Global Veterinaria 21 (1): 35-40, 2019
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2019.35.40

Risk Factors for *Listeria monocytogenes* Contamination in Nigerian Poultry Flock Environment

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Abstract: Poultry production is rising in Nigeria with attendant health concerns to the farmers and the surrounding environments. Microbial contamination from the poultry environments presents with health risks to the poultry. The aim of this study was to identify potential risk factors for *Listeria monocytogenes (L. monocytogenes)* contamination in Nigerian poultry production environment. A total of 71 flocks of poultry farms randomly selected under different settings were investigated for the presence of *L. monocytogenes* contamination. Two hundred and eighty-four swab samples were taken from feed (n=71), water (n=71), dust (n=71) and workers' boots (n=71) and cultured for *L. monocytogenes* isolation using Brilliance™ Selective Listeria Agar and microbial load count with Nutrient Agar. Further identification was done using microscopic and biochemical characterization. Bivariate and logistic regression models were used to assess the association between management practices and the risk of *L. monocytogenes* contamination of the flocks. The prevalence of *L. monocytogenes*-positive flocks was 100.0% with overall prevalence of 90.1% based on samples. The prevalence was highest in feed (95.8%) and least in dust swabs (84.5%). A significant association was observed between *L. monocytogenes* contamination and farm size (p=0.05), but not with feed type (p=0.34), management (p=0.54) and biosecurity type (p=0.74). We recommend stricter hygienic practices especially as farm size increases and when processing and handling poultry feed as feed contamination might be a critical point in the control of *L. monocytogenes* contamination of other materials and in poultry farms.

Key words: *Listeria monocytogenes* · Poultry Environment · Risk Factor · Poultry

INTRODUCTION

Listeria monocytogenes is an important foodborne bacterium that causes listeriosis, a rare but lethal infection in both animals and humans [1, 2]. It is widely distributed in the environment including soil, surface water, plants and infected animals. This ubiquitous nature allows for contamination of numerous food products such as milk and dairy products, raw vegetables, meat and meat products, poultry and poultry products and seafood, which have been reported to be involved in *L. monocytogenes* outbreaks [3]. Consumption of food contaminated by *L. monocytogenes* has been identified as the main transmission route for this pathogen in both humans and animals. Transmission in domestic animals can occur by ingestion of contaminated feed and poor quality silage with pH greater than 5.5, hence the name "silage disease" [4]. Outbreaks usually occur as septicaemia, meningoencephalitis (circling disease) and abortion.

In humans, listeriosis may result in abortion or serious cases of meningitis or encephalitis and even death. In humans, the disease affects primarily pregnant women, newborns, adults with immune-compromised systems and the elderly [5]. Because of the high fatality rate (20–30%), listeriosis ranks among the most frequent causes of death due to foodborne illnesses [6, 7]. In most studies, the contamination of chicken meat occurs during the slaughtering and processing phases [8-10]. *Listeria monocytogenes* contamination can also be due to contamination of raw materials, bacterial spread and ineffective cleaning procedures [11]. It can be found in biofilms on food processing surfaces and the surfaces of equipment and utensils used during food processing, from where food contamination can occur [12, 13].

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In Nigeria, bacterial infections especially due to *L. monocytogenes* constitute a major threat to sustainable animal production and public health. Recently, Ishola *et al*. [14] reported a 100% positive flock prevalence of *L. monocytogenes* among some poultry farms in south-western Nigeria. Considering the fact that controlling *Listeria monocytogenes* remains a formidable task [15], the need to investigate the roles of environmental samples in the transmission of *L. monocytogenes* in poultry farms in south-western Nigeria becomes imperative.

MATERIALS AND METHODS

Study Site: This cross-sectional study was conducted among poultry farms with previous reports of *L. monocytogenes* contaminations [14] in Oyo State, south-western. The farms had different management settings with average capacity of between 1000 and 5000 chickens each. In all, 71 farms were sampled. The purpose of the study including its potential benefits to the farms was explained to the farm owners who then gave their consents.

Sampling Procedure: From these farms, a total of 284 samples were collected comprising feed (n=71), water (n=71), swab samples of dust (n=71) and workers' boot (n=71). A volume of 1ml of peptone water was then dispensed into each of the swab containers to moisten the samples and prevent them from desiccation. Samples collected were properly labeled and packaged in ice packs for transportation to the laboratory. Data on each farm with respect to the type of management, feed type, level of biosecurity measures and farm size were documented. Samples were then taken to the Meat and Milk Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for laboratory processing.

Laboratory Procedure: The isolation of *Listeria monocytogenes* was carried out following earlier described methods by Gibbons *et al*. [16] and Indrawattana *et al*. [17], but with slight modifications. Briefly, peptone water was prepared by dissolving 15g of the powder in 1000mLs distilled water and autoclaving at 121°C for 15min. Nutrient Agar was prepared by dissolving 28g of the powder in 1000mLs of distilled water and autoclaving at 121°C for 15min. Listeria Selective Agar (LSA) (Brilliance™) was prepared by dissolving 33.6g of the powder base in 1000mLs of distilled water, autoclaving for 15min at 121°C, cooling to 40°C and adding LSA antibiotics supplements.

Pre-enrichment and Culture: One milliltre of each homogenized swab sample of dust and boot as well as 1 gram of feed and 1ml of water sample each was transferred into a test tube containing sterile and freshly prepared peptone water (9mL) and incubated at 37°C for 18 hours to revive viable but non-culturable cells. Then, 100µl (0.1mL) each of the peptone broth culture was spread plated on fresh Listeria Selective Agar (LSA) plates and incubated at 37°C for 36-48 hours. Following incubation, discrete bacterial colonies were then counted from the incubated LSA using the colony counter. Counts were transformed to colony forming unit (CFU) [18, 19]. *Listeria monocytogenes* colonies appeared as green colonies with opaque white halos. Discrete *L. monocytogenes* colonies from the LSA plates were then streaked onto freshly prepared LSA plates to obtain pure listeria isolates and the streaked plates were incubated at 37°C for 36-48 hours. Pure *Listeria monocytogenes* isolates were then subjected to various morphological and biochemical studies including Gram staining, Catalase test, Oxidase test and Sugar fermentation test [20].

Gram Staining and Morphological and Biochemical Tests: A smear was made on a clean glass slide by emulsifying a minute amount of a colony of the isolate in a drop of normal saline. The smear was then air-dried and heat-fixed. Crystal violet was added over the fixed culture as primary stain for 60 seconds and then rinsed. Lugol's iodine was then added as a mordant for another 60 seconds and rinsed. It was then rapidly decolourised with alcohol for 5-10 seconds and rinsed. Counterstaining was done with safranin for 30 seconds. It was then rinsed, drained, blotted dry and examined under oil-immersion [21, 22]. In addition, various morphological and biochemical tests including catalase, oxidase and sugar fermentation using Glucose, Mannitol, Sucrose, Maltose, Fructose and Lactose were conducted and phenolphtalein was used as indicator.

Assessment of the Microbial Load on Sample Screened: Serial dilution of each sample was done up to the 6-fold dilutions, using freshly-prepared peptone water. Volume of100ul (0.1mL) each of the 4^{th} and 6^{th} dilutions were then spread plated on Nutrient Agar plates and incubated at 37°C for 18-24 hours for counting. Following incubation,

discrete bacterial colonies were then counted from the incubated nutrient agar plates using the colony counter. Counts were transformed to Colony Forming Unit (CFU) and LogCFU.

Statistical Analysis: Data entry and analysis was done using SPSS version 15. Data obtained were subjected to descriptive statistics and bivariate; and then logistic regression model was carried out to measure association between prevalence of *L. monocytogenes* infection and variables obtained.

RESULTS

Out of the 284 samples from 71 farms screened in this study, an overall prevalence of *L. monocytogenes* contamination was 90.1% (256/284). Among the samples selected, contamination with *L.monocytogenes* was highest in feed (95.8%), followed by boot (93.0%), water (87.3%) and dust swabs (84.5%) (Table 1). Of the 71 farms sampled, all had at least one positive sample yielding a farm prevalence of 100.0%. Based on the variables examined, only farm size ($p = 0.05$), but not feed type ($p=0.34$), management ($p=0.54$) and biosecurity type ($p=0.74$) was significantly associated with *Listeria monocytogenes* contamination (Table 2).

The highest mean LogCfu/ml±SD of 8.46 ± 0.20 was observed in the boots while the lowest value of 8.33 ± 0.47 was in the dust samples. Feed had mean LogCfu/ml±SD of 8.45 ± 0.33 (Table 3). The mean bacteria counts obtained at 10^{-6} dilution were significantly higher ($p = 0.0001$) than those obtained at 10^{-4} dilution when compared across the different samples. The overall Mean ± SD in fourth and sixth-fold dilutions were found to be $7.10 \times 10^6 \pm 5.82 \times 10^6$ and $8.87 \times 10^6 \pm 7.58 \times 10^6$, respectively.

Table 1: Prevalence of *L. monocytogenes* in samples from poultry environments in south-western Nigeria

Sample	Total collected	No positive (%)	No negative (%)
Feed	71	68 (95.8)	3 (4.2)
Boot	71	66 (93.0)	5 (7.0)
Water	71	62 (87.3)	9 (12.7)
Dust	71	60 (84.5)	11 (15.5)
Total	284	256 (90.1)	28 (9.9)

Table 2: Risk factors associated with the prevalence of *L. monocytogenes* detected from poultry environmental samples in south-western Nigeria

Variable	Category	Total screened	No positive (%)	No negative (%)	X^2	P-value
Farm size	<1000	76	64 (84.2)	12 (15.8)		
	1000-5000	160	150 (93.8)	10 (6.2)	5.73	0.05
	>5000	48	42 (87.5)	6 (12.5)		
Feed type	Formulated	212	189 (89.2)	23 (10.8)	0.92	0.34
	Compounded	72	67 (93.1)	5 (6.9)		
Management	Deep litter	188	168 (89.4)	20 (10.6)	0.38	0.54
	Battery cage	96	88 (91.7)	8 (8.3)		
Biosecurity	High	208	186 (89.4)	22 (10.6)		
	Average	44	41 (93.2)	3 (6.8)	0.59	0.75
	Low	32	29 (90.6)	3 (9.4)		

Table 3:Total bacterial counts among the different samples taken

	First Dilution (10^{-4}) Log Cfu/ml			Second Dilution (10^{-6}) Log Cfu/ml			*paired t-test*		
Sample	Min	Max	Mean ± SD	Min	Max	Mean ± SD	T	d_f	*p-value*
Boot	6.15	7.11	6.69±0.20	7.95	8.85	8.46±0.20	96.73	70	0.0001
Dust	5.00	7.10	6.62±0.39	7.00	8.90	8.33±0.47	38.82	70	0.0001
Feed	5.95	7.18	6.70±0.22	7.60	8.87	8.45±0.23	87.97	70	0.0001
Water	6.18	6.99	6.63±0.20	7.78	8.87	8.40±0.26	96.69	70	0.0001

DISCUSSION

There is increasing evidence that contamination of food products with *L. monoctyogenes* is more likely to originate from environmental sources [23], particularly the food processing environment. The complexity of eliminating this organism from the environment is as a result of its unusual growth and survival properties [24, 25] as well as its ability to adhere to various food contact surfaces [26-28]. It suffices to say therefore that very critical attention must be focused on detection of critical control points which serve as risk factors for *L. monocytogenes* in order to reduce or eliminate the potential for cross-contamination of foods from the processing environment [29, 30].

We demonstrated the detection and isolation of *L. monocytogenes* in samples from poultry environment in south-western Nigeria. Our findings reveal high incidences of *L. monocytogenes* in feed (77.5%), dust (66.2%), boots (64.8%) and water samples (60.6%) in this study. While the contamination in feed might suggest that feed meal increases the risk of *L. monocytogenes* transmission in chicken flocks as previously indicated by Aury *et al*. [31], the contamination of water might be due to indiscriminate disposal of refuse materials and sewage effluents in water-ways which is in agreement with previous findings by Olasupo *et al*., & Akano *et al*., [32, 33]. This is similar to findings by Fox, *et al*., & Kuhn and Goebel [34, 35] and which stated that *Listeria* are mainly found in soil, silage and water.

Again, the high incidence of *Listeria monocytogenes* in dust samples could be as a result of cross-contamination from infected birds, personnel or equipment. Given the fact that *Listeria* is hardy and resists cleaning and disinfection, drying and other environmental conditions, it persists and therefore accumulates in the poultry environment which is similar to Adetunji and Adegoke, [36]. *Listeria monocytogenes* counts in boot swab was also very high. This suggests that most poultry attendants' boots are constantly being contaminated with faecal material, soil, silage and water that are on the floor of the poultry house.

Expectedly, *Listeria monocytogenes* contamination was higher in farms with poor biosecurity, soiled litter or generally poor sanitary conditions as well as in farms that shared personnel and equipment with other farms. This suggests likely cross-contamination between farms, an observation similar to Aury *et al*., & Hellstrom *et al*., [31, 37]. Although, the counts were lower compared to farms with poor biosecurity and hygiene, *Listeria monocytogenes* contamination was still found in farms which practised proper bio-security and good sanitary practices. The ability of the organism to form biofilms which are difficult to eliminate via cleaning and sanitizing might be the possible reason for this finding. This is in agreement with Chiarini *et al*., Adetunji and Adegoke & Adetunji and Ishola [15, 36, 38].

In conclusion, the findings of the present study indicate that poultry environmental samples are contaminated with *L. monocytogenes* adhering to contact surfaces in processing environments. The results of this study provide useful insights into identifying control measures for preventing colonization of *Listeria monocytogenes* in poultry meat and processing environments of poultry processing plants. We recommend stricter hygiene practices especially when processing and handling poultry feed as feed contamination might be a critical point in the prevention of contamination of other materials with *Listeria monocytogenes* in poultry farm. In addition, the use of disinfectants as foot dips at the entrance of each pen and in cleaning poultry house materials such as boots, feeding troughs and drinkers is very important to limit the contamination and cross-contamination of poultry environment with *Listeria monocytogenes*.

ACKNOWLEDGEMENTS

We thank the management of the University of Ibadan (UI), Ibadan Nigeria for the Senate Research Grant (SRG/FVM/2010/3A) made available to support this research. The assistance of Mr Ojomo, B. and Mr Okunlade, O. of the Department of Veterinary Public Health and Preventive Medicine, UI during the laboratory analysis is highly appreciated. We are also grateful to the poultry farmers and Poultry Association of Nigeria, Oyo State branch for their cooperation.

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