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DETECTION AND IDENTIFICATION OF SALMONELLA SEROVARS FROM LAYING HEN FARMS IN MOROCCO BY BACTERIOLOGY AND RAPID TESTS

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ABSTRACT

The ubiquitous Salmonellae are one of the most important causes of human foodborne infection. In fact, avian species can be infected either by non-motile Salmonellae (Salmonella gallinarum and Salmonella pullorum) leading to fowl typhoid and pullorum disease, and motile Salmonellae (Non-typhoid group: NTG) causing salmonellosis in chicken and having a zoonotic impact. The present study was conducted from March 2015 to May 2017 in 72 laying hen farms located in different areas in Morocco. In order to determine the prevalence of Salmonella spp. Bacteriological investigations, Salmonella Phage test (SPT) and Polymerase chain reaction to detect invA gene were carried out. A specific PCR to reveal spvC plasmid virulence gene was performed. Bacteriological investigations and SPT revealed that 34.72% (25/72) of sampled farms were infected by Salmonellaspp. Otherwise, invA gene was detected in 24; all of Salmonellaisolates carry spvC virulence plasmid gene. According to the serotyping of 25 Salmonella isolates, 13 were detected as SalmonellaGalinarum, 6 as S. Enteritidis and 6 others were recognized as S.Cyprus, S.Kottbus, S.Curacao, S.Fayed, S.Chomedey, S.Chailley.Finally, we can conclude that avian salmonellosis still an important infection which could have a negative impact on poultry production and also on public health

Keywords: Salmonella; poultry; neglected serovars; food-borne

1. INTRODUCTION

Salmonellosis is an infectious disease of humans and animals, caused by two species of *Salmonella* (*Salmonella* enterica, and *S.* bongori) of the Enterobacteriacea family. Non-typhoidal *Salmonella* are a major cause of foodborne illness throughout the world. The bacteria are generally transmitted to humans through consumption of contaminated food of animal origin, mainly meat, poultry, eggs and milk. In fact Poultry and poultry products have been widely recognized as a major source of *Salmonella* infections in humans, leading to food-borne gastroenteritis [1].

Usually the epidemiological investigations of the foodborne outbreak need microbiological identification and characterization of the involved pathogen. As far as identification of

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Salmonella is based on its isolation from tissues collected aseptically at necropsy, faeces, intestinal swabs, environmental samples, food products and feedstuffs by using conventional bacterial culture methods, followed by various biochemical and serological tests to provide a definitive confirmation of an isolated strain [2]. However, this procedure is both too laborious and time-consuming (requires 3-11 days). Thus the consumer's demand for rapid and sensitive *Salmonella* test has initiated the development of new fast and accurate alternatives to conventional culture methods. Several PCR tests have been developed for the identification of *Salmonella* strains by detection of the *invA* gene that was revealed in almost 2200*Salmonella*serovars, this gene leads to the invasion of epithelial cells of the infected host [3] .The relationship between the *spv* genes, virulence and non-typhoid *Salmonella* bacteremia has been confirmed [4]. *spvC* is one of the five most important *spv* genes; it has phosphothreoninelyase activity and inhibits MAP kinases [5]. There are at least six *Salmonella* serovars containing the virulence plasmid carrying *spvC* genes: *S.* Typhimurium, *S.* Choleraesuis, *S.* Dublin, *S.* Enteritidis, *S.* Gallinarum *and S.* Pullorum[6].

In order to reduce time of diagnosis, an enzyme-linked fluorescent assay (ELFA) using a novel recombinant phage protein-based technology was developed for the detection of *Salmonella* spp. in human and non-human animal food products, environment samples, and feces. All of those new methods, have demonstrated their utility as screening tools for Salmonella, they also contribute to reduce workloads and time.

The present study has been set up in order to estimate the prevalence of different serovars of *Salmonella* in laying hen farms, using conventional methods, PCR and *Salmonella* Phage Test, and to evaluate the prevalence of *spvC* plasmid virulence gene.

2 MATERIALS AND METHODS

2.1 Sample Collection

A total of 72 laying hens farms located in different areas of poultry production in Morocco, (which present 30% of the total of laying hens farms) were sampled from March 2015 to May 2017. For laboratory investigations, 20 healthy birds were selected in each sampled farm. At necropsy, intestinal swabs, liver, spleen, heart and ovary samples were collected aseptically from each bird.

2.2 Salmonella Isolation and Identification procedure

A total of 1440 of each samples type (intestinal swabs, liver, spleen, heart and ovary) collected from necropsy exam were analyzed following the conventional methods for the detection of *Salmonella* using standard guidelines from ISO 6579:2002 (Moroccan standards, 2007) [7] (Figure 1).

This bacteriological procedure is elaborated on four principal stages: pre-enrichment, selective enrichment, selective plating, biochemical confirmation and serotyping.

In fact, 25mg of each sample or intestinal swabs are added in 125 ml of buffered peptone water (BPW) and incubated at 37°C for 18 hours. Then 1 ml of pre-enriched broth was transferred into tube with 10 ml of Muller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn broth, Oxoid®

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agar media) and 0.1ml of the same pre-enriched broth was added into a tube containing 10 ml of Rappaport-Vassiliadis Soya peptone broth (RVS broth, Oxoid® agar media) and incubated at 41.5°C±1° C for 24h ±3h. After selective enrichment, a loop full of culture was involved from each enriched broth onto plate of Xylose Lysine Deoxycholate agar media (XLD agar, Oxoid® agar media) and Brilliant green with red phenol. All the plates were incubated at 37°C for 24h and examined to identify the typical colonies (with black center of *Salmonella* species on XLD plate and transparent and pinkish colonies of Salmonella on brilliant green)

Biochemical confirmation of the presumptive *Salmonella* isolates was carried out with a commercial bacterial identification kit (the Analytical Profile Index System) (API 20E) system (BioMérieux, Marcy l'étoile, France), and tested for motility using manitol-motility media.

Confirmed *Salmonella* isolates were submitted to the National Institute of Hygiene in Rabat for serotyping, according to Kauffmann, White and Le Minor instructions [8]using Biorad Antisera (Bio-Rad®, Marnes La Coquette, France).

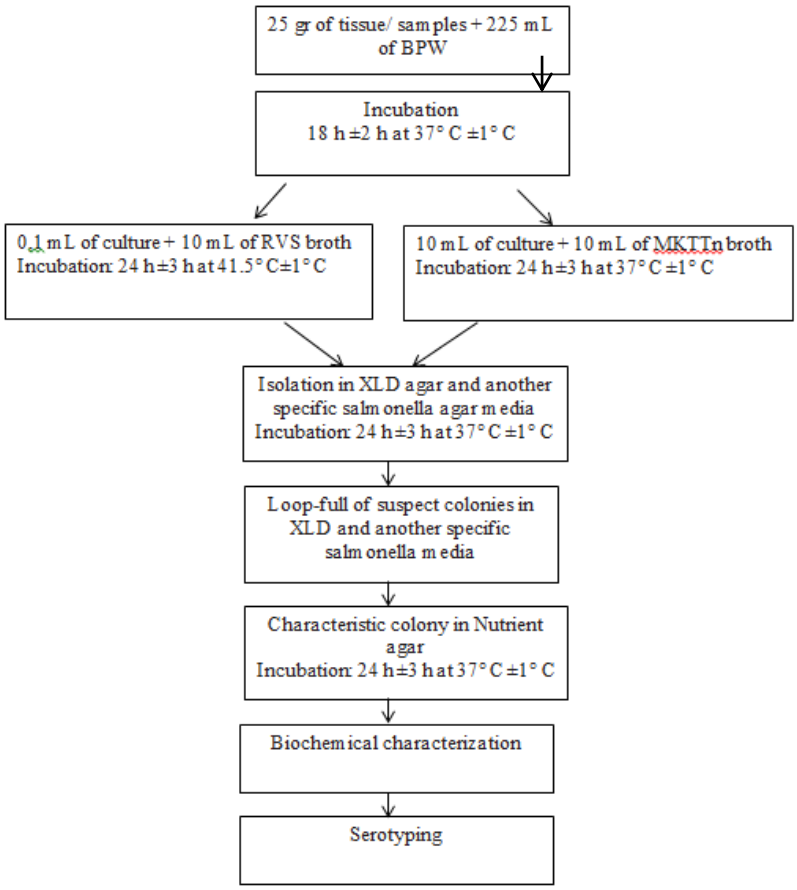

Figure1. Diagram showing procedure for isolation of salmonella species.

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2.3 Salmonella Phage Test

Salmonella Phage test (SPT) is an alternative method that involves an enzyme-linked fluorescent assay (ELFA) using a novel recombinant phage protein-based technology for use with instruments for specific detection of *Salmonella* serovars.

A total of 25 mg of each sample or intestinal swabs were tested using SPT, actually, The protocol consists of an enrichment in buffered peptone water (BPW) supplemented with a Salmonella colored supplement containing Vancomycin to inhibit gram positive bacteria (1 ml/225 ml), after incubated for 18 to 24 hours at 41.5°C ± 1.0°C, a 0.5 ml of enriched broth was added into the first well of the strip containing all the ready-to-use reagents required for the test: washing solution, specific anti-*Salmonella* proteins conjugated with alkaline phosphatase and substrate.

In front of each strip, a specific disposable *Salmonella* Phage Rows SPR® was placed in the VIDAS automate; it's used both for the solid phase and as a pipetting system. The SPR is coated with specific anti-*Salmonella*proteins absorbed on its surface.

All the steps are performed automatically by the VIDAS® analytical module. During the final detection step, the fluorescence intensity of the product from the hydrolysis reaction of the substrate by the conjugate enzyme is measured at 450 nm. It is expressed as a Relative Fluorescence Value (RFV), interpreted by the VIDAS® system as follows:

Test value (TV) = RFV standard/RFV sample

This value was compared to internal references of positive and negative controls (thresholds) and each result was interpreted (positive if TV ≥ 0.25 or negative if < 0.25).

2.4 Molecular detection

The genomic DNA of *Salmonella* isolates was extracted using the Pure Link® Genomic DNA Mini Kit (Invitrogen™ by Life technologies™, Foster City, CA) according to manufacturer's instructions. Two pairs of oligonucleotide primers were used for PCR amplification according to the sequences of the chromosomal *invA* and plasmid *spvC* genes; the nucleotide sequences are listed in Table 1. With this classic duplex PCR, the appearance of one band at 244 bp from the *invA* gene would indicate the presence of *Salmonella* and the revelation of a second band at 571 bp signify that the detected Salmonella carries the *spvC* virulence plasmid gene. PCR mixture was optimized using: 12,5 µl of PCR Master Mix (Thermo fisher Scientific®), 1 µM (of each) of primer pairs, 5 µl of DNA sample, plus Nuclease-free water to have make a total volume of 25µl. The PCR reaction was performed on 30 cycles in peQlab thermal using the thermal cycling conditions: Initial denaturation for 2 min at 95°C, denaturation for 30 s at 94ºC, annealing of primers for 30 s at 56ºC, and primer extension for 2 min at 72 º C. After the last cycle, the mixture was incubated for 10 min at 72ºC. All amplified product were revealed by using electrophoresis on 2% agarose gel pre-stained with ethidium bromide.

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Table 1. Synthetic oligonucleotides used as primers for PCR

Primer	sequence	Gene	Corresponding positions	Localization
*inv*A1	ACAGTGCTCGTTTACGACCTGAAT	*inv*A	244 bp	Chromosome
*inv*A2	AGACGACTGGTACTGATCGATAAT			
*spv*C1	ACTCCTTGCACAACCAAATGCGGA	*spv*C	571 bp	Plasmid
*spv*C2	TGTCTCTGCATTTCGCCACCATCA			

3.RESULTS

Bacteriology

During this study, a farm is considered infected if *Salmonella* is isolated from at least one collected sample. From the 72 apparently health laying hen farms, 34.72% (25/72 positives) were founded positive for *Salmonella* by conventional isolation and identification methods. Motility test revealed 48% (12/25) of *Salmonella* isolates were motile. *Salmonella* was isolated from 23.61% (340/1440) of samples liver, 11.11% of intestinal swabs, 6.94% of collected ovary, 2.77% of analyzed hearts and spleens (Table 2).

Salmonella Phage Test

A perfect concordance was observed between SPT and bacteriology; the SPT assay identified 25 positive laying hen farms.

Serotyping result

According to the results of serotyping for 25 Salmonella isolates, 13 were detected as *Salmonella*Galinarum, 6 as *S.* Enteritidis and 6 others were recognized as *S.* Cyprus, *S.* Kottbus, *S.* Curacao, *S.* Fayed, *S.* Chomedey, *S.* Chailley (Table 2).

PCR detection

The *invA* gene was detected in 24 suspected cultures by generating a PCR product of approximate size 244 bp. However, all of *Salmonella* isolates carried the *spvC* plasmid virulence gene and generated a second band of 571 bp.

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Table 2. Summary of serotyping, PCR and SPT tests results of salmonella isolated from layers poultry tissue and Intestinal swabs samples.

Code of sample	Positive sample type	Motility	Antigenic formula	Serotype	SPT	InvA gene	SpvC gene
Sidiyahya/0615	Liver	+	1,9,12 : g,m: -	*S. Enteritidis*	+	+	+
Ainaouda/0915	Liver, Heart, Spleen	+		*S. Enteritidis*	+	+	+
Tiznit/a0416	Liver	+		*S. Enteritidis*	+	+	+
Eljadia/1116	Intestinal swabs	+		*S. Enteritidis*	+	+	+
Kenitra/0716	Liver, Intestinal swabs	+		*S. Enteritidis*	+	+	+
Salé/0416	Liver, Spleen	+		*S. Enteritidis*	+	+	+
Temara/b1115	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Salé/0216	Liver, Spleen	-		*S.Pullorumserovar Gallinarum*	+	+	+
Tiznit/b0416	Liver	-		*S.Pullorumserovar Gallinarum*	+	+	+
Kenitra/a0516	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Kenitra/b0516	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Kenitra/c0516	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Bouznika/0716	Ovary	-	1,9,12:-:-	*S.Pullorumserovar Gallinarum*	+	+	+
Tiznit/0117	Liver	-		*S.Pullorumserovar Gallinarum*	+	+	+
Temara/1015	Liver, Heart, Ovay	-		*S.Pullorumserovar Gallinarum*	+	+	+
Temara/a1116	Liver, heart	-		*S.Pullorumserovar Gallinarum*	+	+	+
Oujda/1116	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Sidiyahya/0117	Liver	-		*S.Pullorumserovar Gallinarum*	+	+	+
Oujda/0217	Intestinal swabs	-		*S.Pullorumserovar Gallinarum*	+	+	+
Temara/b1116	Liver, Heart	+	*6,8: i: l,w*	*S.Cyprus*	+	+	+
Temara/c1116	Liver	+	*6,8: e,h: 1,5*	*S. Kottbus*	+	+	+
Temara/a1115	Liver, Ovary	+	*6,8: a: 1,6*	*S. Curacao*	+	+	+
Temara/a1215	Liver, Ovary	+	*6,8: l,w :1,2*	*S.Fayed*	+	-	+
Temara/b1215	Liver, Ovary	+	*8,20: z10: e,n,z15*	*S.Chomedey*	+	+	+
Temara/d1115	Liver	+	*6,8: z4, z23: [e,n,z15]*	*S. Chailey*	+	+	+

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4.DISCUSSION

Salmonellosis continues to be one of the most important food-borne illnesses worldwide. As it was reported *Salmonella* serovars is mostly detected from poultry tissues or derivate products and it is known to be higher compared to isolation from other animal species [9,10]. In fact, in addition to non-motile specific avian *Salmonella spp*: *Salmonella*Gallinarum and *Salmonella* Pullorum that cause fowl typhoid and pullorum disease, respectively. Chickens can be infected by motile Salmonellae (paratyphoid group) which cause salmonellosis and have zoonotic significance [11]. Consequently, poultry and products mainly from intensive conditions are recognized as major sources of foodborne salmonellosis, triggering food-borne illness in humans.

In the present study the global prevalence of*Salmonella* isolation from laying hen farms was 34.72% (25 of 72 sampled farms). Out of these isolates, 48% were motile and 52% were identified as non-motile serovars. This prevalence is lower than the prevalence of 76.7% reported in Morocco in 2016 [12] and 73.33 % noted by Saadi [13]. In Turkey, a prevalence of 41.3% was observed by Carli et al., 2001[14] and in Hungry an incidence of 68.2% was reported (European Food Safety Authority)[15]. However, the current prevalence was higher than those mentioned in chicken flocks by authors in others countries: 15.6% in Iran [16]; 28.6 % in Senegal [17]; 25% in Denmark[18]; 21.7 % in Brazil[19]; 6.2% in France [15] ; 2.7% in India [20]; 0.7% in Finland[21] and 0.0% in Sweden [15] . This variation of infection rates might be explained by many factors such as size of samples, type of sampled farm, level of hygiene and biosecurity implemented in farms, category of collected tissues and environmental samples, season of study, geographic location and national strategy applied in countries. In the present study, the rate of *Salmonella* isolation was high in livers (340/1440) and intestinal swabs (160/1440). These results are in agreement with results from studies by Carli et al. 2001[14] and Habtamu et al. 2011[20] . This trend might be due to the virulence mechanism of *Salmonella* serovars, and gives further insight about the most appropriate biological samples to consider for future studies.

A perfect concordance was noted between bacteriology and the SPT ELFA technique for *Salmonella*detection with the same results being found by both procedures. The *Salmonella* Phage Test, based on ELFA technique, could be recognized as a sensitive qualitative method to detect *Salmonella* in animal tissues as mentioned in report of validation study of VIDAS® UP *Salmonella* method (2015)[22]. The SPT assay is a rapid technique that analyzes the sample within 24 h and requires 45 min for automated analysis, and, therefore, it could be considered as an alternative method.

The *invA* gene is recognized as an international standard method for identification of *Salmonella*[23]. In the current study, the *invA* gene was amplified in 24 *Salmonella* isolates. The one remaining sample was detected as a *Salmonella* strain by using bacteriology and ELFA methods. Consequently, for our samples, PCR showed sensitivity of 99.6% and specificity of 100%. Similar results were reported by Shanmugasamy et al., 2011 [24].

The predominant serotypes change over time and differ according to geographical area, condition and period of sampling. In fact *Salmonella* isolates detected in the present study were non-typhoid serovars and different from others identified previously in 2003 and 2016 in Morocco. The National Institute of Hygiene reported that *Salmonella*Gallinarum, *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis were the most prevalent serovars isolated from poultry [25]. In 2016, Ziyate et al. [12] isolated *S.* Enteritidis, *S.* Kentucky, *S.* Infantis, *S.* Typhimurium, *S.*

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Thompson, *S.* Agona and *S.* Amsterdam from laying hen farms. Samples collected for the present study contained 52% (n=13) *Salmonella* Gallinarum, a specific serovar for fowl.

Although the vaccination of laying hens was authorized since 2002 by Office National de Sécurité Sanitaire des produits Alimentaires, ONSSA, *Salmonella* Gallinarum is still detected on sampled farms, which reveals the importance of establishing other measures such as biosecurity and control of efficacy of vaccination.

According to the serotyping, 24% (n=6) of the isolates were identified as *S.* Enteritidis, an ubiquitous serovar responsible for food-borne illness, and all over the world it is the most often isolated serotype. This finding is lower than the observation noted by Ziyate et al. (37.5%)[12]and higher than published results in France (6.3%) [15]. The present study resulted in the identification of six serovars for the first time in Morocco; *Salmonella* Fayed (n=1) was detected from the liver and ovary collected from poultry. This serovar was isolated for the first time in 1945 in Fayed, Egypt from a German prisoner of war who complained of acute diarrhea followed by bacterial endocarditis [26, 27]. Wan-Ling et al., [28] have reported that only 0.01 to 2.7 % out of 87 cases of bacterial endocarditis diagnosed from de 1976 to 2014 were due to non-typhoid *Salmonella*serovars.

Salmonella Chailey was detected in only one sample. The first isolation of this serovar was performed in 1951 from a little girl in Chailey hospital in England [27], and in 2012, it was isolated for the first time in New Zealand from patient who had recently traveled to the Philippines [29].

One isolate was identified as *S.* Kottbus. This non-typhoid serovar has been detected in many countries. In 2001, 24 humans cases were reported in California, 6 in Arizona, 1 in Colorado and 1 in New Mexico. All patients had consumed alfalfa sprouts seeds imported from Australia in 2000 [30]. In France, 883, 855, 703 and 367 *Salmonella*Kottbus were isolated from poultry in 2005, 2007, 2011 and 2012, respectively [31]. In 2006, a gastroenteritis outbreak in infants due to *S.* Kottbus caused by bottled water was reported in Gran Canaria [32]. In 2013, 335 *S.* Kottbus were detected in Germany in different samples collected from poultry farms and meat, cattle and Humans [33]. *Salmonella*Chomedey was detected in one farm. This ubiquitous serovar was isolated in Gambia from 844 healthy kids [34] and in Burkina Faso from sheep [35]. *Salmonella* Curacao, which was isolated from South America in 1955 [36] was detected in our study and isolated from the liver and ovary. *Salmonella*Cyprus (n=1) was isolated for the first time from a waterway in Virginia [37].

All of 25 serovars carried the *spvC* gene responsible for plasmid virulence. This observation was different from what was reported by Ziyate et al. in 2016[12]. Since *spvC* is not detectable in all *Salmonella*isolates [38], detection of this plasmid gene is related to host and sample [39]. The *spvC* gene is higher in non-human animal serovars and could be affected by transfer of plasmid virulence between humans and other animals [40].

Those results reveal possibilities of cross contamination between humans, other animals and the environment, which may be accentuated by lack of biosecurity measures. Also, it is important to consider some *Salmonella* serovars as emerging strains.

5.CONCLUSION

This study showed the presence of *Salmonellaspp* among poultry samples collected from laying hen in Morocco. Detected serovars could lead to economic losses in poultry production and have

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an important public health threat, especially the emergence of neglected serovars detected for the first time in Morocco.

Salmonella detection methods used in the present study were equivalent, the conventional technique is certainly sensitive and specific. In order to optimize testing time, *Salmonella* phage test might be a good alternative method. Molecular techniques have an added value for the Moroccan context by identifying virulence genes.

REFERENCES

[1] [1]. Kumar K (2012) Development of a multiplex PCR technique for detection of *Salmonella* in food samples. Biotechnol Lett. 3(1): 32-33.
[2] [2]. World Health Organization for Animal Health-Office International des Epizooties – OIE. 2008. Available: http://www.oie.int/fr/ .
[3] [3]. Darwin KH, Miller VL (1999) Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin. Microbiol. Rev. 12: 405-428.
[4] [4]. Guiney G, Fang FC, Krause M, Libby S, Buchmeier NA, Fierer J (1995) Biology and clinical significance of virulence plasmids in *Salmonella* serovars. Clin. Infect. Dis. 21(Suppl.2):146–151.
[5] [5]. Guiney G, Joshua F (2011) The role of the *spv* genes in *Salmonella* pathogenesis. Front. Cell. Infect. Microbiol., (2011) 2 :129
[6] [6]. Jeníková G, Pazlarová J, Demnerová K (2000) Detection of *Salmonella* in food samples by the combination of immunomagnetic separation and PCR assay. Internatl microbiol (2000) 3:225–229.
[7] [7]. Moroccan Standards (2007) Microbiology of food and animal feeding stuffs, horizontal method for the detection of *Salmonella* serovars.
[8] [8]. Le Minor L, Popoff MY (1987) Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, 146 pages.
[9] [9]. Davies RH, Breslin M (2003) Observations on *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. Veterinary Records, 152: 283-287.
[10] [10]. Poppe C (2000) *Salmonella* infections in the domestic fowl. In : Wray C. and Wray A. *Salmonella* in domestic animals. CAB International, New York, US. 107-132.
[11] [11]. Hossain MS, Chowdhury EH, Islam MM, Haider MG, Hossain MM (2006) Avian *Salmonella* infection: isolation and identification of organisms and histopathological study. Bangl. J. Vet. Med. 4 (1): 07–12.
[12] [12]. Ziyate N, Karraouan B, Kadiri A, Darkaoui S, Soulaymani A, Bouchrif B (2016) Prevalence and antimicrobial resistance of *Salmonella* isolates in Moroccan laying hen farms. J. Appl. Poult. Res. 25:539–546.
[13] [13]. Saadi S (2011) Etude de la prévalence des salmonelles dans les élevages de poules pondeuses au Maroc. [Thèse de Doctorat vétérinaire of Institut Agronomique et vétérinaire Hassan II].
[14] [14]. Carli KT, Eyigor A, Caner, V (2001) Prevalence of *Salmonella* serovars in chicken in Turkey. J. Food Prot. 64(11): 1832–1835.

[15] [15]. European Food Safety Authority EFSA (2007) Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of Gallus gallus. The EFSA Journal 97:1-85.

[16] [16]. Salehi Zahraei T, Mahzounieh M, Saeedzadeh A (2005) Detection of *invA* Gene in Isolated *Salmonella* from Broilers by PCR Method. Int. J. Poult. Sci. 4 (8): 557-559.

[17] [17]. Cardinale E, Tall F, Guèye EF, Cisse M, Salvat G (2004) Risk factors for *Salmonella* Enterica subsp. Enterica infection in Senegalese broiler-chicken flocks. Prev. Vet. Med. 63: 151–161.

[18] [18]. Chadfield M, Skov M, Christensen J, Madsen M, Bisgaard M (2001) An epidemiological study of *Salmonella* Enterica serovar 4, 12 : b: in broiler-chicken in Denmark. Vet. Microbiol. 82(3): 233–247.

[19] [19]. Tavechio AT, Ghilardi AC, Peresi JT (2002) *Salmonella* serotypes isolated from non-human sources in Sao Paulo Brazil from 1996 through 2000. J. Food Prot. 65(6) : 1041–1044.

[20] [20]. Habtamu TM, Rajesh R, Kulip D, Kumar AR (2011). Isolation, Identification and polymerase chain reaction (PCR) Detection of *Salmonella* Species from Field Materials of Poultry Origin. Int. J. Microbiol Res 2 (2): 135-142.

[21] [21]. Anon A (1998) *Salmonella* in livestock production Weybridge: Veterinary Laboratories Agency, Ministry of Agriculture, Fisheries and Food, Welsh Office Agriculture Department, Scottish Office Agriculture and Fisheries Department.

[22] [22]. Summary report, EN ISO 16140 validation study of the VIDAS® UP *Salmonella* method (VIDAS ® SPT) for the detection of *Salmonella* spp. Validation of alternative analytical methods Application. food microbiology, BIO 12/32 – 10/11, Version 0 December 22, 2015.

[23] [23]. Malorny B, Hoorfar J, Bunge C, Helmuth R (2003) Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. Appl. Environ. Microbiol. (69): 290-296.

[24] [24]. Shanmugasamy M, Velayutham T, Rajeswar J (2011) *Inv A* gene specific PCR for detection of *Salmonella* from broilers. Veterinary World, Vol.4 No.12 December 2011.

[25] [25]. Programme "Global Salm-Surv" de l'OMS "Global Salm-Surv". Note d'information INFOSAN No. 6/2005. Available: http://www.who.int/foodsafety/fs_management/No_06_GSS_Oct05_fr.pdf.

[26] [26]. Anderson ES, Anderson HJ, Taylor J (1947) A new *Salmonella* (Salm. Fayed) which caused fatal endocarditis in man. J. Pathol., 59: 533–537.

[27] [27]. Lapage SP, Taylor J, Nicewonger CR, Phillips AG (1966) New serotypes of *Salmonella* identified before 1964 at the *Salmonella* reference laboratory, Colindale. Int J Syst Bacteriol. Vol. 16, (3): 253-297.

[28]

[29] [28]. Wan-Ling C, Chia-Wen, L, Ming-Chi L, Nan-Yao L, Ching-Chi L, Wen-Chien K (2016) *Salmonella* infective endocarditis. J Microbiol Immunol Infect Volume 49: 313-320.

[30] [29]. Public health surveillance, information for New zealand public health action (2012) No. 12 December, Human *Salmonella* isolates.

[31] [30]. Winthrop KL, Palumbo MS, Farrar JA, Mohle-boetani JC, Abbott S, Beatty ME, Inami G, Werner AB (2003) Alfalfa Sprouts and *Salmonella* Kottbus Infection: A Multistate

Outbreak following Inadequate Seed Disinfection with Heat and Chlorine. J. Food Protect, Vol. 66, (1) :13–17.

[32] [31]. Lailler R, Moury F, Leclerc V, Bohnert M, Cadel-Six S, Brisabois A (2015) Surveillance de *Salmonella* dans la chaîne alimentaire pour la détection d'émergences en France. Bulletin épidémiologique, santé animale et alimentation n° 68/Spécial Vigilance sur la chaîne alimentaire.

[33] [32]. Palmera-Suárez R, Garcia P, Garcia A, Barrasa A, Herrera D (2007) *Salmonella* Kottbus outbreak in infants in Gran Canaria (Spain), caused by bottled water. Euro Surveill. 12(28): 32-35.

[34] [33]. Toboldt AB, Erhard Tietze C, Reiner Helmuth A, Ernst Junker A, Angelika Fruth C, Malorny AB (2014) Molecular epidemiology of *Salmonella* Enterica serovar Kottbus isolated in Germany from humans, food and animals. Vet Microbiol 170: 97–108.

[35] [34]. Dione M, Geerts S, Antonio M, Adegbola R (2010) Epidemiology of Non-Typhoidal *Salmonella* (NTS) in Humans and animals in the Gambia and Senegal. Tropicultura, vol 28, (4):253-256.

[36] [35]. Kagambèga A, Haukka K, Lienemann T, Aulu L, Traoré AS, Barro N, Siitonen A (2013) Prevalence and characterization of *Salmonella* Enterica from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. BMC Microbiology, 13:253.

[37] [36]. Edwards PR, Rutten FJ, MC-Whorter AC (1955) Four new *Salmonella* types isolated in Curacao. Antonie van Leewen-Hoek, Amst., 21 : 80-82.

[38] [37]. Timothy M (2012) Identifying pathogenic *Salmonella* serotypes isolated from Prince Edward County, VA waterways via multiplex PCR analysis. Biological and Environmental Sciences, Research, Volume 5. Available: http://blogs.longwood.edu/incite/2013/02/14/identifying-pathogenic-*Salmonella*-serotypes-isolated-from-prince-edward-county-va-waterways-via-multiplex-pcr-analysis/

[39] [38]. Oliveira SD, Rodenbusch CR, Michael GB, Cardoso MIR, Canal CW, Brandelli A (2003) Detection of virulence genes in *Salmonella* Enteritidis isolates from different sources. Brazilian Journal Microbiol. 34(1):123-124.

[40]

[41] [39]. Amini K, Salehi TZ, Nikbakht G, Ranjbar R, Amini J, Ashrafganjooei SB (2010) Molecular detection of *invA* and *spv* virulence genes in *Salmonella* Enteritidis isolated from human and animals in Iran. Afr. J. Microbiol. Res. 4(21): 2202-2210.

[42] [40]. Derakhshandeh A, Khoshbakht R, Firouzi, R (2013) Association of three plasmid-encoded *spv* genes among different *Salmonella* serotypes isolated from different origins. Indian J Microbiol. 53(1):106–110.