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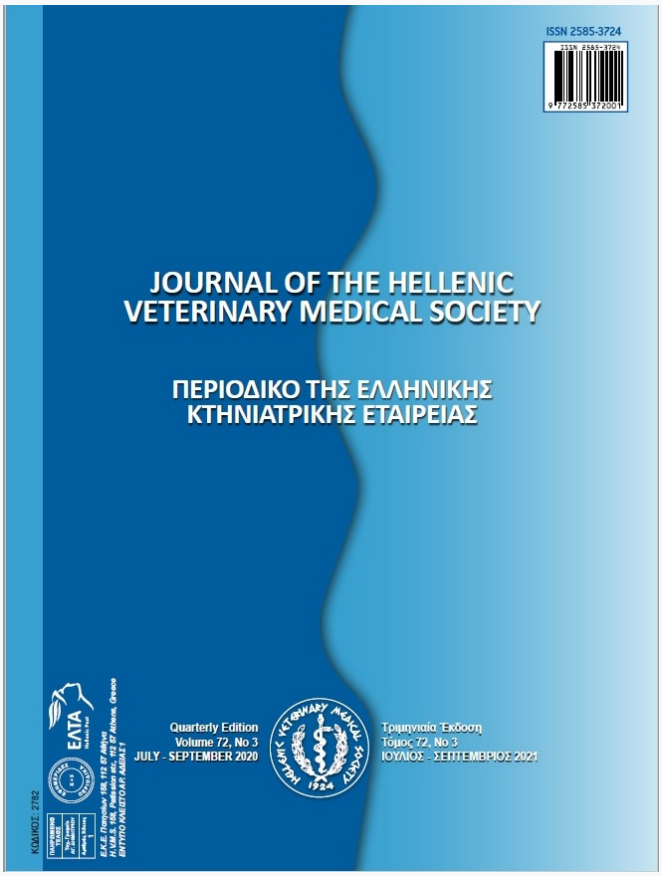

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Research article
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Seroprevalence and risk factors associated with *Salmonella* Dublin presence in Algerian dairy farms

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ABSTRACT: *Salmonella* Dublin is a causative agent of a gastrointestinal bacterial infection prevalent in many cattle herds worldwide. Hence, the goal of this research was to evaluate the prevalence of *Salmonella* Dublin carriage in fecal and milk samples from dairy cattle from Algeria, and to investigate potential risk factors associated with the presence of *S*. Dublin antibodies. A total of 307 cows from 39 farms were analyzed in this study. Bacteriological and immunological methods were used to isolate and detect *S.* Dublin antibodies in feces and cow's milk. Antimicrobial susceptibility testing was performed using the disc diffusion method. Logistic regression was used to study risk factors associated with *S*. Dublin antibodies. The bacteriological results showed the absence of*S*. Dublin and a prevalence of 0.97 %(3/307) (IC 95% 0 - 2.08)for *S*. Mbandaka. The immunological analysis of milk by the ELISA technique showed a prevalence of 36.33% (95% CI 30.44 - 42.22) for *S*. Dublin. Final multivariate regression models showed that the breed, the region and introduction of purchased cattle were associated with the presence of *S.* Dublin antibodies. This study is the first that reports the seroprevalence and risk factors associated with *S*. Dublin infection in Algeria and could be considered as a comparison point for further studies in Algeria.

***Keywords*:** Cattle, Milk, Risk factors, *Salmonella* Dublin, Seroprevalence.

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INTRODUCTION

Salmonella infections are a major concern for the various animal productions and for public health(Agren et al., 2016). Salmonellosis is one of the most common diseases in cattle but also poses a significant zoonotic risk (Camart-Périéet al., 2007). Cattle is the main reservoir of *Salmonella enterica* subsp. *Enterica* serovar Dublin (*Salmonella* Dublin) which is considered to be the most frequent cause of *Salmonella* infection in cattle (Henderson and Mason, 2017).

These carrier animals are responsible for propagating infection in dairy herds via *S.* Dublin shedding in feces and milk (Holschbach and Peek, 2018),and are transmitted to humans, usually through the consumption of beef meat and cow's milk (Molla et al., 2003; Rodrigez-Rivera et al., 2014).Veterinarians have also been infected from skin contact with the bacteria, especially following obstetric maneuvers and inseminators (Visser, 1998). In addition, *S.* Dublin is the serovar of most economic concern, because of its particularly invasive nature, causing acute diarrhea and mortality, mainly observed in calves between 2 weeks to 3 months of age, septicemia and reproductive disorders, including abortions. Moreover, with this serovar, some animals remain infected for life without manifesting clinical signs (asymptomatic carriers) (Radostits et al., 2007).Therefore, the presence of these asymptomatic carriers of *S.* Dublin in cattle herds is a major concern because they shed the bacteria continuously or intermittently for years in milk and/or faeces, resulting in environmental contamination and infections in other animals (Holschbach and Peek, 2018). However, the use of bacteriological examination for the detection of the Dublin serovar has lower sensitivity rate compared with serological methods (Nielsen, 2013; Nyman et al., 2013). Therefore, the most used tests for *S.* Dublin detection include enzyme-linked immunosorbent assays (ELISAs) used for the detection of immunoglobulins against *S.* Dublin in serum and in milk samples, and bacteriological culture of fecal samples (Veling et al., 2002; Nielsen and Ersbøll, 2004). In Algeria, the prevalence of *S.* Dublin has not yet been studied.To date,only two studies were published about *S.* Dublin in Algeria (Ayachi et al., 2012; Derdour et al., 2017),but no study was done on risk factors associated with the presence of *S.* Dublin antibodies. Therefore, the aims of our work were (i) to investigate the prevalence of *S.* Dublin carriage in dairy cattle, (ii) to identify potential risk factors that could be associated with the presence of *Salmonella* Dublin antibodies, and (iii) to compare the ELISA test with bacteriological methods in detection of serovar Dublin from the dairy herd.

MATERIALS AND METHODS

Study area

This study was carried out in Khenchela region. This region is located in the east of Algeria, and it is characterized by a large number of cattle (4478 cows in 2018), and a promising milk sector (27 million liters of milk per year). The altitude range is from 1050 to 1710 meters and the daily average temperature ranges from -2°C to 42°C.

Sampling

We calculated the sample size using the formula for simple random samples recommended by Thrusfield (2007):

$$n = (1.96)^2 \frac{Pexp(1-Pexp)}{d^2}$$

where n = required sample size; Pexp = expected prevalence; d = desired absolute precision; 1.96 was the Z value for the selected confidence level (95%). According to this formula, the minimum sample size for an infinite population was 139 cows using an expected individual prevalence of 10% (according previous studies in this region), a desired absolute precision of 5% and a confidence level of 95%. The sample size was increased to 307 in order to increase the absolute precision and compensate for 5% attrition. A total of 39 farms were randomly selected, from which, 307 fecal samples were taken and analyzed. About 25g of individual fecal samples of cows were collected directly from the rectum using disposable gloves, and then stored in sterile pots. Samples were then sent for analysis on the same day. On the other hand, milk from 256 cows (10 mL) among the 307 cows selected for bacteriological analysis, was collected in vacutainer tubes and stored at -80°C until serological analysis (Fifty-one cows were in the dry period, and they were not included in milk sampling).

The minimum number of cattle to be tested on each farm was established as 10 (Cannon and Roe, 1982), the number of cattle to be sampled on each farm was defined on the basis of the total number of cattle in the farm: the farm consisted of less than 10 cattle, in which case all cattle were harvested or the farm contained more than 10 cattle and, in this case, at least 10 individuals were taken.

Questionnaire survey

During this study, a questionnaire was established-to determine potential risk factors. The variables included as potential risk factors at the farm level were as follows: Farm location (El Hamma, Baghai, El Mahmal, Kais, Remila), age (between 2 to 10 year), breed (Montbéliarde, Holstein, crossed breed, Brown Swiss, Fleckvieh, Normande, Limousin), general hygiene (good, average, bad), introduction of new purchased animals (yes/no), water supply (networks,-drilling), water quality (bad/clean), gestation (yes/no), gestation stage (between 1 to 9 month), parity(uniparous, multiparous), clinical signs at the time of collection(diarrhea, mastitis, respiratory problem, arthritis, eye infection, no sign, abortion (yes/no),stage of abortion (between1-9 month)).

BACTERIOLOGICAL CULTURE

Isolation of *Salmonella* spp.

The isolation was performed according to the AFNOR standard (NF U: 47-100) (2007). 25g of individual fecal samples were mixed with 225 mLof buffered peptone water (Condalab, Spain)and incubated for 24h at 37°C. Then, 1 mL of the pre-enriched culture was transferred to Müller Kauffmann Tetrathionate-novobiocin broth (Bio-Rad, France) and 0.1mLof the same pre-enriched culture was transferred to Modified Semisolid Rappaport Vassiliadis Medium (MSRV;Condalab, Madrid, Spain)and incubated at 37°C and 42°C for 24h respectively. A loopful from each culture was streaked into selective xylose-lysine-deoxycholate agar (Condalab,Spain) and Hektoenagar plates (HK; InstitutPasteur Algeria (IPA)),and incubated at 37°C for 24h. The initial biochemical tests were performed on a 24h pure culture using Triple Sugar Iron (TSI; IPA) agar slant, indole urea reagent (IPA), Lysine Decarboxylase (LDC; IPA) reagent and ortho-NitroPhenyl-β-galactoside (ONPG; IPA).Then, the API 20E system (BioMérieux, France)**.**

Serotyping of *Salmonella*

Salmonella serovars were identified serologically by slide agglutination test using diagnostic polyvalent and monovalent O and H *Salmonella* antisera (Bio-Rad, France),according to Kauffman-White scheme (Grimont and weill, 2007).

Antimicrobial susceptibility testing

The agar disk diffusion method was used to determine the antimicrobial susceptibility patterns of *Salmonella* isolates according to the Clinical and Laboratory Standards Institute guidelines, (CLSI)(2018) Using Mueller- Hinton agar (IPA, Algiers, Algeria). The isolates were tested for the following antibiotics (disk content): ampicillin (10 µg), piperacillin (100 µg), ticarcillin (75 µg), amoxicillin/clavulanate (20 µg/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), imipenem (10 µg), sulfonamides(300 µg), trimethoprim (5 µg), cortrimoxazol (25µg), nalidixic acid (30 µg), norfloxacin(10 µg), ciprofloxacin (5 µg),colistin (10 µg), furans (300 µg), chloramphenicol (30 µg) and tetracycline (30 µg), the results were evaluated after 24h of incubation at 35°C.

ELISA TEST

The ELISA test is based on the detection of antibodies against *Salmonella* lipopolysaccharide (LPS) antigens, and it was performed according to the manufacturer's instructions (PrioCHECK *Salmonella* Antibody ELISA Dublin; Thermo Fisher Scientific, Waltham, MA). Milk samples were heated for one hour at 37°C. Briefly, the upper layer of fat was pulled out, and the undiluted skim milk samples were inoculated in 96 microtiter plate and the optical density (OD) was measured at 450nm using ELISA reader (Bio-Rad, USA).

STATISTICAL ANALYSIS

Statistical differences in proportions were compared using the Chi-square test. The association between the presence of serovar Dublin in milk and possible risk factors was tested using logistic regression (SPSS software version 20). The farm was included as random effect due to repeated measurements, P value equal to or less than 0.25 during simple regression were forwarded to multiple regression analysis, and only variables with P value ≤ 0.05 were included in the final model of risk factors. Specificity, sensitivity, Kappa, McNemar test and confidence intervals were calculated with the use of Winepiscope 2.0. Values of$P< 0.001$ and $P<0.05$ were considered as statistically significant.

RESULTS

Bacterial isolation and serotyping of *Salmonella* isolates

Three out of 307 (0.97%) collected fecal samples were positive for *Salmonella*, all the three serotyped *Salmonella* were *Salmonella* Mbandaka. However, serovar Dublin was not found in any fecal cultures.

Antibiotic susceptibility testing

The antimicrobial susceptibility pattern of the three isolates indicated that all isolates were susceptible to the all antibiotics used.

Serology of milk samples

The ELISA results showed, that out of the 256 milk sample examined, 93 (36.33%) were positive at 95% with a confidence interval between30.44 to 42.22for *S.* Dublin antibodies in milk samples, while 163 (63.67%) were found negative. The difference of *S.* Dublin individual seroprevalence between regions (municipalities) was statistically significant (P<0.05) (Table1).The comparison of the prevalence of *S.* Dublin using bacteriological methods (0%) and ELISA (36.33%) indicated clearly that those methods were significantly different (P<0.01). The capability to detect a positive animal is significantly higher for ELISA.

Risk-factors analysis

Risk factors (Table 2) with P≤ 0.25 in the univariable analysis (Univariable regression results table is included as supplementary material S1) were included in the final model of regression: Age, hygiene, gestation, stage of gestation, parity, and clinical signs at the time of collection. Cows from the Remila region were less susceptible of having *Salmonella* antibodies in milk, than cows in El Hamma region (OR=0.027, IC: 0.003-0.256), and the introduction of new purchased animals reduced the risk of having *Salmonella* antibodies in milk (OR=0.06, IC: 0.008-0.510). However, Brown Swiss cows were 15 times more susceptible of having *Salmonella* Dublin antibodies in milk than the Montbeliarde (OR= 15.66, IC: 1.679-146.15).

DISCUSSION

Diseases caused by *Salmonella* spp. constitute a real problem of public health and animal production in the world (Smith et al., 2004). *S.* Dublin is a serotype adapted and concern to cattle in several countries due to its ability to induce abortions, reduced milk production and its significant economic losses (Visser et al., 1997).

Table 1. Individual serological prevalence of *Salmonella* Dublin in milk by region

Region	Farm	Samples (%)	Seropositive	Prevalence % 95% CI[a]	P value
El hamma	14	105 (41.01)	42	40 (30.63- 49.37)	
Baghai	2	12 (4.68)	7	58.33 (30.44-86.23)	
El mahmal	6	24 (9.37)	19	79.77 (62.92-95.41)	
Kais	11	69 (26.95)	17	24.64 (14.47-34.81)	< 0.0001[b]
Remila	6	46 (17.96)	8	17.39 (6.44-28.34)	
Total	39	256	93	36.33 (30.44-42.22)	

[a]Confidence interval (95%CI),[b]< 0.0001: The results are very significant in every single region.

Table 2. Final multivariable logistic regression model; for identifying the association between risk factors and the presence of *Salmonella* Dublin in milk

Risk factors	Level	OR[a]	95% CI[b]	Pvalue
Cow breed	Montbéliarde	-[c]	-	
	Brown Swiss	15.66	1.679-146.15	0.016
Region	El Hamma	-	-	-
	Remila	0.027	0.003-0.256	0.002
the introduction of purchased cattle into a farm	Yes	0.06	0.008-0.510	0.010
	No	-	-	-

[a]Odds ratio at cow level (OR), [b]Confidence interval (95%CI), [c]Reference Category

In this study, based on the Bacterial isolation, three *Salmonella* spp. were isolated from 307 fecal samples (0.97%), similar results were previously reported in Spain (0.9%) (Adesiyun et al., 1996), Egypt (0.97%) (Mohamed et al*.,* 2011), Iran (1.25%) (Halimi et al.,2014), and in Turkey (1.74%) (Hadimli et al., 2017). However, the prevalence was much higher in other countries such as the USA (10.1%)(Cummings et al., 2010), Ethiopia (7.6%) (Eguale et al., 2016), and in Ivory Coast (20%) (Yao et al., 2017).These differences could be explained by seasonal variation in *Salmonella* shedding of animals, other factors such as herd size and age could be responsible for these differences (Fossler et al., 2005). Moreover, most of the farms visited in the current study had small herd size, and *Salmonella* fecal shedding by cattle is commonly intermittent (Warnick et al., 2003;Cummings et al., 2010). Moreover, the region can also influence the frequency of isolation from one study to another (Callaway et al., 2005).

In our study, *S*. Dublin was not detected. However, the isolates detected in fecal samples belonged to Mbandaka serovar, this serovar is not frequently reported from cattle. Nevertheless, in one study conducted in the USA, it was found to be one of the most prevalent serovars at slaughter houses (Wells et al., 2001), which can indicate that *S.*Mbandaka can colonize cattle and could be transmitted to the slaughterhouse environment.

Milk collected from 256 cows was analyzed by ELISA serology to evaluate the presence of serovar *S.* Dublin. A positivity rate of 36.32%(93/256) was recorded; this prevalence was similar to that found in Ireland (49%) (Doherty et al., 2013). However, our results were higher than those found in the USA (14.1%) (Smith et al., 1989), Denmark (11%) (Nielsen, 2009) and in Sweden(3%) (Agren et al., 2015). The differences in the seroprevalence rates of *S.* Dublin in milk from dairy cows may also be attributed to the geographical location and herd size that can influence significantly the seroprevalence of salmonellosis in the dairy cattle (Kabagambe et al., 2000).

The comparison between the direct detection technique of *S.* Dublin (Fecal culture), and the indirect detection technique (ELISA test), shows different results,by the absence of this bacteria in fecal culture, and the presence of its antibodies in milk, which can indicate that the bacteriological method is less sensitive than the immunological method.Nevertheless, the two methods indicated two different results. The bacteriological method showed the presence of alive *Salmonella* in feces (at least one bacteria per 25 g). On the other hand, ELISA detected the presence of anti-*Salmonella* antibodies in milk. This can indicate that *Salmonella* antibodies will persist even in absence of alive *Salmonella* in cows. These results were similar to those reported by Nielsen (2013) who found a low number of *S.* Dublin, and they were isolated from 0.7% (46/6614) of dairy cattle. The immunological method is based on the presence of specific antibodies in milk, the persistence and the level of detectable antibodies seems to be higher than the presence and amount of *S*. Dublin in feces.*S*. Dublin in feces also might be caused by the existence of latent carries with persistent antibodies and intermittent shedding of *S*. Dublin in feces (Smith et al., 1989; House et al., 1993).Therefore, bacteriological culture tests are not ideal, because of their lack of sensitivity (Nielsen and Dohoo, 2012). However, some differences in detection limits may be found between types of *Salmonella* and between feces types, *S*. Dublin may have a poor analytical sensitivity than other types of *Salmonella*, and its detection limits in cow feces may be higher, because of some factors such as structure of the fecal matter and competing ruminal microflora(Nielsen and Dohoo, 2012). Moreover, the sensitivity of the bacteriological culture tests are known to be best for recently infected animals (1-15 days post-infection), untreated, diseased animals and carrier cows during the peripartum period where shedding is most likely to occur due to stress following for instance hormonal changes (Nielsen et al., 2004).

The study of risk factors allowed the identification of some factors that can be associated with the presence of *S.* Dublin antibodies in milk. A strong association was found between the Brown Swiss cows and the presence of *S*. Dublin antibodies in milk,these cows are characterized by a low milk production level. However, they are rich in protein content which create a good environment for bacteria proliferation (De Marchi et al.,2007).

Moreover, the Remila region was more likely to be infected with *S.* Dublin than El Hamma region, this finding is in agreement with a study conducted in Wales and North-west England (Davison et al., 2006), and another study in USA (Ruzante et al., 2010) that showed that differences between regions can be found. In addition, there was a significantly negative association between the introduction of new purchased ani-

mals and the presence of *S.* Dublin antibodies in milk. Where the purchase of animals reduced the amount of antibodies in milk, which is not in accordance with other studies who found that the purchase of animals is a significant risk factor for the development of *Salmonella* infections in herds (Van Schaik et al., 2002; Nielsen and Dohoo, 2012).

CONCLUSIONS

We have detected *S.* Dublin antibodies from 36.33% of milk samples, indicating that it is widely distributed in the region of Khenchela. Moreover, we have found thatthe indirect method (ELISA test) is more sensitive that the direct method (bacteriological culture) for the detection of *S.* Dublin. Moreover, the region, the breed and the purchase of new animalsare important risk factors associated with the presence of *S.* Dublin antibodies in milk.This work could be considered as pioneer and a comparison point for further studies in Algeria. However, additional epidemiological data using more cattle herds are needed to determine the distribution of these serovars in Algeria.

CONFLICT OF INTEREST

We have no conflict of interests to declare

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