodies to PPR (Kardjadj et al., 2015b), using a competitive ELISA (c-ELISA) according to manufacturer's instructions (ID Screen® PPR Competition, ID vet, Montpellier, France). This diagnostic kit detects antibodies against the nucleoprotein of PPRV with sensitivity and specificity of 94.5% and 99.4%, respectively (Libeau et al., 1995).

Similarly, a competitive enzyme-linked immune-sorbent assay (c-ELISA) was performed for BTV antibodies using a commercially c-ELISA Kit (Veterinary Medical Research and Development Laboratory, USDA Pullman, WA, USA). The sera were screened for IgG anti-VP7-BTV antibodies as described by the manufacturer's specifications. The test sensitivity and specificity were 100% and 99%, respectively according to the manufacturer's specification.

The assay was performed in a 96-well antigen coated microplates. The incubations were performed for 15 min at room temperature (21 ± 2 °C). The plates were washed three times with the provided washing buffer. Briefly, aliquots of 25 μl test sera as well as positive and negative controls sera were transferred undiluted to the BTV antigen coated plates. After incubation, the plates were washed, and 25 μl of antibody-peroxidase conjugate were added to each well. The plate was then incubated at 15 min at room temperature. The plates were then washed and 50 μl the substrate was added to each well. The reaction was stopped using 50 μl of the stopping solution. The results were read using ELISA reader set at 630 nm. A diagnosis was made when the test samples produced an optical density <50% of the mean of the negative controls. The test samples were considered negative if the optical density ≥50% of the mean of the negative controls.

Additionally, all serum samples were screened using Rose Bengal Plat Test (RBPT) according to the procedures (Lilidale®). Briefly, 75 μl of serum was mixed with 25 μl antigen, on the plate and shacked. After 4 min of gentle shacking, any visible agglutination was considered as positive.

2.4. Data collection and statistical analysis

A questionnaire was administered to small ruminant owners (farmers) in all selected flocks by personal interview with 100%

retrieval. The questionnaire contained information about abortion history in the flock during the last two (2) years, the number of small ruminants as well as females per flock and flock management risk factor attributes including size (small flocks <100 heads, large flocks >100 heads), grazing system (sedentary and transhumant flocks), history of contact with other flock (yes or no), and type of farming; sheep flock (containing only sheep) and mixed flock (containing both sheep and goats). There was no goat alone rearing flocks encountered during the study.

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. If substantial changes (i.e., higher than 20%) were observed in the regression coefficients, this was considered as indicative of confounding. The calculations were performed using SPSS software version 20.0.

Confidence Interval (CI) at 95% = $P \pm$ Pa, where P is the obtained Prevalence and Pa is the Absolute Precision 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: $\text{Pa} = 2 \times \sqrt{(1 + \text{pm}) \times \frac{P(1-P)}{\text{nm}}}$ where p is the within-class coefficient, n is the flock size and m is the mean of sampled animals within each flock (Toma et al., 2009).

3. Results and discussion

The epidemiology of infectious agents causing abortion in Algeria is not well investigated. The report of Hamza and Bouyoucef, (2013) only revealed abortion from sheep and goats using questionnaire study without looking at the causal agents. Furthermore, with the outbreak of PPR in Algeria and it national survey (Kardjadj et al., 2015a,b), the same samples were utilized for the detection of antibodies to some of the infectious agents (PPR, BTV and Brucella), which were highlighted by farmers as a problem at that time. However, there was no much information from Algeria for *Toxoplasma gondii, Chlamydia abortus, Coxiella burnetii* and *Campylobacter* spp.

The main objective of this study is to investigate the prevalence of abortion in Algeria during a national survey and correlates it possible association with infectious agents and managerial risk factors. The survey processed data including region, flock size, grazing system, contact with other flocks and seropositivity to PPRV, BTV and Brucellosis.

The present study showed a flock size ranging from 38 to 2500 and a mean of 127 small ruminants per flock. The average number of females per flock was 82 ranging from 21 to 1400. From the questionnaire analysis, there were reports of abortion in 113 of the 150 flocks sampled and an overall flock prevalence of small ruminant's abortion as 75.33% [95% CI71.72–78.94%]. Our results were lower than those of Hamza and Bouyoucef (2013) who reported abortion prevalence of 90%. Arguably, this disparity in higher percentage compared to our present study maybe due to fact that ours was a national survey compared to one region (central region of Algeria) where the previous authors investigated. Holler (2012) in the USA reported that abortion rates varies among producers, production systems and management styles, but a rate much higher than 5–8% is usually deemed unacceptable and worthy of investigation. Therefore, identification of the risk factors associated with abortion can aid in optimizing herd reproductive efficiency.

However, in our study the information about abortion was obtained by a questionnaire and this means that bias may be produced. The abortion history was asked for the last two years because, of previous study by Hamza and Bouyoucef, (2013) assessing the risk of abortion in 2012 and there is no much information from 2013 and 2014. Furthermore, small ruminants farming in Algeria is extensive to semi intensive with intensive farming newly introduced into the country, so therefore breeding is random with nothing like lambing season.

Our findings revealed that abortion is widely prevalent in small ruminant's flock in all the studied regions; the region's prevalence ranged from 40 to 88.2%. Nevertheless the univariable analysis for risk factors associated with the prevalence of abortions in Algerian small ruminant flocks (Table 1) illustrated that the differences among studied regions was statistically significant ($P = 0.002$). Prevalence in the north-western and the steppe region of Algeria was significantly higher than in other studied region.

Surprisingly, the univariable analysis for management risk factors associated with abortion in Algerian small ruminant flocks attributes (Table 1) indicated no statistically significant links among flock size ($P = 0.574$), type of farming ($P = 0.443$), grazing system ($P = 0.117$) and contact with other flocks ($P = 0.245$). Arguably, this may be due to the fact that the abortion prevalence was very high covering all managerial flock system. Although, previous researchers' showed that small ruminant's abortion history was influenced by flock size, mixed farming, grazing system and contact with other flocks (Mellado et al., 2006; Gebremedhin et al., 2013) and attributed this fact to the environmental stress and inadequate feeding regimes of the herd. High density atmosphere increases the chances of contracting the contagious pathogen causing abortion. Furthermore, Entrican, (2009) reported that animals in large flocks also receive inadequate attention from managers as compared to small flocks which are easier to monitor and control.

Our results also showed that BTV infection was widely prevalent in small ruminant's flocks in all the studied regions with an overall flock seroprevalence 13.33% (20/150) [95% IC 9.86–16.8]. Such a seroprevalence suggests that the disease is well distributed within all the geographical areas and infect perhaps most of the susceptible animals in affected flocks. The highest seropositivity to BTV was from the northern region (Table 3) and could be attributed to climatic factors that favor the maintenance and recirculation of the BTV in its vertebrate and non-vertebrate vector hosts. Comparatively, Sahara and the Steppe reported a low seroprevalence; this could be mainly due to the hot and dry climatic conditions of these regions which are unfavorable for the survival and maintenance of the life cycle of the insect vector. Climatic factors play an important role in the occurrence of BTV infection in animals because they influence the size of the vector populations (Tabachnick, 2004; Maclachlan, 2010). Although BTV infection cause abortion in small ruminants flocks (Radostits et al., 2007; Mellor et al., 2008), the univariable analysis for BTV infection associated with the prevalence of abortions in Algerian small ruminant flocks (Table 1) illustrated non significant association ($P = 1.000$).

On the contrary, the serological evidence of Brucellosis infection (due to *Brucella melitensis*) was observed to be very low compared to PPR and BTV infection. Five (05) flocks out of 150 were positives for Brucellosis infection (Table 3) accounting for 3.33% flock prevalence [95% IC 2.41–4.25] suggesting a significant enhancement in brucellosis sanitary statute. Furthermore, the multivariate analysis showed that vaccination decreased the chances of brucellosis infections in flocks by 25 times compared to non-vaccinated flocks.

In addition Kardjadj and Ben-Mahdi (2014) reported a significant improvement of small ruminant brucellosis sanitary status in

Table 1
Univariate analysis of the abortions prevalence in Algerian small ruminant flocks.

Variables	No. of flocks sampled	No. of flocks with abortion	%. of flocks with abortion	*P-value*
Region				
North-Central	20	12	60%	0.002*
North-Western	17	15	88.2%	
North-Eastern	23	14	60.9%	
Steppe	80	68	85%	
Sahara	10	4	40%	
Flock size				
Small	69	50	72.5%	0.574
Large	81	63	77.8%	
Type of farming				
Sheep	99	77	77.8%	0.443
Mixed	51	36	70.6%	
Grazing system				
Sedentary	118	85	72%	0.117*
Transhument	32	28	87.5%	
Contact with other flock				
No	36	24	66.7	0.245
Yes	114	89	78.1	
PPR positivity				
No	86	55	64%	<0.001*
Yes	64	58	90.6%	
BTV positivity				
No	130	98	75.4%	1.000
Yes	20	15	75%	
Brucellosis positivity				
No	145	108	74.5%	0.334
Yes	5	5	100%	

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

Table 2
Risk factors of the abortions in Algerian small ruminant flocks.

Risk factors	Logistic regression coefficient	Standard error	Wald	Odds ratio	95% CI	*P*-value
Steppe region	2.333	1.063	4.820	10.313	1.284–82.809	0.028
North-western region	2.440	0.799	9.336	11.474	2.399–54.887	0.002
PPR positivity	1.828	0.526	12.099	6.221	2.221–17.427	0.001

Hosmer and Lemeshow chi-square = 0.770; $P = 0.979$.

Table 3
Flock seroprevalence for BT, brucellosis and PPR infection in the five regions.

Variables	North-Central	North-Western	North-Eastern	Steppe	Sahara
BT flock overall	3/20 (15%)	3/17 (17.64%)	6/23 (26.08%)	7/80 (8.75%)	1/10 (10%)
Brucellosis flock overall	1/20 (5%)	0/17 (0%)	1/23 (4.34%)	1/80 (1.25%)	1/10 (10%)
PPR flock overall Kardjadj et al. (2015b)	9/20 (45%)	11/17 (64.7%)	7/23 (30.4%)	33/80 (41.2%)	4/10 (40%)

the steppe region eight (08) years after the Algerian state adopted the Rev-1 vaccination in 2006 as a prophylactic approach.

Large number of Algerian unpublished studies had suggested an association between *Brucella* seropositivity and abortion in Algerian small ruminant's flocks. However, our study revealed no significant association ($P = 0.334$) between abortion history and brucellosis infection (Table 1).

We equally correlated flock history of abortion with PPR flocks seropositivity, since PPR has been recently linked to small ruminant's abortion in Turkey (Güler et al., 2014), when PPR virus was detected by RT-PCR and isolated from an aborted sheep fetus that was bacteriologically negative (culture, molecular, and serological methods). Furthermore Truong et al. (2014) observed a lesion at the uterus level in sheep and goats following an experimental PPRV infection.

Subsequently, Kardjadj et al. (2015b) described overall apparent flock seroprevalence of PPR was 42.66% (64/150) and showed a relatively uniform distribution of PPR seroprevalence among the five studied regions (Table 3), suggesting a widespread distribution and endemic establishment of PPR in Algerian small ruminant population.

From our study, significant association was found ($P < 0.001$) between PPR flocks seropositivity and abortion history in Algerian small ruminant's flocks (Table 1). Furthermore the risk factor analysis using multivariable logistic regression also showed (Table 2) that the presence of PPRV infection in small ruminants flock amplified the odds by 6 times [95% CI 2.221–17.427; $P = 0.001$]. Abubakar et al. (2008) has previously suggested a possible association of PPR virus with abortion in goats based on serology in Pakistan. In addition, clinical and pathological findings such as abortions/stillbirths (Toplu, 2004), and secondary bacterial infectious agents have been frequently observed in PPR affected flocks probably as a result of the immunosuppressive effects of the virus (Diallo et al., 2007).

The risk factor analysis using multivariable logistic regression did recognize the north-western and the steppe region as a risk factor for abortion in Algerian small ruminant's flocks (Table 2). The odds of flock abortion was 11.47 [95% CI 2.39–54.88; $P = 0.002$] and 10.31 [95% CI 1.28–82.88; $P = 0.028$] times higher in north-western and steppe regions respectively compared to other region. Arguably, this disparity in higher percentage compared to other region could be related to the high prevalence of PPR in the north-western region and to flocks/animal's density in the steppe region, considering that in the steppe region lives more than 52% of the Algerian small ruminants' population.

4. Conclusion

Our results revealed PPR to be chiefly linked to abortion in small ruminants in Algeria. Although, BTV and Brucellosis are not statistically a risk factor from our analysis but their detection is a pointer to the need for their control to enhance productivity. Therefore an effective vaccination and control program is advocated for small ruminants in Algeria so as to prevent the spread of the disease among small ruminants.

Conflict of interest

The authors declare no conflict of interest.

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