SEROLOGICAL STUDY OF *SALMONELLA* DUBLIN CARRIAGE IN COWS IN KHENCHELA, ALGERIA

Hezil Djamila[1,2*], H. Benseghir[2.3], S. Zaidi[2] , R. Zineddine[2], N. Zaatout[3], A. Msela[4], F. Ghalmi[2]

[1]Department of Biology, Faculty of Sciences, M'Hamed Bougara University, Boumerdes 035000, Algeria
[2]Research Laboratory Management of Local Animal Resources, Higher National Veterinary School of Algeria, ENSV, Algeria
[3]Department of Microbiology, Faculty of Natural and Life Sciences, University of Batna2, Algeria.
[4]Faculty of Biological and Agronomic Sciences, Mouloud Mammeri University of Tizi Ouzou, Algeria.
**Corresponding author: d.hezil@univ-boumerdes.dz*

Abstract. The objective of this study is to estimate the seroprevalence of *S.* Dublin infection in healthy cows in the Khenchela region and to identify potential risk factors that may be associated with the presence of *S.* Dublin antibodies. 194 cows sera from 35 farms were tested using an enzyme-linked indirect immunosorbent assay (ELISA) and the results showed a prevalence of 9.23% (18/194, 95% CI 5.17-13.29). The logistic regression model indicates that none of the factors tested were found to be significantly associated with *S.* Dublin seropositivity. We concluded that *S.* Dublin circulates in cattle farms in the Khenchela region of Algeria. In addition, we recommend the implementation of hygiene practices and biosecurity measures on farms to reduce the spread of infection and the use of vaccination in animals and people at risk.

Keywords: Cows, *S.* Dublin, Sera, ELISA, Risk factors

INTRODUCTION

Salmonellosis is one of the most common diseases in cattle. They cause high rates of disease and mortality. All *Salmonella* found in cattle have the potential to spread to humans. Among the 2600 serotypes that exist (Huang et al., 2020), *Salmonella enterica* serovar Dublin (*S.* Dublin) a serotype adapted to cattle, is considered the most common cause of *Salmonella* infection in cattle (Visser et al., 1997). In addition, *S.* Dublin causes significant economic losses in calves and young animals, abortions and reproductive disorders in adults (Henderson and Mason, 2017). Transmission of *S.* Dublin to humans occurs primarily through consumption of beef and cow's milk (Humphrey et al., 2000).An epidemiological study of *S.* Dublin infection in humans was conducted in the United States and showed that between 2005 and 2013, 78% of infected persons were hospitalized and 4.2% died (Harvey et al., 2017).

The *S.* Dublin is an emerging disease in cattle farming. Once it enters the farm, this bacterium can persist for a long time (Nielsen et al., 2004). 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). The slow growth of Dublin serovar in common culture media makes its detection difficult (Nielsen, 2013). However, laboratory tests, mainly serological

methods, are used for the detection of immunoglobulins against this serovar in serum and milk (Veling et al., 2002; Nielsen and Ersbøll, 2005).

Purchase of cattle, direct contact with other cattle, especially grazing, and Neighbouring farms being seropositive are risk factors for introduction of infection (Wedderkopp et al., 2001; Van Schaik et al., 2002). Increasing herd size, increasing surface water area, can either aggravate the disease or increase the susceptibility of cattle to *Salmonella* infections (Vaessen et al., 1998). Despite its importance, the status of cattle caused by *Salmonella* spp. and particularly *S*. Dublin in Algeria is unknown. Therefore, the main objectives of this study are to estimate the seroprevalence of *S*. Dublin infection in apparently healthy cows in the Khenchela region, and to identify the risk factors associated with its seropositivity.

MATERIAL AND METHODS

Study area: The Wilaya of Khenchela (35° 25′ 55″ N, 7° 08′ 40″ E), is located 500 km south-east of the capital Algiers. It belongs to the natural area of the Eastern Highlands, and is located in the extreme south of it. It is constituted of 8 Daïras and 21 communes The wilaya of Khenchela covers an area of 9 811 km², a large part of which is used for agriculture. The wilaya of Khenchela has approximately 10885 heads of cattle including 4478 heads of modern dairy cows. The production of cow's milk is 27806260 liters per year (Agricultural Services Direction of Khenchela, 2019). The farming method is generally semi-intensive. The animals are fed hay, bran and grass during the grazing season. The grazing season runs from March to December with variations according to climatic conditions. The combination of cattle breeding with sheep and goats is common in this region.

Sampling mode: A descriptive epidemiological survey was carried out using a well-structured questionnaire addressed to the breeders of the selected farms. The questionnaire covered 35 cattle farms and a total of 194 randomly selected cows. This questionnaire was used to analyze potential risk factors related to S. Dublin infection.

Farms were selected randomly from a list of cattle breeders in the Wilaya of Khenchela (Fig.1). The aim was to have a homogeneous distribution of the selected farms in the study area. Subsequently, the number of cattle to be sampled from each farm was defined according to the total number of cattle present. When the farm had less than 10 cattle, all cattle were sampled. When the farm contained more than 10 cattle, the number of individuals sampled was at least 10 (Table1). The goal was to have a sample representing at least 10% of all cattle present on the farms visited (Cannon and Roe, 1982).

The variables included as potential risk factors at the operating 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 (between 1-9 month)).

Table 1

Summary of the number of farms and cows sampled by municipality.

Municipalities	N° herds	Number of cows sampled
El hamma	14	74
Baghai	2	9
El mahmal	6	31
Kais	8	58
Remila	5	22
Total	35	194

Collection of samples and conservation: This study was conducted during the period from December 2017 to May 2018. A total of 35 farms were randomly selected from which 194 blood samples were collected (from different farms in Khenchela region).

Blood samples were collected from the tail vein. An amount of 5 to 10 mL was recovered in sterile, vacuum-packed tubes. The tubes were then numbered. The blood samples were transported in a cooler at 4°C to the microbiology laboratory at the CHU of the wilaya of Khenchela where the collected blood was centrifuged for 5 to 10 minutes at 3000 rpm. The resulting sera were immediately transferred to eppendorf® tubes and stored in a freezer at -20°C.

Detection of antibodies directed against *S*. Dublin (The ELISA test): The ELISA test, based on the detection of antibodies directed against *Salmonella* lipopolysaccharide (LPS) antigens, was performed according to the manufacturer's instructions (PrioCHECK® *Salmonella* Ab bovine Dublin; Thermo Fisher Scientific, Waltham, MA). It is a test designed for the *in vitro* detection of *Salmonella*-specific antibodies in bovine milk and serum.

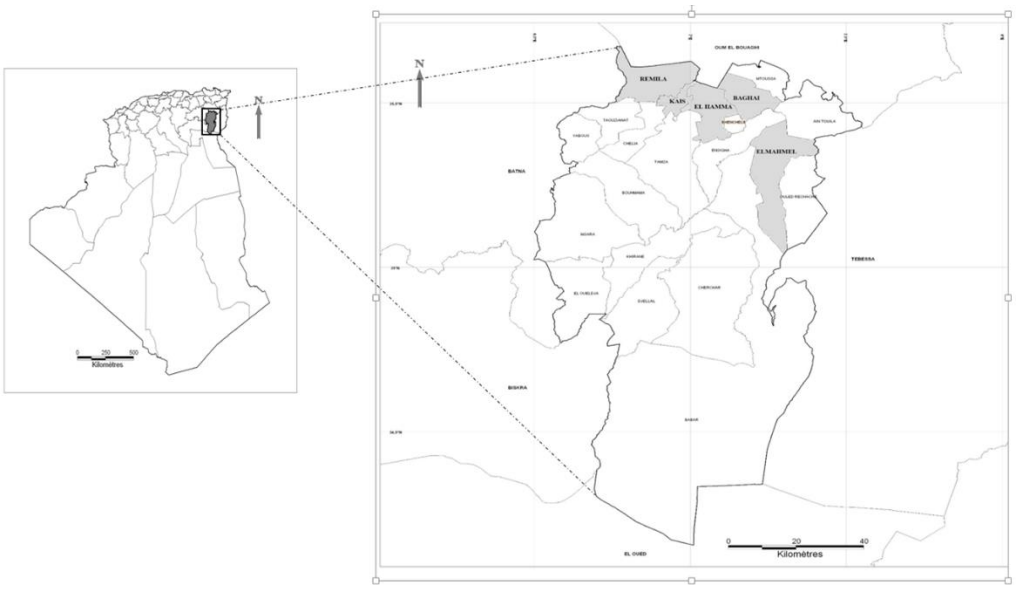

Fig .1. Location of regions selected for sampling

A 1/20 pre-dilution was performed for the sera in an unsensitized blank plate by mixing 10 µL of sera with 190 µL of dilution buffer. After the following the protocol prescribed by the manufacturer. Optical densities were recorded using a plate reader (BIO RAD, USA) using a 450 nm filter.

The result of each sample was expressed as percent positivity (% PP), which was calculated according to the formula (1).

$$\text{Formula (1) : PP} = \left[\frac{\text{Corrected OD450 test sample}}{\text{Corrected OD450 Positive Control}} \times 100 \right] - 10$$

Samples with a % PP(= ≥ 35%) were considered positive; and those less than 35% were considered negative. Doubtful results were considered negative in this study.

Statistical analysis

Proportions were compared using the Chi-square test. The significant association of risk factors potentially associated with *S.* Dublin seropositivity was evaluated in two steps: Univariable and multivariable analyzes (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.

RESULTS

Seroprevalence of *S.* Dublin: The results showed that of the 194 cows, 18 tested positive for *S.* Dublin , with an individual prevalence rate of 9.23% (95% CI 5.17–13.29), *S.* Dublin and the location of the tested cows (p > 0.05). However, the highest rate of seropositivity is observed in the east of the wilaya of Khenchela in the commune of El mahmal (16.13%) and the absence of anti-*Salmonella* Dublin antibodies is noted in cows from farms located in the commune of Remila (Table 2).

Table 2

The seroprevalence of *S.* Dublin in cows in the five municipalities of the wilaya of Khenchela.

Municipalitie	Farm	Samples (%)	Number of positive samples	Number of negative samples	Seroprevalence (%) IC 95%	*P value*
El Hamma	14	75 (38.66)	6	69	8 (1.86-14.14)	0,38
Baghai	2	9 (4.63)	1	8	11,11 (0- 31.64)	
El Mahmal	6	31 (15.97)	5	26	16,13 (3.18-29.08)	
Kais	8	58 (29.89)	6	52	10,34 (2.51-18.08)	
Remila	5	21 (10.82)	0	21	0	
Total	35	194	18	176	9,23 (5.17-13.29)	

[a]Confidence interval (95%CI), P probability.

Risk factors associated with *S*. Dublin infection in cows in the wilaya of Khenchela: Table 3 presents the results of univariate logistic regression analysis of the risk factors associated with the presence of *S*. Dublin in cow sera from the Khenchela region.The logistic regression model indicates that none of the factors tested were found to be significantly associated with *S.* Dublin seropositivity in cow sera.

Table 3

Univariate logistic regression Analysis of risk factors associated with the presence of *S*. Dublin

Variable	Numbre	Seroprevalence % IC	OR[a]	Odds Ratio[b] (95% CI)	*P* value
Region					
El Hamma	75	8 (1.86 -14.14)	0.526	0.025-11.261	0.679
Baghai	9	11,11(0- 31.64)	0.212	0.004-12.426	0.453
El Mahmal	31	16,13 (3.18- 29.08)	0.202	0.008-5.401	0.338
Kais	58	10,34 (2.17-13.29)	0.295	0.009-9.190	0.484
Remila	21	0	[c] -	-	-
Age (year)					
2 to 4	66	13,64 (5.36 -21.92)	1.524	0.085-27.379	0.774
5 to 7	155	7.83	1.303	0.178-9.546	0.262
8 to10	13	0	-	-	-
Breed					
Montbeliarde	93	6,45 (1.45 -11.44)	0.294	0.000-292.685	0.726
Holstein	72	11,11 (3.85 -18.37)	0.250	0.000-259.454	0.694
Crossed breeds	7	0	0.900	0.000-862.974	0.988
Fleckvieh	3	33,33 (0- 86.68)	0.120	0.000-223.656	0.579
Normande	13	15,38 (0-35.00)	0.020	0.000-31.224	0.294
Limousine	3	0	0.168	0.000-680.579	0.672
Brown of the Alps	3	33,33 (0 -86.68)	-	-	-
Quality of Hygiene					
Good	34	5 ,88 (0- 13.79)	2.850	0.077-105.330	0.567
Average	115	11,30 (5.52 -17.09)	0.807	0.156-4.183	0.797
Bad	45	6,67 (0-13.95)	-	-	-
Source of water					
Drilling	188	9,57 (5.37- 13.78)	0.105	0.000-56.679	0.737
Networks	6	0	-	-	-
Quality of water					
Bad	111	7,21 (2.40-12.02)	0.671	0.144-3.118	0.608
Clean	83	12,05 (5.04- 19.05)	0.671	0.144-3.118	0.608
Introduction of new animals purchased from the farm					
yes	153	7,19 (3.10- 13.29)	3.415	0.598-19.499	0.166

No	41	17,07(5.56-28.59)	-	-	-
Gestation					
yes	131	11,45 (6.00- 16.90)	1.831	0.006-604.513	0.837
No	63	4,76 (5.17 -13,29)	-	-	-
Stage of gestation (month)					
Absence	131	11,45 (6.00-16.90)	1.860	0.005-664.067	0.835
1 to 3	30	10 (0 -20.74)	0.567	0.058-5.556	0.624
4 to 6	53	15,09 (5.46- 24.73)	0.391	0.083-1.834	0.232
7 to 9	48	8 ,33 (0.51-16.15)	-	-	-
Parity					
Multiparous	153	8,90 (5.09 -14.52)	1.473	0.097-22.382	0.779
Uniparous	41	7,32 (0 -15.29)	-	-	-
Clinical signs at the time of collection					
No sign	185	9,19 (5.03-13.35)	0.331	0.00-585.801	0.857
Diarrhea	1	0	0.434	0.00-130.578	0.924
mastitis	3	0	1.925	0.00-167.127	0.925
Respiratory problem	3	33,33 (0- 86.68)	0.018	0.00-529.084	0.530
Arthritis	1	0	1.143	0.00-333.304	0.988
Ocular	1	0	-	-	-
Abortion					
No	184	0	0.120	0.00-102.322	0.760
yes	10	9,78 (5.49-14.08)	-	-	-
Stage of abortion (month)					
Absence	184	0	2.130	0.00-576.684	0.920
de 7 to 9	5	0	1.264	0.00-361.817	0.954
de 4 to 6	5	0	-	-	-

[a]Odds ratio at cow level (OR), [b]Confidence interval (95%CI), [c] Modality of reference.

DISCUSSION

This epidemiological survey is the first study in Algeria elucidating the risk factors associated with the prevalence of *S.* Dublin in a representative number of randomly selected cows. The main objective is to clarify the epidemiology of bovine salmonellosis due to *S.* Dublin. In the present research, 18 out of 194 cows are seropositive for *S.* Dublin, a prevalence of 9.27%. This seroprevalence is close to that published in Denmark with a rate of 8% (45/587) (Hoorfar et al., 1994), in the Netherlands with a rate of 12.3% (Veling et al., 2002). On the other hand, our seroprevalence was lower than those reported in Denmark (25-35%) (Nielsen, 2013). These differences in the seroprevalence obtained can be explained by the different serological tests used, the threshold values and the sampling methods applied. These factors make it difficult to compare seroprevalence rates between countries and regions. Differences in results may also be related to hygiene management, herd size

and the presence of other diseases in the herd. This can also be explained by differences in endemic situations that may be related to variations in infectious doses and immunity levels in different age groups (Nielsen, 2013).

Concerning the factors associated with *S.* Dublin infection in cows is necessary for a good understanding of its epidemiology, as well as its implications in terms of control strategies adapted to local conditions.In addition, data on the factors that may contribute to the occurrence of *S.* Dublin in the cattle herd are scarce and deserve more attention. Thus, a number of factors potentially related to *S.* Dublin infection in cows were analyzed in our study. The logistic regression model indicates that none of the factors tested were found to be significantly associated with *S.* Dublin seropositivity in cow sera.

The disease caused by *S.* Dublin is considered a multifactorial disease (Nielsen, 2003). *S.* Dublin is a host-adapted strain and infected cattle therefore represent the main risk to a naïve herd. Purchase of cattle, direct contact with other cattle, especially grazing, and Neighbouring farms being seropositive are risk factors for introducing infection (Wedderkopp et al., 2001; Van Schaik et al., 2002). Farms that do not have biosecurity measures in place for trade visitors are more likely to experience an epidemic (Van Schaik et al., 2002). Increasing herd size, increasing surface water area, and the presence of liver flukes on the farm increase the risk of infection, can either aggravate the disease or increase the susceptibility of cattle to *Salmonella* infections (Vaessen et al., 1998).

Concerning the geographical distribution, we could not find a link between the prevalence of *S.* Dublin and the geographical location of the cows ($p>0,05$). However, a study conducted in Wales and northwest England (Davison et al .,2006), and another study conducted in the United States (Ruzante et al., 2010) found a significant association between the presence of *S.* Dublin and geographical distribution. These two studies show that differences in prevalences between regions can be observed. Indeed, seroprevalence in cows can vary widely between countries, or even between regions of the same country.

It is difficult to compare the regional results with the results of other studies because different parameters come into play. It is clear that regional differences in *Salmonella* occurrence may exist, but there is a lack of current evidence that these regional differences appear consistently over time.

As far as the breed is concerned, the *S.* Dublin has not revealed any significant relationship according to breed. However, the highest rate of seropositivity is observed by the Fleckvieh and Brown of the Alps breed with a rate of 33.33%, due to their susceptibility to diseases and to the fact that it is a breed that does not tolerate the breeding conditions practiced in our country. However, the interpretation of these observations is sometimes difficult because other factors can intervene such as breeding practices that vary from one breed to another.

The analysis of the age factor shows that there is no significant association between age and seropositivity with respect to *S.* Dublin *(*$p > 0.05$). The higher rate is observed in cows between 2 and 4 years of age (13.64%). This is consistent with the results of other studies suggesting that heifers and younger cows had significantly higher risks of becoming carriers of *S.* Dublin (Nielsen, 2003; Nielsen et al., 2004).

The purchase of new animals is generally considered a major risk factor for the introduction of infectious diseases, including *Salmonella* (Vaessen et al 1998; Van

Schaik et al., 2002; Nielsen et al., 2007; Nielsen and Dohoo, 2012). In the present study, statistical analysis showed no significant difference between the introduction of new animals and *Salmonella* positivity. Indeed, it has been shown that previously uninfected animals may be contaminated during transport if they are in contact with a latent carrier animal whose infection is reactivated by transport stress (Gronstol et al., 1974).

Source and quality of water showed no significant relationship with positivity at *S.* Dublin. However, there are numerous reports of *Salmonella* isolation from rivers and streams and once the water supply is contaminated, rapid spread of infection can occur. Williams (1975) found that a number of cases of *S.* Dublin infection have been associated with streams contaminated by grazing animals and farm effluents. Vaessen et al. (1998) also found an increase in *S.* Dublin infection in cattle when cattle have direct access to a contaminated stream. In addition, *Salmonella* has often been reported in river and stream samples (Wray and Davies, 2000). Therefore, surface water should generally be considered a risk factor for *Salmonella* infections because of the potential for surface water contamination from runoff of fertilized manure from fields or from animals defecating in water (Pelzer, 1989).

The results obtained for the gestational status factor did not show any difference between pregnant and non-pregnant cows. On the other hand, a difference in seroprevalence according to the stage of pregnancy was observed. Indeed, cows around mid-gestation (4-6 months) are often less seropositive (15.09%: 95% CI 5.46 - 24.73).

In general, cows infected with *S.* Dublin do not develop clinical signs, but some latent animals may retain the bacterium in their lymph nodes and tonsils for a long time. These latent carriers may become active carriers following stress, particularly during gestation, and play an important role in the spread of infection within and between herds (La Ragione et al., 2013; Holschbach and Peek, 2018). Indeed, the authors found that changes in the immune response through pregnancy or hormonal imbalance can reactivate *S.* Dublin carriers and increase significantly towards the end of gestation. In addition, stress is an important risk factor in the pathogenesis of infection development. The stress hypothesis is further supported by a study in which dexamethasone injections in experimentally infected animals were used to induce long-term *S.* Dublin mammary gland carriers (Spier et al., 1991; Nielsen, 2003).

Thus, several other studies have shown that cows can be infected and excrete the organisms to a higher degree when they are stressed, in particular at parturition (Kemal, 2014).

The analysis of the parity factor, firm showed no significant relationship with positivity in *S.* Dublin ($p > 0.05$). Indeed, multiparous cows were more positive to *S.* Dublin infection with a rate of 8.90% compared to uniparous cows with a rate of 7.32%. However, Nielsen (2013) suggested that the seroprevalence of *S.* Dublin becomes more stable with age. This finding appears to be related to the fact that primiparous cows are immunologically naïve compared to multiparous cows that have developed immunity strong enough to prevent recurrence.

Concerns the cow having aborted factor; if we consider the analysis according to whether or not the seropositive cow had an abortion, no significant association is found ($p > 0.05$). Indeed, the seroprevalence in aborted cows is 9.78% (95% CI: 5.49-14.08), compared to 0% seroprevalence in non-abortioned cows. These results

corroborate with data in the literature (Hinton 1971 and 1974; Nielsen, 2003). Hall and Jones (1977) found that *S.* Dublin multiplied rapidly in the connective tissue of the cotyledons just prior to abortion, resulting in placental destruction and hormonal changes, which trigger abortion.

However, the results on risk factors should be interpreted with prudence, because other pathogens responsible for abortion in cows such as *Brucella abortus, Neospora caninum, Listeria monocytogenes and L. interrogans* serovar Hardjo exist.

No significant relationship was found between the presence of clinical signs at the time of sampling and seropositivity. In addition, a study by Chaturvedi and Sharma (1981) indicates that seroprevalence may be higher in cattle persistently infected with *S.* Dublin without clinical signs than in herds with clinical problems. However, an animal infected with another pathogen may also be immunologically weak and develop salmonellosis following infection with *S.* Dublin (Vaessen et al., 1998). Aitken et al. (1976) found that *Fasciolase hepatica* increased susceptibility to *S.* Dublin infection. Similarly, in calves, combined *Salmonella* and bovine viral diarrhea virus (BVDV) infection was more severe than *Salmonella* infection alone (Wray and Roeder, 1987). Severe disease was observed in a group of pregnant dairy heifers that had BVDV and *S.* Typhimurium DT104 infection (Penny et al., 1996). On the other hand, Morisse and Cotte (1994) found no association between BVDV and *F. hepatica* infections with salmonellosis, as both agents had identical prevalence in infected and control herds.

CONCLUSION

Our seroprevalence study for *S.* Dublin showed that this bacterium is widespread in cattle farms in the Khenchela region. Univariate analysis indicated a lack of association between seroprevalence of *S.* Dublin and risk factors tested in cows in the Khenchela region. Indeed, the percentage of detection of *S.* Dublin was higher in the parameters breed (Fleckvieh and Brown of the Alps), age (between 2 and 4 years), poor hygiene conditions this shows that these factors probably favour contamination by *S.* Dublin. Parity (multiparous) and non-aborting cows were more positive for *S.* Dublin infection.

Therefore, we recommend the implementation of hygienic practices and biosecurity measures on farms to reduce the spread of infection and the use of vaccination in animals and people at risk. We also recommend extensive epidemiological investigations in animals and humans to better understand, control and evaluate the real prevalence of salmonellosis in Algeria.

REFERENCES

1. Aitken, M.M., Jones, P.W., Hall, G.A., Hughes, D.L. (1976). The effect of fascioliasis on susceptibility of cattle to Salmonella Dublin. British Veterinary Journal, 132, 119-120.Cannon, R.M., Roe, R.T. (1982). Livestock Diseases Surveys: A Field Manual for Veterinarians. Canberra: Australian Bureau of Animal health.

2. Carrique-Mas, J.J., Willmington, J.A., Papadopoulou, C., Watson, E.N., Davies, R.H.(2010). Salmonella infection in cattle in Great Britain, 2003 to 2008. Veterinary Record, 167(15), 560-565.

3. Chaturvedi, G.C., Sharma, V.K. (1981). Cell-mediated immunoprotection in calves immunized with rough Salmonella dublin. British Veterinary Journal, 137, 421-430.

4. Davison, H.C., Sayers, A.R., Smith, R.P., Pascoe, S.J ., Davies, R.H ., Weaver, J.P., Evans, S.J . (2006). Risk factors associated with the salmonella status of dairy farms in England and Wales. Vet Rec,159 (26), 871-80.

5. Gronstol, H., Osborne, A.D., Pethiyagoda, S. (1974). Experimental salmonella infection in calves.2. Virulence and the spread of infection .J Hyg, 72 (2), 163-168.

6. Hall, G. A., Jones, P. W. (1977).Study of the pathogenesis of experimental Salmonella Dublin abortion in cattle. J Cow PATH, 87.

7. Harvey, R. R. Friedman, C.R., Stacy, M. C, Michael, J., Barrett, A.K, Beth, T., Jason, P.F., Patricia, M. G., Allison, C. B. (2017). Epidemiology of Salmonella enterica Serotype Dublin Infections among Humans, United States, 1968-2013. Emerging Infectious Diseases, 23, 9.

8. Henderson, K., Mason, C. (2017). Diagnosis and control of Salmonella Dublin in dairy herds. In Practice, 158-168.

9. Hinton, M.H. (1971). Salmonella abortion in cattle. Veterinary Bulletin, 41, 973-980.

10. Holschbach, C.L., Peek. (2018). Salmonella in Dairy Cattle. Vet Clin Food Anim, https://doi.org/10.1016/j.cvfa.2017.10.005

11. Hoorfar, J., Feld, N.C., Schirmer, A.L., Bitsch, V., Lind, P. (1994). Serodiagnosis of Salmonella dublin infection in Danish dairy herds using O-antigen based enzyme-linked immunosorbent assay (Published erratum appears in Can. J. Vet. Res, (1995), 59 p. 25. Canadian Journal of Veterinary Research, 58, 268-274.

12. Huang, K., Fresno, A.H., Skov, S., Olsen, J.E. (2020). Dynamics and Outcome of Macrophage Interaction Between Salmonella Gallinarum, Salmonella Typhimurium, and Salmonella Dublin and Macrophages From Chicken and Cattle. Front Cell Infect Microbiol, 9, 420.

13. Humphrey, T., Wray, C., Wray, A. (2000). Public-health aspects of Salmonella infection. In: C. Wray and A. Wray (Eds.), Salmonella in Domestic Animals, CABI Publishing (pp. 245-263) .New York.

14. Kemal, J.A. (2014). Review on the Public Health Importance of Bovine Salmonellosis. Veterinar Sci Technolo, 5, 2.

15. LA Ragione, R., Metcalfe, H., Villarreal-Ramos, B., Werling, D. (2013). Salmonella Infection in Cattle, in: Salmonella in domestic animals. Paul A. Barrow and Ulrich Methner (p. 233-263).

16. Morisse, J.P., Cotte, J.P. (1994). Evaluation of some risks factors in bovine salmonellosis. Vet Res, 25(2-3), 185-191.

17. Nazer, A.H.K., Osborne, A.D. (1977). Experimental Salmonella Dublin Infection in Calves. Br Vet J, 133, 388-398.

18. Nielsen L.R., Dohoo, I. (2012). Survival analysis of factors affecting incidence risk of Salmonella Dublin in Danish dairy herds during a 7-year surveillance period. Prev Vet Med , 107(3-4), 160-169.

19. Nielsen, L.R., Ersbøll, A.K. (2005). Factors associated with variation in bulk-tank-milk Salmonella Dublin ELISA ODC% in dairy herds. Prev Vet Med , 68,165-79.

20. Nielsen, L.R., Schukken, Y.H., Gröhn, Y.T., Ersbøll, A.K. (2004). Salmonella Dublin infection in dairy cattle: risk factors for becoming a carrier. Prev Vet Med, 65, 47- 62.

21. Nielsen, L .R.(2013).Salmonella Dublin in cattle Epidemiology, design and evaluation of surveillance and eradication programmes. These for obtain the grade de Doctor Veterinary. Faculty of health and medical sciences universit y of Copenhagen, 398p.

22. Nielsen, L.R., Toft, N., Ersbøll, A.K. (2004). Evaluation of an indirect serum ELISA and a bacteriological faecal culture test for diagnosis of Salmonella serotype Dublin in cattle using latent class models. Journal of Applied Microbiology, 96, 311-319.

23. Nielsen, L. R., Kudahl, A. B., Østergaard, S. 2012. Age-structured dynamic, stochastic and mechanistic simulation model of Salmonella Dublin infection within dairy herds. Preventive Veterinary Medicine, 105(1-2), 59-74

24. Nielsen, L. R., Warnick, L. D., Greiner, M.(2007). Risk Factors for Changing Test Classification in the Danish Surveillance Program for Salmonella in Dairy Herds. Journal of Dairy Science, 90(6), 2815-2825.

25. Pelzer, K.D. (1989). Salmonellosis. J Am Vet Med Assoc, 195, 456-463.

26. Penny, C.D., Low, J.C., Nettleton, P.F., Scott, P.R., Sargison, N.D., Strachan, W.D., Honeyman, P.C. (1996). Concurrent bovine viral diarrhoea virus and Salmonella Typhimurium DT104 infection in a group of pregnant dairy heifers. Veterinary Record, 138, 485-489.

27. Ruzante, J.M., Lombard, J.E., Wagner, B., Fossler, C.P., Karns, J.S., Van Kessel, J.A.S., Gardner, I.A. (2010). Factors Associated with Salmonella Presence in Environmental Samples and Bulk Tank Milk from US Dairies. Zoonoses Public Health, 57, e217-25.

28. Segall, T., Lindberg, A.A. (1991). Experimental oral Salmonella dublin infection in calves: a bacteriological and pathological study. J Vet Med B, 38, 169-184.

29. Spier, S.J., Smith, B.P., Cullor, J.S., Olander H.J., Roden L.D., Dilling, G.W .(1990). Persistent experimental Salmonella Dublin intramammary infection in dairy cows. J Vet Intern Med, 5(6), 341-350.

30. Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A.M., Klunder, T.(1998). Risk factors for Salmonella Dublin infection on dairy farms. Vet Q, 20, 97-99.

31. Van Schaik, G, Schukken, Y.H, Nielen, M, Dijkhuizen, A.A, Barkema, H.W, Benedictus, G. (2002). Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev Vet Med, 54, 279-289.

32. Veling, J., Barkema, H.W., Van Der Schans, J., Van Zijderveld, F., Verhoeff, J. (2002). Her-level diagnosis for Salmonella enterica spp. enterica serovar Dublin in bovine dairy herds. Prev Vet Med, 31- 42.

33. Veling ,J., Barkema ,H.W., Van Der Schans, J., Van Zijderveld ,F., Verhoeff, J. (2002). Her-level diagnosis for Salmonella enterica spp. enterica serovar Dublin in bovine dairy herds. Prev Vet Med, 31- 42.

34. Visser, S.C., Veling, J., Dijkhuizen, A.A., Huirne, R.B.M. (1997). Economic losses due to Salmonella Dublin in dairy cattle. In: Proceedings of the Dutch/Danish Symposium on Animal Health and Management Economics, Copenhagen, Dina Notat: pp143-151.

35. Wedderkopp, A., Stroger, U., Lind, P. (2001). Salmonella Dublin in Danish dairy herds: Frequency of change to positive serological status in bulk tank milk ELISA in relation to serostatus of neighbouring farms. Acta Vet.Scand, 42, 295-302.

36. Williams B.M. 1975. Environmental considerations in salmonellosis. Vet Rec .96, 318-21.

37. Wray C., Davies R.H. 2000. Salmonella Infection in Cattle. In: Wray C, Wray A eds. Salmonella in domestic Animals Oxan, UK : CABI Publishing, pp.169-190.

38. Wray, C., Roeder, P.L. (1987). Effect of bovine virus-diarrhoea-mucosal disease infection on Salmonella infections in calves. Res Vet Sci, 42, 213-218.