Hezil et al. *Afr. J. Clin. Exper. Microbiol.* 2021; 22 (2): 211 - 222 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Apr 2021; Vol.22 No.2
AJCEM/2089. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Salmonella Dublin associated with abortion in dairy cattle in Algiers and comparison of different diagnostic methods

*[1]Hezil, Dj., [2]Zaidi, S., [1]Benseghir, H., [1]Zineddine, R., [3]Benamrouche, N., and [1]Ghalmi, F.

[1]Research Laboratory Management of Local Animal Resources, Higher National Veterinary School, El Alia, Oued Smar, 1615, Algiers, Algeria
[2]Higher National Veterinary School, El Alia, Oued Smar, 1615, Algiers, Algeria
[3]Laboratory of Enterobacteria and other related bacteria, Institute Pasteur of Algeria
*Correspondence to: d.hezil@etude.ensv.dz

Abstract:

Background: In cattle, many serotypes of *Salmonella enterica* are responsible for a wide variety of clinical manifestations, which can cause considerable economic loss. Some serotypes can cause cows to abort sporadically, such as the Dublin serotype. This study was carried out on different cattle farms in the Algiers region to determine the prevalence of *Salmonella* Dublin using bacteriological and immunological methods.
Methodology: The prevalence of *Salmonella* was determined by bacteriological analysis in accordance with the reference method AFNOR NF U 47-100 on faecal samples collected from 184 cattle belonging to 19 different farms, and serotyping for *S.* Dublin. Immunological analysis by enzyme-linked immunosorbent assay (ELISA) for *S.* Dublin was carried out on milk samples collected from 91 cattle. A survey of case (n=5) and control (n=14) farms for comparative analysis was performed to demonstrate a link between abortion in cows and prevalence of *S.* Dublin with both bacteriological and immunological methods. Sensitivity, specificity, Cohen Kappa coefficient, McNemar test odds ratios, and confidence intervals were calculated using Winepiscope 2.0 and StatA 9.1 software, and $p<0.05$ was considered as statistically significant.
Results: The bacteriological results showed a prevalence of 7.6% (95%CI: 3-10), for *Salmonella* and serotyping revealed a prevalence for *S.* Dublin of 2.7%. The immunological analysis of milk by the ELISA technique revealed a prevalence of 13.2% (95%CI: 5-20) for *S.* Dublin. The comparative study between immunological results from milk and bacteriological results from faeces for detecting *S.* Dublin showed poor agreement between the two tests (k=0.25), with enzyme immunoassay being significantly more sensitive than the bacteriological test ($p<0.05$). The results of the survey did not demonstrate a clear association between bacteriological detection of *S.* Dublin in faeces and abortion in cows (OR=8.66, 95%CI: 0.58-130.12). However, with the immunological analysis of milk for *S.* Dublin, there was a significant positive association (OR=62.33, 95%CI: 2.13-18.22) between a positive antibody response to *S.* Dublin in milk and the presence of abortions on the farm.
Conclusion: In view of these results, we can conclude that *Salmonella* infections should systematically feature in the differential diagnosis of abortions in dairy cattle in Algeria.

Keywords: *S.* Dublin, cattle, faeces, milk, abortion, immunology, bacteriology, Algiers

Received Oct 24, 2020; Revised Jan 2, 2021; Accepted Jan 3, 2021

Salmonella Dublin associée à l'avortement chez les bovins laitiers à Alger et comparaison de différentes méthodes de diagnostic

*[1]Hezil, Dj., [2]Zaidi, S., [1]Benseghir, H., [1]Zineddine, R., [3]Benamrouche, N., et [1]Ghalmi, F

[1]Laboratoire de Recherche Gestion des Ressources Animales Locales, Ecole Nationale Supérieure Vétérinaire, El Alia, Oued Smar, 1615, Alger, Algérie.
[2]Ecole Nationale Supérieure Vétérinaire, El Alia, Oued Smar, 1615, Alger, Algérie
[3]Laboratoire d'entérobactéries et autres bactéries apparentées, Institut Pasteur d'Algérie
*Correspondance à: d.hezil@etude.ensv.dz

Résumé:

Contexte: Chez les bovins, de nombreux sérotypes de *Salmonella enterica* sont responsables d'une grande variété de manifestations cliniques, ce qui peut entraîner des pertes économiques considérables. Certains sérotypes peuvent provoquer des avortements sporadiques chez les vaches, comme le sérotype Dublin. Cette étude a été réalisée dans différents élevages bovins de la région d'Alger pour déterminer la prévalence de *Salmonella* Dublin à l'aide de méthodes bactériologiques et immunologiques.

Méthodologie: La prévalence de *Salmonella* a été déterminée par analyse bactériologique selon la méthode de référence AFNOR NF U 47-100 sur des échantillons fécaux prélevés sur 184 bovins appartenant à 19 exploitations différentes, et sérotypage pour *S.* Dublin. Une analyse immunologique par dosage immunoenzymatique (ELISA) pour *S.* Dublin a été réalisée sur des échantillons de lait prélevés sur 91 bovins. Une enquête sur des cas (n=5) et des fermes témoins (n=14) pour une analyse comparative a été réalisée pour démontrer un lien entre l'avortement chez les vaches et la prévalence de *S.* Dublin avec des méthodes bactériologiques et immunologiques. La sensibilité, la spécificité, le coefficient Cohen Kappa, les Odds ratios du test de McNemar et les intervalles de confiance ont été calculés à l'aide des logiciels Winepiscope 2.0 et StatA 9.1, et $p<0,05$ a été considéré comme statistiquement significatif.
Résultats: Les résultats bactériologiques ont montré une prévalence de 7,6% (IC 95%: 3-10), pour *Salmonella* et le sérotypage a révélé une prévalence pour *S.* Dublin de 2,7%. L'analyse immunologique du lait par la technique ELISA a révélé une prévalence de 13,2% (IC à 95%: 5-20) pour *S.* Dublin. L'étude comparative entre les résultats immunologiques du lait et les résultats bactériologiques des fèces pour la détection de *S.* Dublin a montré une mauvaise concordance entre les deux tests (k=0,25), le dosage immunoenzymatique étant significativement plus sensible que le test bactériologique ($p<0,05$). Les résultats de l'enquête n'ont pas démontré une association claire entre la détection bactériologique de S. Dublin dans les fèces et l'avortement chez les vaches (OR = 8,66, IC à 95%: 0,58-130,12). Cependant, avec l'analyse immunologique du lait pour *S.* Dublin, il y avait une association positive significative (OR=62,33, IC 95%: 2,13-18,22) entre une réponse anticorps positive à S. ferme.
Conclusion: Au vu de ces résultats, nous pouvons conclure que les infections à *Salmonella* devraient systématiquement figurer dans le diagnostic différentiel des avortements chez les bovins laitiers en Algérie.

Mots clés: *S.* Dublin, bovins, fèces, lait, avortement, immunologie, bactériologie, Alger

Introduction:

Salmonella infections are major concern in animal husbandry and public health. Ruminants, in particular, cattle are victims of salmonellosis, which has serious economic consequences on animal production (1). Cattle are the main reservoir for *Salmonella enterica* subsp *enterica* serovar Dublin (*Salmonella* Dublin) which is considered to be the most common cause of *Salmonella* infections in cattle (2). *S.* Dublin is the serotype of most economic concern due to its particularly invasive nature, causing diarrhoea, sepsis, and mortality, mainly in calves aged 2 weeks to 3 months, as well as affecting reproduction, and causing abortions in cattle (3). As a host-adapted strain in cattle, animals infected with *S.* Dublin can become a chronic subclinical reservoir that has the potential to excrete large numbers of bacteria in the environment. These reservoirs also play an important role in maintaining infection within a herd by excreting the germ not only in faeces, but also in milk and colostrum (4). This serotype can be difficult to detect due to asymptomatic carrier status with intermittent bacteraemia and shedding (4,5).

Several studies have shown that bacteriological method for detection of *S.* Dublin in infected cattle suffers limitations in terms of sensitivity compared to serological methods (6). Therefore, the most widely used tests for the detection of *S.* Dublin include the enzyme-linked immunosorbent assays (ELISA) in the serum and in milk (7,8,9). Despite its importance, this disease has so far been very little studied in the Algerian context, and the epidemiology of *S.* Dublin infections in cattle remains largely unknown, either in terms of the prevalence of the infection or its impact on abortions on the farms. The objective of this study is to provide information on the epidemiological situation of this disease in Algeria and particularly in the Wilaya of Algiers.

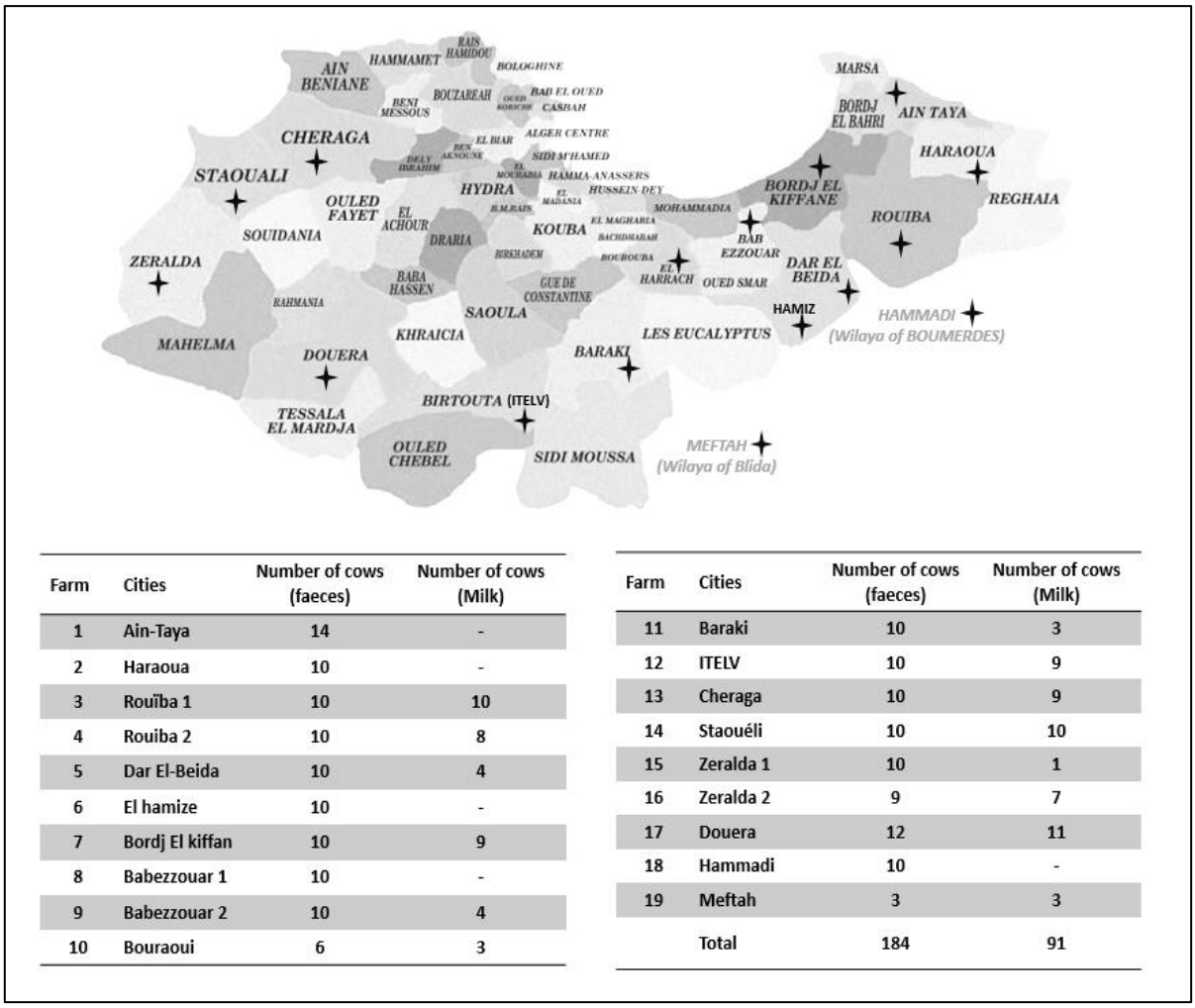
Materials and method:

Study area and sampling technique

We carried out our samples in different regions of the Wilaya of Algiers (Fig. 1). The region studied has 1,281 breeders with 13,115 herd of cattle, including 7,514 herds of dairy cows (10). The selection of farms was done by random sampling method, using a list of cattle breeders in the Wilaya of Algiers, to ensure homogeneous distribution of the farms in the study area. Subsequently, the number of cattle to be included in the study from each farm was defined according to the total number of cattle present. When the farm had less than 10 cattle, all cattle were included. When the farm contained more than 10 cattle, the number of cattle included was at least 10, the objective of which was to have a sample representing at least 10% of all cattle present in the farms selected (11,12).

Survey of 'case' and 'control' farms

A pre-validated epidemiological questionnaire was administered to herd owners from the selected 19 farms; 5 as 'case' and 14 as 'control' farms. The questionnaire was interviewer-administered on the day of sample collection to determine whether or not the cattle in the farm had experienced abortion episodes. The questionnaire contained information related to the herds visited (management system, type and size of herd) and the cows enrolled (breed, age, pregnancy, month of gestation, history of abortion, stage of pregnancy at which abortion occurred, pathological history and clinical signs observed at the time of sampling). 'Case' farms were those where episodes of abortions had occurred in the last 5 years, a phenomenon not observed in the 'control' farms. A farm was considered positive if at least one animal was positive.



Farm	Cities	Number of cows (faeces)	Number of cows (Milk)
1	Ain-Taya	14	-
2	Haraoua	10	-
3	Rouïba 1	10	10
4	Rouiba 2	10	8
5	Dar El-Beida	10	4
6	El hamize	10	-
7	Bordj El kiffan	10	9
8	Babezzouar 1	10	-
9	Babezzouar 2	10	4
10	Bouraoui	6	3

Farm	Cities	Number of cows (faeces)	Number of cows (Milk)
11	Baraki	10	3
12	ITELV	10	9
13	Cheraga	10	9
14	Staouéli	10	10
15	Zeralda 1	10	1
16	Zeralda 2	9	7
17	Douera	12	11
18	Hammadi	10	-
19	Meftah	3	3
	Total	184	91

Fig. 1: Location of farms studied and number of cows sampled from each farm

Sample collection

Faeces were collected from the rectum of 184 cows; 43 from the 5 'case' farms and 141 from the 14 'control' farms. The faeces were stored in sterile jar with a capacity of 100 ml, and then sent for laboratory analysis on the same day. In addition, milk samples were collected from 91 of the 184 cows; 34 from the 5 'case' farms and 57 from 9 of the 14 'control' farms and then transferred to sterile tubes with a capacity of 10 ml. The samples were stored in a cool place at -20°C and analyzed in the microbiology laboratory of the National Veterinary School of El Alia (ENSV), Oued Smar, Algeria.

Bacteriological analysis

This method was based on the application of the Association française de normalisation (AFNOR), NF U 47-100 standard (18). This technique is a standard method of research by isolation and identification of any specified serovar(s) of *Salmonella* in the environment of animal production (Fig 2) as itemized in the following steps;

Pre-enrichment with buffered peptone water:

25g of faeces were added to 250ml of buffered peptone water (Pasteur Institute of Algeria, EPT) at room temperature and incubated for 18 (±2) hours in an incubator set at 37°C.

Enrichment in liquid and semi-solid media:

This step allowed the growth and selection of bacteria of the genus *Salmonella*, with the use of two media selective enrichments in parallels; MSRV (Modified Semi-solid Rappaport-Vassiliadis) medium (BioRad, France) and MKTTn (Müller-Kauffmann Tetrathionate) medium (Bio-Rad, Marnes-La-Coquette, France). Three drops (total of about 0.1mL) of the pre-enrichment broth were transferred and inoculated on the semi-solid agar dishes of the MSRV medium. The medium was supplemented with novobiocin solution before pouring into the Petri dishes to inhibit the growth of Gram-positive bacteria. The plates were incubated at 41.5°C (±1) for 24 hours cover up, and then examined. If the migration is greater than 20mm

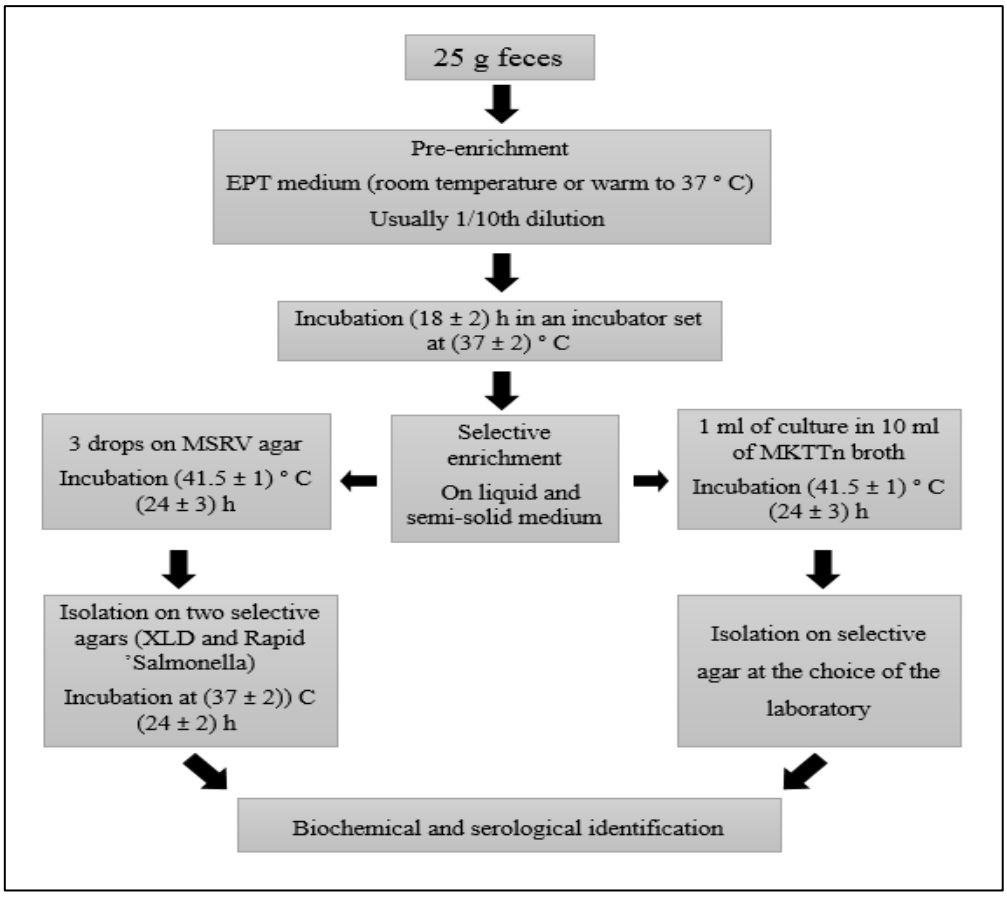


Fig 2: Analysis diagram according to the reference method AFNOR, NF U 47-100 (18)

from the point of inoculation, an inoculum was taken from the periphery of the migration zone and then inoculated on the RAPID *Salmonella* chromogenic medium (Bio-Rad, Marnes-La-Coquette, France) and the XLD medium (XLD: Condalab, Madrid, Spain) by an appropriate isolation technique. For the MKTTn medium, 1ml of the pre-enrichment broth was transferred to a 10 ml tube of MKTTn broth, and then incubated at 41.5°C (±1) for 24 (±3) hours.

Isolation of *Salmonella*

Each typical *Salmonella* colony was taken from each of the selective media (XLD and chromogenic agar). The typical *Salmonella* colony appeared red with black centers on XLD and on RAPID *Salmonella* chromogenic medium, *Salmonella* formed characteristic magenta colonies. The colonies recovered were then purified on nutrient agar (GN: Pasteur Institute of Algeria) after incubation at 37°C for 18-24 hours.

Identification and serotyping of *Salmonella*

Confirmation of suspected *Salmonella* colonies was carried out using Triple Sugar Iron agar (TSI: Pasteur Institute of Algeria) and API 20E gallery (BioMérieux, France). *Salmonella* serovars were identified by serotyping with slide agglutination reaction using diagnostic polyvalent and monovalent O and H *Salmonella* antisera according to Kauffman-White scheme (13).

Serological analysis on milk samples

For the detection of specific antibodies against *S.* Dublin, we used an indirect ELISA test for the detection of antibodies directed against the O antigen (part of the lipo-polysaccharide LPS); 1, 9 and 12 of S. Dublin, and performed according to the manufacturer's instructions (Prio CHECK *Salmonella* Antibody ELISA Dublin; Thermo Fisher Scientific, Waltham, MA). Briefly, milk samples were first heated for one hour at 37°C. The upper layer of fat was pulled out, and the undiluted skim milk samples were inoculated in microtiter plate and the optical density (OD) was measured at 450 nm using ELISA reader (Bio-Rad, USA).

Statistical analysis

Sensitivity, specificity, accuracy, Cohen Kappa coefficient, McNemar test Odds ratios and confidence intervals were calculated for comparison of bacteriological and immunological methods using Winepiscope 2.0 and Stat A 9.1 softwares. $P<0.05$ was considered as statistically significant.

Results:

Prevalence of *Salmonella* spp and *S.* Dublin by bacteriological analysis of the cattle faeces

The results obtained show that of the 184 faecal samples, 14 (7.6%) were positive for *Salmonella* spp., and 5 (2.7%) were positive for *S.* Dublin. Of the 19 farms studied, *Salmonella* spp was isolated in 6 (31.6%) and *S.* Dublin in 3 (15.8%) (Table 1).

Seroprevalence of *S.* Dublin by ELISA assay on the cow milk

Of the 19 selected farms, 14 were analyzed for antibodies to *S.* Dublin in the milk samples of 91 cows. Twelve milk samples of cow were positive for *S.* Dublin, which represents a prevalence of 13.2% (Table 2).

Table 1: Prevalence of *Salmonella* spp and *S.* Dublin in the farms by bacteriological results of faeces

Farm/Municipality	Number of cattle	*Salmonella* spp (%)	*S.* Dublin (%)
Babezzouar 1	10	6 (60.0)	3 (30.0)
Babezzouar 2	10	1 (10.0)	1 (10.0)
Bordj El kifane	10	1 (10.0)	-
Bouraoui	6	1 (16.7)	-
ITELV	10	4 (40.0)	-
Meftah	3	1 (33.3)	1 (33.3)
Other 13 farms	135	-	-
Total	184	14 (7.6%)	5 (2.7%)

Table 2: Prevalence of *S.* Dublin by bacteriological method on faeces and immunological method on milk of the cattle from the various farms/municipality

Farm/Municipality	Bacteriological test on faeces		Immunological test on milk	
	Number of cattle	Positive for *S.* Dublin	Number of cows	Positive for *S.* Dublin
Rouiba 2	10	0	8	0
Babezzouar 1	10	3	0	0
Babezzouar 2	10	1	4	3
Bordj El kifane	10	0	9	1
Cheraga	10	0	9	3
Staouéli	10	0	10	1
ITELV	10	0	9	2
Meftah	3	1	3	2
Other farms	111	0	39	0
Total	184	5 (2.7%)	91	12 (13.2%)

Comparison of the bacteriological and serological methods of S. **Dublin detection**

The immunological results of 91 milk samples from 184 cows were compared with bacteriological results of 184 faeces from the same cows (Table 3). The results obtained showed that bacteriological analysis had a sensitivity of 16%, specificity of 100% and accuracy of 89%, compared to the immunological assay. The Cohen's Kappa coefficient of 0.25 and McNemar test of 0.004 showed that the two methods gave significantly different values (p <0.05).

Results of survey with respect to bacteriological results of S. **Dublin** ***at the farm level***

The association between exposure to *S.* Dublin and the presence of abortions on the farm as calculated is shown in Table 4. The survey shows farm exposure rate of 40% for the case farms compared to 7.14% for the control farms. However, given the low number of farms tested, the Odds ratio was not significantly different from 1 (p=0.12). As a result, there was no association between *S.* Dublin exposure and the presence of abortions in the case and control farms.

Results of survey with respect to bacteriological results of S. **Dublin** ***for individual cattle***

From the survey at individual cattle level, the calculation of the Odds Ratio revealed a value of 2.2 (Table 5), which is not significantly different from 1 (p=0.38). Analysis of the table shows cattle exposure rate of 4.65% for the case farms, and 2.12% for the control farms. As a result, there was no association between *S.* Dublin in cattle and presence of abortion in case and control farms.

Table 3: Comparison of bacteriology and immunology methods (as gold standard) for identification of *S.* Dublin

		Immunology		**Total**
		Positive	Negative	
Bacteriology	Positive	2	0	2
	Negative	10	79	89
Total		12	79	91

95% CI (4.56 – 17.41); p = 0.0020

Table 4: Result of the survey for bacteriological identification of *S.* Dublin in case and control farms

	Farms	**Case**	**Control**	**Total**
Bacteriology	Positive	2	1	3
	Negative	3	13	16
	Total	5	14	19
	Exposure rate	40%	7.14%	
	Odd	0.66	0.07	
	Odds Ratio (95% CI)	8.66 (0.58- 130.12)		

Table 5: Results of the survey for bacteriological identification of *S.* Dublin in the cattle from case and control farms

Bacteriology	**Animals**	**Cattle from case farms**	**Cattle from control farms**	**Total**
	Positive	2	3	5
	Negative	41	138	179
	Total	43	141	184
	Exposure rate	4.65%	2.12%	
	Odd	0.04	0.02	
	Odds Ratio (95% CI)	2.2 (0.36-13.88)		

Results of survey with respect to immunological results of S.* Dublin *in milk at farm level

The survey at the farm level revealed exposure rate in the case farms to be 100%, in contrast to the control farms which was 11.11%. The OR ratio normally has an infinite value due to the presence of zero. In this case, 0.5 was added to all the values according to Deeks and Higgins, and Addis et al., (14,15). With this modification, we obtain an OR value of 62.33 (2.13-1822) (Table 6), which was significantly different from 1 (p<0.05). As a result, there was a positive association between farm exposure with *S.* Dublin antibody presence in milk and the presence of abortions on the farms.

Results of survey with respect to immunological results of S.* Dublin *in milk at individual cattle level

From the survey of the individual cattle, it revealed the OR of 26.78 (Table 7), which was significantly different from 1 (p< 0.01). As a result, there was positive association between positive *S.* Dublin antibody presence in milk of individual cattle and presence of abortions on the farm. This was further underscored by the *S.* Dublin exposure rate of 32.35% for the cattle in the case farms compared to 1.75% for the cattle in the control farms.

Table 6: Results of the survey for immunological identification of *S.* Dublin in case and control farms

ELISA PrioCHECK	Farms	Case farms	Control farms	Total
	Positive	5 (5.5)*	1 (1.5)*	6
	Negative	0 (0.5)*	8 (8.5)*	8
	Total	5	9	14
	Exposure rate	100%	11.11%	
	Odd	∞ (11)*	0.12 (0.176)*	
	Odds Ratio (95% CI)	62.33 (2.13-1822)		

*The numbers in brackets are the modified values for the calculation of the Odds ratio as described above

Table 7: Results of the survey for immunological identification of *S.* Dublin in the milk of cattle from case and control farms

ELISA PrioCHECK	Animals	Cattle from case farms	Cattle from control farms	Total
	Positive	11	1	12
	Negative	23	56	79
	Total	34	57	91
	Exposure rate	32.35%	1.75%	
	Odd	0.47	0.01	
	Odds Ratio (95% CI)	26.78 (3.27-219.57)		

Table 8: Different studies around the world illustrating the prevalence of *S.* Dublin in milk

Country	Number of samples (Milk) at		*S.* Dublin Prevalence	References
	Herd	Cattle		
California (USA)	/		14.1%	(49)
California (USA)	/		3.5%	(49)
Denmark	1464		9.9%	(51)
Pays-Bas	79		54.5%	(8)
Denmark	4326	/	11%	(52)
Ireland	158	/	49% (78)	(53)
Suede	/	1069	17%	(54)
Suede	/	4683	3% (142)	(55)
New York (USA)	4896	5219	1% (50/5219) 0.9% (46/4896)	(56)

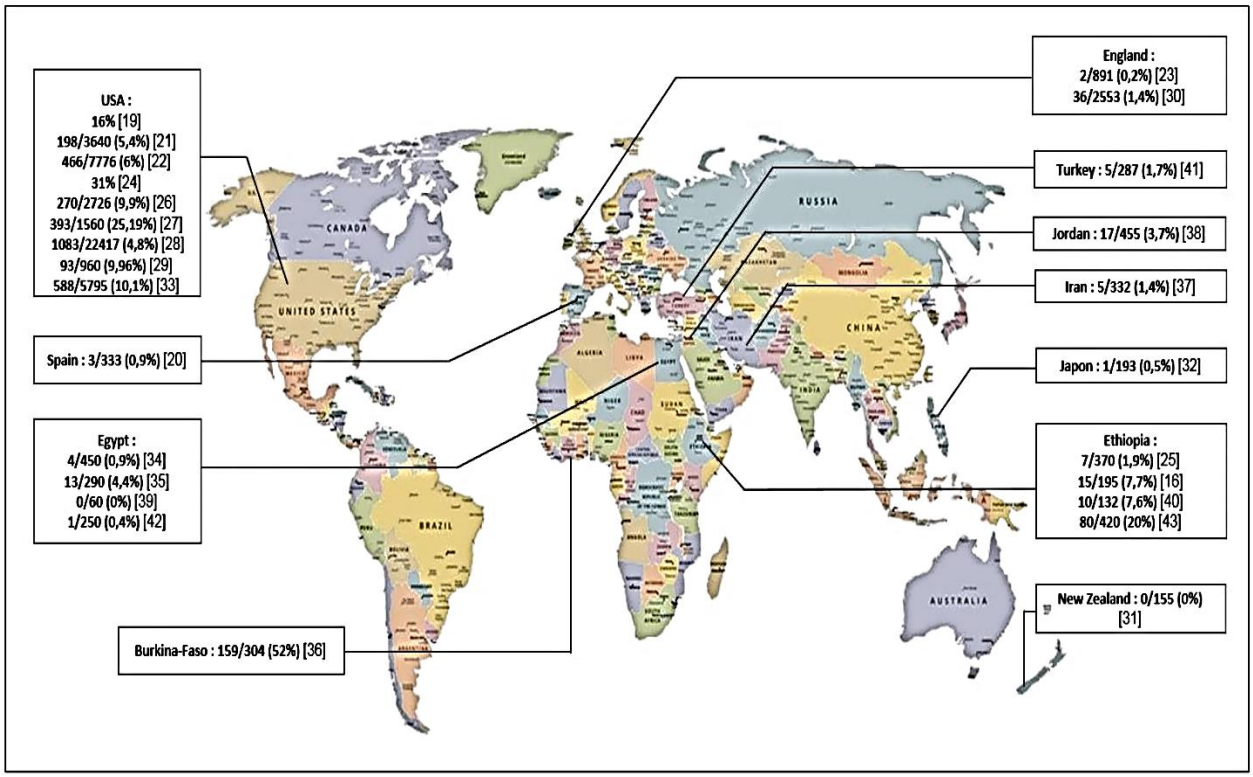


Fig 3: Studies around the world illustrating the prevalence of faecal excretion of *Salmonella* spp in cattle

Discussion:

Salmonellosis remains a significant public health problem around the world, particularly in developing countries (16). In addition, *Salmonella* are emerging pathogens responsible for many diseases in cattle. *S*. Dublin infection is of concern in several countries because of its ability to cause abortions and reduced milk production, as well as the significant economic losses it causes (17). Of the 184 faecal samples analyzed by the AFNOR NF U 100-47 reference method (18) in our study, 14 were positive for *Salmonella* spp or an overall prevalence of 7.60%, while the positivity rate for *S.* Dublin was 2.71%. Numerous epidemiological studies carried out worldwide on faecal excretion of *Salmonella* spp in cattle (16,19-43) showed prevalence of between 0% and 52% (Fig 3). These differences in the prevalence of *Salmonella* could be explained by the seasonal variation in faecal excretion of *Salmonella* in animals. Some studies showed that *Salmonella* excretion was highest in cows sampled from spring through summer (February through September) (22,29) while *Salmonella* excretion in cows sampled during the winter was found to be low (44). Likewise, the serotype and prevalence of the *Salmonella* serotype may vary from farm to farm and within the same farm from one sampling period to another (44). Other factors that could be responsible for the wide differences include size and age of the herd, region which can influence the frequency of isolation from one study to another (29), clinical condition of the animals, amount of sample used, individual laboratory skills, differences in culture methods, presence of inhibitory factors in faeces contaminated with other microorganisms, and differences in the data collected from the population studied (45).

The absence of *Salmonella* in healthy adult cattle may be explained by the fact that the bacteria is not detectable in some samples which contain small number of organisms (39). In addition, it is important to note that the detection limit for the enrichment methodology is approximately 1 CFU/g of faeces. Therefore, a negative result does not necessarily indicate that the animal is negative, but simply that the *Salmonella* population is present at less than 1 CFU/g of faeces (29). In addition, none of the farms included in our study reported clinical salmonellosis cases before taking the sample. The prevalence of salmonellosis in animals is difficult to assess due to the lack of an epidemiological surveillance system in place, which is the case in most developing countries. In Algeria, only few studies have been carried out on the presence of *Salmonella* spp in

lactating cows on dairy farms.

The prevalence of *S.* Dublin serotype from faeces was 2.71% (5 of 184), which is similar to the study carried out in Denmark with a rate ranging from 0.3 to 2.8% (46), and 1% of 393 samples in the USA study (27). The Dublin serotype was weakly detected in these studies, despite being the most frequently excreted serotype in the faeces of cows. Nevertheless, some authors have reported higher prevalence, in a study carried out in Denmark with a prevalence of 6%-14% in 4531 faecal samples (47), and by Pacer et al., in California who reported prevalence of Dublin serotype of 10.7% among 16% of *Salmonella* detected (19). It should be noted that *S.* Dublin is the most frequently isolated serotype in Danish cattle and is responsible for the economic losses reported in infected herds. As a result, a national surveillance program was launched in Denmark in October 2002 which lowered the prevalence by 12% in 2009 (48).

In this study, the positivity rate of *S*. Dublin from milk collected from 91 cows was 13.18%. Numerous studies on prevalence of *S.* Dublin in milk in cattle conducted worldwide (8, 49-56), show prevalence rate between 0.9% and 54.5% (Table 8). The differences in the prevalence rates from these studies may be explained by differences in geographical locations and herd size. These two parameters can significantly influence the seroprevalence of salmonellosis in dairy cattle (57). The comparison between direct detection of *S.* Dublin by faecal culture and indirect detection by ELISA test on milk samples in our study gave different results. The sensitivity of faecal detection of *Salmonella* was low compared to detection of antibodies directed against the bacterium, which are present in the milk. This may be explained by the fact that the duration of the Dublin antibodies presence in the milk is longer compared to the duration of excretion of Dublin serovar in the faeces. The existence of latent carriers with persistent antibody titers and intermittent or even absent excretion of Dublin serovar in faeces may also influence the results (49,50). Therefore, we can say that the bacteriological method is less sensitive than the immunological method. Some other studies have shown that bacteriological culture methods for the detection of *S.* Dublin in infected animals suffer from severe limitations in terms of sensitivity (6, 58).

The sensitivity was very low at around 16% in our study, which is similar to that reported by Nielsen and al., (47) with a sensitivity of 6-14% and that reported by Nielsen (46) with a sensitivity of 20%. Dublin serovar is difficult to detect because of its poor growth in commonly used culture media (46,59). The most common technique used to detect *Salmonella* is the traditional microbiological technique but this detection method is insensitive due to the large number of Gram-negative organisms present in the faeces, which often hamper the isolation of *Salmonella* colonies. In addition, these methods are generally labor intensive and time consuming, requiring a minimum of 4 to 6 days, thus increasing the risk of transmission of this pathogen (60). It has also been reported that culture methods show low sensitivity following low level contamination (61). Diagnostic laboratories use enrichment media to promote *Salmonella* growth and inhibit other faecal flora. Enriched samples are then spread over *Salmonella* selective media, and suspect colonies are tested using a series of biochemical tests and *Salmonella* antisera. In the case of active carriers of *S*. Dublin or other serotypes, faecal crops grown three times at intervals of 7 to 14 days are recommended to confirm the diagnosis (62). Bacteriological culture of large numbers of individual faecal samples is however expensive and time consuming (26).

Morphological descriptions and biochemical tests can also produce ambiguous results (63). Bacteriological culture tests can lead to suboptimal detection of excretors with many false negative results (64). They require collecting samples repeatedly over a long period of time to differentiate acutely infected animals from persistently infected animals. The substantial economic cost of this procedure necessitates the use of a less expensive and easier method to detect persistently infected animals (50). *Salmonella* serotyping is generally performed by reference laboratories, and is based on the identification of somatic (O) and flagellar (H) antigens using specific sera according to the Kauffmann-While Le Minor scheme (65). It could therefore be a difficult task as it requires many antisera and expertise to interpret the results of agglutination, not to mention that serotyping is also laborious, complicated and very time consuming. It should be noted that, carriers are easier to detect by use of the serological ELISA technique than acutely infected animals. The sensitivity of ELISA is therefore much higher for carriers than acutely infected animals (66). Carriers frequently have consistently elevated levels of immunoglobulins in serum and milk (67,68).

In order to verify whether *S.* Dublin is a cause of abortion in cows, we conducted a survey of 'case' and 'control' farms with *S.* Dublin positivity as an exposure factor. The results of the survey at the farm level based on bacteriological analysis for *S*. Dublin, show an exposure rate of 40% for the 'case' farms compared to 7.14% for the 'control' farms, however the Odds ratio was not significantly different from 1. Therefore, there was no association between *S.* Dublin positivity and

the presence of abortions on the farms, hence we suggest that more farms would have to be tested. For a disease prevalence of 10%, at least 86 farms should be tested for a relative risk of 4 with a proportion of one to three controls per case (69). The results of the survey of individual cattle based on bacteriological analysis gave an Odds Ratio value of 2.2, which was not significantly different from 1 (p=0.24), and analysis showed an exposure rate of 4.65% for cattle in the 'case' farms, and 2.12% in the 'control' farms, which implied no association between *S.* Dublin positivity in the cattle on the farms and abortions.

The results of the survey of 'case' and 'control' farms based on immunological analysis of milk at the farm level, gave an OR of 62.33 (95%CI: 2.13-1822), which value was significantly different from 1 (p<0.05), indicating an association between *S.* Dublin seropositivity in milk and the presence of abortions on the farms. This was further underscored by the exposure rate of 100% of the 'case' farms compared to only 11.11% in the 'control' farms. The results of the survey for individual cattle in both 'case' and 'control' farms gave an Odds Ratio of 26.78, which was significantly different from 1 (p<0.01) indicating an association between *S.* Dublin seropositivity in milk and the presence of abortions on the farms. This was further underscored by the exposure rate of 32.35% for the cattle in the 'case' farms compared to only 1.75% in the 'control' farms. From these results, we concluded that there was a clear association between *S*. Dublin seropositivity and the presence of abortions. A similar study carried out on cattle from the Algiers region, also demonstrated the existence of a close relationship between *S*. Dublin seropositivity and presence of abortions with an Odds ratio of 14.12, an exposure rate of 4.9% for the case farms, and 0.4% for the control farms (70). Indeed, several other studies have demonstrated the abortive effect of *S*. Dublin in cows (4,58,71-74).

Conclusion:

This study provided new information on bovine salmonellosis and particularly on salmonellosis caused by *S*. Dublin serovar among cattle in Algeria. *S*. Dublin identification pose challenges during laboratory diagnostic process. The use of more sensitive and less expensive method is important in order to monitor this pathogen. As *S.* Dublin is associated with abortions in cattle, we recommend that it should be systematically included in the differential diagnosis of abortions in cows in Algeria.

Acknowledgements:

The authors appreciate with thanks the Higher National Veterinary School in Algiers, for the resources made available by them, and the veterinarians of the Algiers region for their participation in providing the samples.

References:

1. Camart-Périé, A., Nemann, Y., and Dufour, B. Salmonelloses bovines: actualités - bovine salmonellosis: state of the art. Point Veterinaire. 2007; 274: 32-37.
2. Vaillant, V., Haeghebaert, S., Desenclos, J. C., et al. Outbreak of *Salmonella* Dublin infection in France, November-December 1995. Euro Surveill 1996; 1: 9-10.
3. Henderson, K., and Mason, C. Diagnosis and control of *Salmonella* Dublin in dairy herds. In Practice. 2017; 39 (4): 158-168.
4. Holschbach, C. L., and Peek, S. F. Salmonella in dairy cattle. Vet Clin North Am Food Anim Pract. 2018; 34 (1): 133-154.
5. Goodman, L. B., McDonough, P. L., Anderson, R. R., et al. Detection of *Salmonella* spp. in veterinary samples by combining selective enrichment and real-time PCR. J Vet Diagn Invest. 2017; 29 (6): 844-851.
6. Richardson, A. Serological responses of *Salmonella* Dublin carrier cows. Br Vet J. 1973; 129 (4): liii-lv.
7. Robertsson, J. A. Humoral antibody responses to experimental and spontaneous *Salmonella* infections in cattle measured by ELISA. Zentralbl Veterinarmed B. 1984; 31: 367-380.
8. Veling, J., Barkema, H. W., Van der Schans. J., et al. Herd-level diagnosis for *Salmonella enterica* subsp. *enterica* Serovar Dublin infection in bovine dairy herds. Prev Vet Med. 2002; 53: 31-42.
9. Nielsen, L. R., and Ersboll, A. K. Factors associated with variation in bulk-tank-milk *Salmonella* Dublin ELISA ODC% in dairy herds. Prev Vet Med. 2005; 68:165-79.
10. DSA. Direction des services Agricoles Algérie; 2003.
11. Cannon, R. M., and Roe, R. T. Livestock Diseases Surveys: A Field Manual for Veterinarians. Canberra: Australian Bureau of Animal health. 1982.
12. Ghalmi, F., China, B., Ghalmi, A., et al. Study of the risk factors associated with *Neospora caninum* seroprevalence in Algerian cattle populations. Res Vet Sci. 2012; 93 (2): 655-661.
13. Grimont, P. A. D., and Weill, F. X. Short textbook of antigenic formulas of the Salmonella serovars. 9 rd Ed: WHO collaborating centre for reference and research on Salmonella. Institut Pasteur, 75724 Paris Cedex 15, France, 2007.
14. Pagano, M., and Gauvreau, K. Principles of Biostatistics (2nd ed.), Pacific Grove (CA): Duxbury Thompson Learning, 2000.
15. Deeks, J. J., and Higgins, J. P. T. Statistical algorithms in Review Manager 5 Statistical Methods Group of The Cochrane Collaboration. 2010; P:11.
16. Addis, Z., Kebede, N., Sisay, Z., et al. Prevalence and antimicrobial resistance of Salmonella isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. BMC Infect Dis. 2011; 11 (1). https://doi.org/10.1186/1471-2334-11-222

17. Visser, S. C., Veling, J., Dijkhuizen, A. A., et al. 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. 1997; 56: 143-151.
18. Association française de normalisation (AFNOR), NF U47-100. Standard Methods of analysis in animal health - Search by isolation and identification of any specified serovar or serovar(s) of Salmonella in the environment of animal production, Paris. 2007.
19. Pacer, R. E., Spika, J. S., Thurmond, M. C., et al. Prévalence de Salmonella et de multiples Salmonella résistantes aux antimicrobiens dans les laiteries de Californie. JAVMA 1989; 195: 59-63.
20. Adesiyun, A. A, Webb, L. A., Romain, H. et al. Prevalence of *Salmonella*, *Listeria monocytogenes, Campylobacter* spp*., Yersinia enterocolitica and Cryptosporidium* spp. in bulk milk, cows' faeces and effluents of dairy farms in Trinidad. Rev Elev Med Vet Pays Trop. 1996; 49: 303-309.
21. Wells, S. J., Fedorka-Cray, P. J., Dargatz, D. A., et al. Fecal shedding of *Salmonella* ssp. By dairy cows on farm and at cull cow markets. J Food Prot, 2001; 64:3-11
22. Huston C. L., Wittum, T. E., Love, B. C., et al. Prevalence of fecal shedding of *Salmonella* spp. in dairy herds. JAVMA. 2002; 220: 645-649.
23. Davies, R. H., Dalziel, R., Gibbens, J. C., et al. National survey for *Salmonella* in pigs, cattle and sheep at slaughter in Great Britain (1999-2000). J Appl Microbiol. 2004; 96 (4): 750-760.
24. Fitzgerald, A. C., Edrington, T. S., Looper, M. L., et al. Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. Letters Appl Microbiol. 2003; 37 (5): 392-398.
25. Molla, B., Alemayehu, D., and Salah, W. Sources and distribution of Salmonella serotypes isolated from food animals, slaughterhouse personnel and retail meat product in Ethiopia: 1997-2002. Ethiop J Hlth Dev. 2003; 17: 63-70.
26. Warnick, L. D., Kaneene, J. B., Ruegg, P. L., et al. Evaluation of herd sampling for *Salmonella* isolation on midwest and northeast US dairy farms. Prev Vet Med. 2003; 60: 195-206.
27. Edrington, T. S., Schultz, C. L., Bischoff, K. M., et al. Antimicrobial resistance and serotype prevalence of Salmonella isolated from dairy cattle in the southwestern United States. Microb Drug Resist. 2004; 10 (1): 51-56.
28. Fossler, C. P., Wells, S. J., Kaneene, J. B., et al. Prevalence of Salmonella in a multi-state study of conventional and organic dairy farms. JAVMA 2004; 225: 567-573.
29. Callaway, T. R., Keen, J. E., Edrington, T. S., et al. Fecal prevalence and diversity of *Salmonella* species in lactating dairy cattle in four states. J Dairy Sci. 2005; 88: 3603-3608.
30. Milnes, A. S., Stewart, I., Clifto- Hadley, F. A., et al. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica* in cattle, sheep and pigs at slaughter in Great Britain during 2003. Epidemiol Infect. 2008; 136 (6): 739-751.
31. Moriarty, E. M., Sinton, L. W., Mackenzie, M. L., Karki, N., Wood, D. R. A. survey of enteric bacteria and protozoans in fresh bovine faeces on New Zealand dairy farms. J Appl Microbiol. 2008; 105 (6): 2015-2025.
32. Ishihara, K., Takahashi, T., Morioka, A., et al. National surveillance of *Salmonella enterica* in food-producing animals in Japan. Acta Veterinaria Scandinavica. 2009; 51 (1): 35.
33. Cummings, K. J., Warnick, L. D., Elton, M., et al. The Effect of clinical outbreaks of salmonellosis on the prevalence of fecal Salmonella shedding among dairy cattle in New York. Foodborne Pathog Dis. 2010; 7: 815-823.
34. Mohamed, O. N., Farid, A. F, Abaza, A. F., et al. Fecal Shedding of Non-typhoidal Salmonella Species in Dairy Cattle and their Attendants in Alexandria Suburbs. J Am Sci. 2011; 7: 623-631.
35. Moussa, I. M., Ashgan, M. H., Mahmoud, M. H., et al. Rapid detection and characterization of *Salmonella* Enterica serovars by multiplex polymerase chain reaction assay. Afr J Biotechnol. 2012; 11: 3452-3458.
36. Kagambèga, A., Lienemann, T., Aulu, L., et al. Prevalence and characterization of *Salmonella enterica* from the faeces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human Salmonella isolates. BMC Microbiol. 2013; 13 (1): 253.
37. Halimi, H. A, Seifi, H. A., Rad, M. Bovine salmonellosis in Northeast of Iran: Frequency, genetic fingerprinting and antimicrobial resistance patterns of Salmonella spp. Asian Pac J Trop Biomed. 2014; 4:1-7.
38. Tarazi, Y. H., and Abo-Shehada, M. N. Herd- and individual-level prevalences of and risk factors for *Salmonella* spp. fecal shedding in dairy farms in Al-Dhulail Valley, Jordan. Trop Anim Hlth Prod. 2015; 47 (7): 1241-1248.
39. Abd El-Rahman, A. M., Mahmoud, A. A., Khadr Adel, M., et al. Some Studies on *Salmonella* Enterica Associated with Diarrhea in Cattle. Alexandria J Veter Sci. 2016; 48 (2): 54-60.
40. Eguale, T., Engidawork, E., Gebreyes, W.A., et al. Faecal prevalence, serotype distribution and antimicrobial resistance of Salmonella in dairy cattle in central Ethiopia. BMC Microbiol. 2016; 16: 20.
41. Hadimli, H. H., Pinarkara, Y., Sakmanoğlu, A., et al. Serotypes of Salmonella isolated from feces of cattle, buffalo, and camel and sensitivities to antibiotics in Turkey. Turk J Vet Anim Sci. 2017; 41:193-198.
42. Ahmed, L. M., Sayed, A. S. M., ElKader, H. A. A., et al. Phylogenetic analysis of Salmonella species isolated from cows, buffaloes, and humans based on gyrB gene sequences. Trop Anim Hlth Prod. 2020; 52 (3): 1487-1492.
43. Julien, C. K., René, Y. K., Komissiri, D., et al. Phenotype and Molecular Characterization of Antibiotic Resistance of Salmonella spp. from Cattle in Abidjan District, Côte d'Ivoire. J Adv Microbiol. 2019; 18 (4): 1-10.
44. Edrington, T. S., Hume, M. E., Looper, M. L., et al. Variation in the faecal shedding of Salmonella and *E. coli* O157:H7 in lactating dairy cattle and examination of Salmonella genotypes using pulsed-field gel electrophoresis. Lett Appl Microbiol. 2004; 38 (5): 366-372.
45. Hassan, L., Mohammed, H. O. McDonough, P. L., et al. A Cross-Sectional Study on the Prevalence of *Listeria monocytogenes* and *Salmonella* in New York Dairy Herds. J Dairy Sci. 2000; 83: 2441-2447.
46. Nielsen, L. R. *Salmonella* Dublin faecal excretion probabilities in cattle with different temporal antibody profiles in 14 endemically infected dairy herds. Epidemiol Infect. 2013; 141: 1937-1944.
47. Nielsen, L. R., Toft, N., and Ersboll, A. K. Evaluation of an indirect serum ELISA and a bacteriological faecal culture test for diagnosis of *Salmonella* serotype Dublin in cattle using latent class models. J ApplMicrobiol. 2004; 96: 311-319.
48. Ersboll, A. K., and Nielsen, L. R. Spatial patterns in surveillance data during control of *Salmonella* Dublin in bovine dairy herds in Jutland, Denmark 2003-2009. Spat Spatiotemporal Epidemiol. 2011; 2 (3): 195-204.
49. Smith, B. P., Oliver, D. G., Singh, P., et al. Detection of *Salmonella* Dublin mammary gland infection in carrier cows, using an enzyme-linked immunosorbent assay for antibody in milk or serum. Am J Vet Res. 1989; 50: 1352-1360.

50. House, J. K., Smith, B. P., Dilling, G. W., et al. immunosorbent assay for serologic detection of *Salmonella* Dublin carriers on a large dairy. Am J Vet Res. 1993; 54:1391-1399.
51. Wedderkopp, A., Strøger, U., Bitsch, V., et al. Testing of bulk tank milk for *Salmonella* Dublin infection in Danish dairy herds.Can J Vet Res. 2000; 65 (1): 15-21.
52. Nielsen, L. R. Overview of pathogenesis, epidemiology and diagnostic tools necessary for successful surveillance and eradication of *Salmonella* Dublin from the Danish cattle population: prize assignment "Professor Dr.med.h.c. C. O. Jensens Mindefond". Department of Large Animal Sciences, University of Copenhagen, 2009.
53. Doherty, E., Sayers, R. O., and Grady, L. Temporal trends in bulk milk antibodies to *Salmonella*, *Neospora caninum*, and *Leptospira interrogans* serovar hardjo in Irish dairy herds. Prev Vet Med. 2013; 109: 343-348.
54. Nyman, A. K. J., Ågren, E. C., Bergström, K., and Wahlström, H. Evaluation of the specificity of three enzyme-linked immunosorbent assays for detection of antibodies against Salmonella in bovine bulk milk. Acta Vet Scand. 2013; 55: 5.
55. Agren, E. C., Johansson, J., Frössling, J., Sternberg-Lewerin, S. Factors affecting costs for on-farm control of Salmonella in Swedish dairy herds. Acta Vet Scand. 2015; 57: 28.
56. Cummings, K. J., Virkler, P. D., Wagner, B., et al. Herd-level prevalence of *Salmonella* Dublin among New York dairy farms based on antibody testing of bulk tank milk. Zoonoses Publ Hlth. 2018; 65 (8): 1003-1007.
57. Kabagambe, E. K., Wells, S. J., Garber, L. P., et al. Risk factors for fecal shedding of Salmonella. Prev Vet Med. 2000; 43: 177-194.
58. Hinton, M. *Salmonella* Dublin abortion in cattle: studies on the clinical aspects of the condition. Br Vet J. 1974; 130 (6): 556-563.
59. Wray, C., and Sojka, W. J. *Salmonella* Dublin Infection of Calves: Use of Small Doses to Simulate Natural Infection on the Farm. J Hyg. 1981; 87: 501-509.
60. Andrews, W. H., and Hammack, T. S. Food sampling and preparation of sample homogenate. Bacteriological Analytical Manual Online. 2003. http://www.cfsan.fda.gov.
61. D'Aoust, J. Y., Sewell, A. M., and Warburton, D. W. Une comparaison des méthodes culturales standard pour la détection des salmonelles d'origine alimentaire, Int J Food Microbiol. 1992; 16 (1): 41-50.
62. La Ragione, R., Metcalfe, H., Villarreal-Ramos, B., et al. *Salmonella* Infection in Cattle, In: Barrow, P. A., and Methner, U. Salmonella in domestic animals. 2013: 233-263.
63. Xiong, D., Song, L., Tao, J., et al. An Efficient Multiplex PCR-Based Assay as a Novel Tool for Accurate Inter-Serovar Discrimination of Salmo-*nella* Enteritidis, *S.* Pullorum/Gallinarum and *S.* Dublin. Front Microbiol. 2017; 8: 420.
64. Nielsen, N. R., and Dohoo, I. Survival analysis of factors affecting incidence risk of Salmonella Dublin in Danish dairy herds during a 7-year surveillance period. Prev Vet Med. 2012; 107: 160-169.
65. Majchrzak, M., Krzyzanowska, A., Kubiak, A. B., et al. TRS-based PCR as a potential tool for inter-serovar discrimination of *Salmonella* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow, *S.* Hadar, *S.* Newport and *S.* Anatum. Mol Biol Rep. 2014; 41: 121-7132.
66. Nielsen, L. R. *Salmonella* Dublin in Dairy Cattle: use of diagnostic tests for investigation of risk factors and infection dynamics. Thesis, Copenhagen, 2003: 208
67. Spier, S. J., Smith, B. P., Cullor, J. S., et al. Persistent experimental *Salmonella* Dublin intramammary infection in dairy cows. J Vet Intern Med. 1990; 5 (6): 341-350.
68. Smith, B. P., House, J. K., Dilling, G. W., et al. Identification of Salmonella Dublin carrier cattle. In: Proceedings of Salmonella and Salmonellosis. Ploufragan, France, 1992; 15-17: 225-230.
69. Toma, B., Dufour, B., Bénet, J. J., et al. Epidémiologie appliquée à la lutte contre les maladies animales transmissibles majeures, 4th edition, AEEMA Maison-Alfort, France. 2001: 696.
70. Derdour, S. Y., Hafsi, F., Azzag, N., et al. Prevalence of the main infectious causes of abortion in dairy cattle in Algeria. J Vet Res. 2017; 61: 337-343.
71. Smith, B. P. Salmonellosis in ruminants. In: Smith B. P. (editor). Large animal internal medicine. 4th edition. Mosby; St. Louis (MO), 2009; 877-881.
72. Carrique-Mas, J. J., Willmington, J. A., Papadopoulou, C., et al. Salmonella infection in cattle in Great Britain, 2003 to 2008. Vet Rec. 2010; 167: 560-565.
73. Costa, L. F., Paixão, T. A., Tsolis, R. M., et al. Salmonellosis in cattle: Advantages of being an experimental model. Res Veter Sci..2012; 93 (1): 1–6.
74. Peek, S. F., Cummings, K. J., and McGuirk, S. M. Infectious diseases of the gastrointestinal tract. In: Peek, S. F., Divers, T. J., (editors). Diseases of dairy cattle. 3rd edition. Elsevier; St. Louis (MO) 2017.