# Risk factors of *Salmonella* infection in laying hens in Menoua Division, Western region of Cameroon (Central Africa)

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ABSTRACT

*Salmonella* infections in poultry farms are overlooked in many African countries; yet these infections are mostly zoonotic with impact on both poultry industry and public health. Considering the impact of *Salmonella* in laying hens, and the role of laying hens as a source of *Salmonella* outbreak in human, knowledge of the status of *Salmonella* on laying hen farms as well as the factors influencing the presence of *Salmonella* is important. In a cross sectional study, cloacal swabs were collected from 270 commercial laying hens on 27 farms located in Menoua Division. These samples were cultured on standard media. A questionnaire was used to collect information on animals, farms and farmer's characteristics. The prevalence of *Salmonella* was 93.34%; three zoonotic isolates namely *S*. Enteritidis (75.90%), *S*. Paratyphi (11.90%), and *S*. Typhimurium (5.60%) were identified. The location of farms was significantly associated with presence of *Salmonella*, and the risk of infection was 10-fold higher in Nkong-ni than Santchou (p < 0.05). Other potential risk factors such as flock size, age of the farm (infrastructure), or water source were not associated with *Salmonella* infection. The prophylactic measures against avian diseases in the country must include measures against *Salmonella* to protect poultry industry and public health.

## 1. Introduction

The continual growth of human population worldwide is subject to a variety of challenges, one of which is the demand for healthy food including meat and eggs. According to the Food and Agricultural Organization of the United Nations (FAO), the protein requirement for an individual is estimated as 33 kg/habitant/year [1]. But in Cameroon, meat and meat products only provide 12.7 kg/hab/year, with poultry sector contributing only 4.1 kg/hab/year [1,2]. Such a low performance contrasts with reports from other areas of the world like Asia where poultry industry substantially contributes to meat supply [3]. A number of constraints are known to limit poultry productivity... In Cameroon specifically, the greatest challenge in poultry production is related to health issues, as ascertained by the great number of prophylactic measures against poultry diseases [4]. In spite of the prophylactic measures in place, disease outbreaks of unknown origin are frequently reported on the field by animal farmers. An example of this is the on-going epidemic of highly pathogenic avian influenza (HPAI) which has led to economic disaster due to either the high mortalities in affected farms or the systemic slaughter without compensation, of the entire flock in affected farms.

*Salmonella* infections are not included among the diseases the prophylaxis plan is against in Cameroon, though these infections are a potential cause of acute and chronic disease outbreaks on the farms. [5]. Salmonellosis is a significant zoonotic disease with a considerable economic impact on the poultry industry, including egg layer production. Though chicken are thought to be chronic carriers of *Salmonella* [6] in poultry industry, the economic losses from *Salmonella* are both direct (high morbidity and mortality) and indirect (slow growth rate, drop in egg laying, poor egg quality, and poor chick viability among others) [5,7,8]. In humans, *Salmonella* is one of the most prevalent foodborne pathogen, causing acute gastroenteritis/diarrhea, fever, vomiting, and sometimes even death [6,9]. Outbreaks of salmonellosis in humans have often been associated with the consumption of contaminated raw chicken meat, eggs, beef, pork, seafood, and vegetables [10] with chicken meat and eggs being the main sources of *Salmonella* [11–13].

Despite the adverse impact of *Salmonella* on poultry and egg production, and also on the consumer's health, there is no basic information regarding the epidemiology of *Salmonella* in poultry in the country. Therefore, the objective of this study was to investigate the prevalence of *Salmonella* and the risk factors associated with the infection in laying

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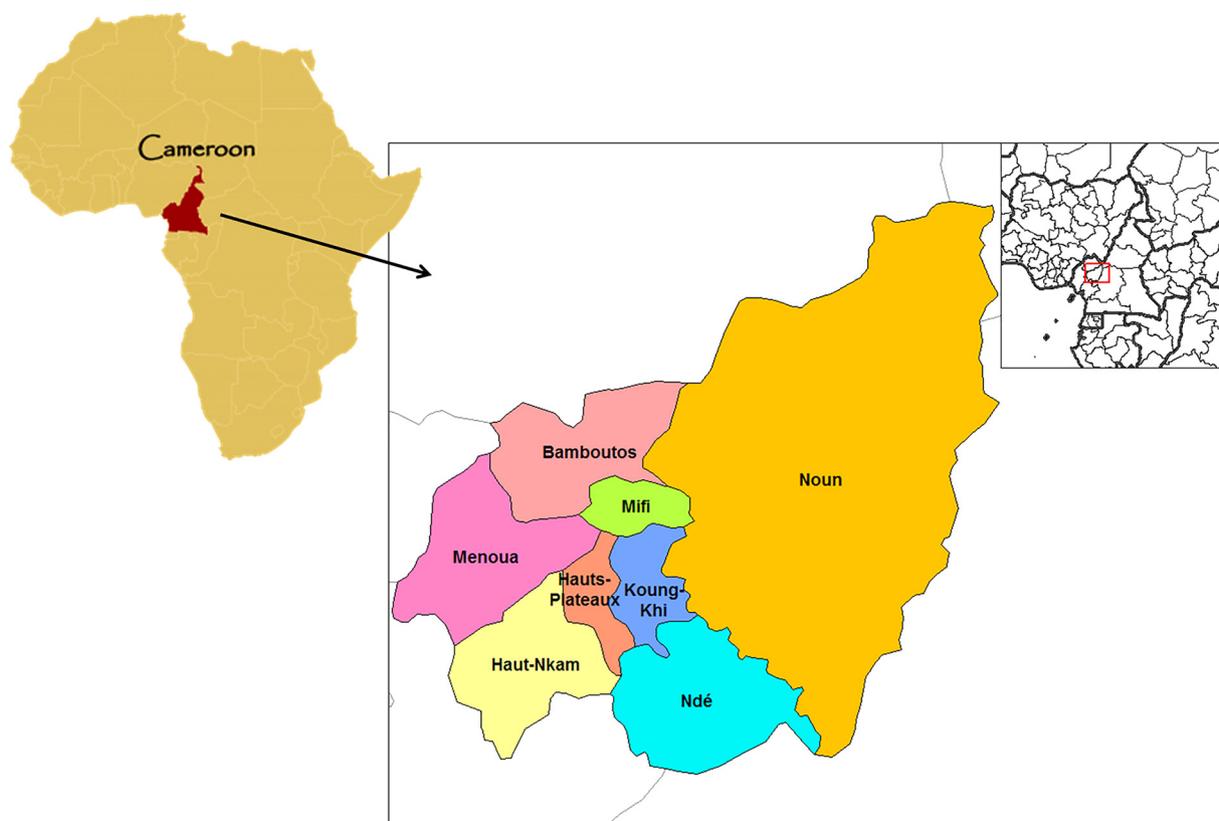  *E-mail address:* mkouam2003@yahoo.fr (M.K. Kouam).

**Fig. 1.** Map of the West region of Cameroon showing Menoua Division.

hens in Cameroon.

## 2. Material and methods

### 2.1. Study area

The study was carried out in Menoua Division of the West Region of Cameroon (Fig. 1) from May to July 2016. Animal husbandry in the Division consist of rearing small and large ruminants, cavies, pigs, rabbits, broilers and laying hens among others. The West region is the greatest poultry and egg production area in Cameroon, and even in Central Africa, with 53,992 tons of eggs out of 63,382 tons in total produced country-wide [4]. The housing system adopted by farmers is the simple deep-litter housing system, with the house floor covered with wood shavings upon which laying hens freely move.

### 2.2. Sample collection

Basic information on the location of farms was provided by the veterinary health service of the District. Then the snow-ball technique (the technique consists of identifying the next farm from the information obtained from a previously identified farm) was used to identify the farms to be included in the study. The technique was applied due to the absence of an official registry for poultry farmers. Once a farm was identified, the owner was contacted and only volunteer farmers participated in the study. Due to rumors of bird flu epizootic in the country during that period, many farmers were reluctant to take part in the study and did not welcome visitors on their farms.

On farm sampling consisted of 10 random cloacal swabs from 10 hens evenly selected throughout the house. From each hen, a cloacal swab was taken by inserting a cotton-tipped swab into the cloaca, taking care to avoid contact with the surrounding feathers and the skin. Hens were all in the laying stage of their life at the time of sampling. Then swabs were placed into a sterile plastic bag which in turn was

placed into a leak proof cool box and transported to the laboratory under ambient conditions. The samples were incubated for bacteriological analysis on the day of sampling.

### 2.3. Bacteriological analysis

The samples were analyzed using the classical method as previously described [14]. Each cloacal swab was placed into 9 mL of buffered peptone water and homogenized. Afterwards, the sample was incubated at $37 \pm 1$ °C for 24 h. Next, 1 mL of the pre-enrichment culture was inoculated into selenite cystine broth and incubated at $37° \pm 1$ °C for 24 h, before plating out onto a selective agar. A drop of the selective enrichment culture was plated onto *Salmonella-Shigella* (SS) agar. Plates were incubated in an inverted position for 24 h at $37 \pm 1$ °C, and *Salmonella*-positive plates, based on presence of typical colonies, were recorded. *Salmonella*-like colonies were biochemically confirmed using three media: Hajna Kliger, indole urea and Simmons citrate broth. The colonies were inoculated into sterile tubes containing 3 mL of these media, and incubated over night at $37 \pm 1$ °C for 24 h. The motility test was further performed. Indeed, the isolates were microscopically characterized based on the difference in their motility. *S.* Typhimurium is the fastest, followed by *S.* Enteritidis. *S.* Paratyphi is the slowest of the three. Bacteria were definitely identified using the identifying chart.

### 2.4. Questionnaire design

The questionnaire was filled in following an on-farm interview at the time of sampling. Questions related to farmer, farm and flock characteristics. Farmer's characteristics consisted of age, sex, and training in animal husbandry. Farm characteristics were geographic location, farm age, and water source. Flock data investigated was the flock size. The questionnaire was pre-tested and adjusted accordingly.

## 2.5. Statistical analysis

Descriptive statistics were used to summarize the data. The relationship between the risk factors and *Salmonella* infection was evaluated by the multivariate logistic regression. An animal was classified as infected if the sample was positive for any *Salmonella* isolate. A positive test was coded as 1 and the negative test as 0. Firstly, all potential risk factors were tested using the univariate analysis. Secondly, variables with p-value < 0.3 were selected and included in the final multivariate logistic regression. The significance level was set at p < 0.05. All statistics were performed using SPSS statistical package (version 13.0, SPSS Inc., USA).

## 3. Results

A total of 27 commercial laying farms corresponding to 27 flocks were sampled in the Division. The mean flock size was 2337.04 ± 1420.809 individuals, ranging from 500 to 7000 hens. The age of the infrastructure varied between 1 to 40 years, with the mean age being 11.352 ± 10.25. Of the 27 flocks, 270 hens were sampled; all the 27 flocks were positive for *Salmonella*, and the prevalence in hens was 93.34% (252 out of 270). Three zoonotic isolates were identified in all the flocks and in all the localities of the study area (Table 1). The predominant isolate was *Salmonella* Enteritidis (75.90), followed by *S*. Paratyphi (11.90%). *S*. Typhimurium was the isolate with the lowest prevalence (5.60%).

All the three isolates were taken into account in risk factor analysis. Thus, the factor found to be associated with *Salmonella* was farm location within the Division (Table 2). The risk of *Salmonella* presence in laying hen flocks was found to be 10-fold higher in Nkong-ni than Santchou (p < 0.05). Other factors such as flock size, age of the farm (infrastructure), or water source were not associated with *Salmonella* infection in the area.

## 4. Discussion

This study reports for the first time the occurrence of *Salmonella* in laying hen farms in Cameroon, and particularly the zoonotic isolates *S*. Enteritidis, *S*. Paratyphi and *S*. Typhimurium. A previous study in Cameroon [15] indicated the occurrence of *Salmonella* in wild birds (*Ploceuscucullatus* sp).

The prevalence of *Salmonella* infection was high in this study compared with the results obtained in similar studies in Algeria [16,17]. The high prevalence reported may be linked to poor biosecurity measures on farms; indeed rodents and insects reported as the most important contamination source of *Salmonella* on farms [18], were found in all the farms visited. In addition, the vertical mode of *Salmonella* transmission from infected breeder hens to progeny through eggs, might have contributed to the high prevalence recorded. Considering the fact that estimates obtained are an indication of the number of birds shedding *Salmonella* rather than the accurate indication of the number of birds infected with *Salmonella*, the real prevalence might be higher than the estimate obtained in this study.

Three zoonotic isolates were identified in this study. The finding that *S*. Enteritidis was the most prevalent isolate agrees with the results of Carli et al. [19] and Aysegul et al. [16] in Turkey as well as Suresh et al. [20] in India, showing that *S*. Enteritidis was the most recorded isolate among laying hens. Conversely, other isolates such as Hadar, Heidelberg or Bareilly were reported to be the most frequent serovars on other farms [21,22]. The difference in the distribution of isolates could be explained by differences in local parameters such as climate and types of animals being reared. Both *S*. Enteritidis and *S*. Typhimurium have been demonstrated to have the ability to colonize the reproductive tract of hens [23]. This ability which makes possible the internal contamination of the offspring justifies the impact of these isolates in public health, and poultry and egg industry. Therefore, the occurrence of these isolates on local farms is a threat to poultry industry, since they cause morbidity and mortality [24] which result in reduced productivity and economic losses. Also, hens infected with *Salmonella* have been shown to have a decreased egg production which did not improve within 2 weeks post-infection [23]. Considering the damage induced by *Salmonella* on poultry and egg industry, it is unfortunate to point out the fact that local prophylactic measures on farms do not include measures against *Salmonella* infection but exclusively focus on other infectious diseases (Marek disease, Gumboro disease, New-castle disease, and coccidiosis among others) for which the clinical signs are more alarming. These findings suggest that salmonellosis control measures must be implemented on farms. A diverse assortment of *Salmonella* control measures implemented in numerous locations around the world could be adapted in Cameroon.. *S*. Paratyphi known to be adapted to human hosts is scarcely reported in birds. The finding of this serotype in laying hens, with a higher prevalence than in *S*. Typhimurium is an indication that hens should henceforth be considered as an important reservoir for this *Salmonella*. It is also possible that the appearance of the serovar Paratyphi in chickens indicates transmission into poultry flocks from human sources (workers, fecal-contaminated waste, and fecal-contaminated water among others). Also, as the serotypes found in this study have been isolated in many instances of food intoxication in man [9,25], poultry farm workers as well as the end consumer of poultry meat, egg or egg products from the sudy area are all at risk of contamination with *Salmonella*. These results suggest that *Salmonella* might be incriminated as one of the main causes of human and animal gastroenteritis documented in the country [26,27]. With the absence of poultry abattoir in the country added to the fact that eggs are consumed uncooked in salad dishes by some people, the risk of *Salmonella* transmission is exacerbated. Indeed, slaughter in areas other than a dedicated poultry slaughterhouse does not guaranty the hygiene of meat since *Salmonella* contamination of meat is likely to occur in unhygienic slaughter conditions, when a slaughter site is not protected from human and animal intrusions, and from fecal contamination. Furthermore, *S*. Typhimurium seems to be widespread and widely distributed in livestock in Cameroon, as it has been reported in cattle and pigs in Buea in the Southwest region of Cameroon [27]. *S*. paratyphi and *S*. Typhimurium have been isolated in humans still in Buea [28] confirming the suspicion that *Salmonella* is involved in gasteroenteritis both in humans and animals in the country. Given that isolates can be further subdivided by a number of methods including phage typing, genotyping, and antibiotic resistance profiles, it is necessary to investigate a possible difference between human and chicken serovars using the aforementioned methods. The transportation means was not ideal in this study, which could result in poor recovery and even loss of some serovars. Though Selenite F, a suitable transport medium for studies looking for *Samonella* in poultry was not used, the negative impact on the recovering rate of *Salmonella* was minimized by transporting the samples in a cool box. Indeed, poultry specific salmonellae (*S*. Gallinarum and *S*. Pullorum) are readily overgrown by competitor organisms in non-selective broths but will grow if seleinte F broth, which is inhibitory for many bacteria, is used.

**Table 1**
Distribution of *Salmonella* isolates per locality in Menoua division.

| Locality | | *Salmonella* isolates | | | |
|---|---|---|---|---|---|
| | N | Enteritidis n (%) | Paratyphi n (%) | Typhimurium n (%) | Total |
| Dshang | 90 | 70 (77.80) | 9 (10.60) | 3 (3.30) | 82 (91.11) |
| Nkong-ni | 160 | 123 (76.90) | 22 (13.80) | 10 (6.30) | 155 (96.88) |
| Santchou | 20 | 12 (60.00) | 1 (5.00) | 2 (10.00) | 15(75.00) |
| Total | 270 | 205(75.90) | 32 (11,90) | 15(5.60) | 252 (93.34) |

N = number of samples examined; n = number positive; (%) = prevalence in percentage.

**Table 2**

Logistic regression analysis of risk factors for *Salmonella* infection in laying hen farms in Menoua Division. Results are expressed in terms of odd ratio (OR) and 95% confidence interval (CI).

| Factor | N | n | (%) | Univariate analysis | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|---|
| | | | | OR | p-value | OR | 95% CI | p-value |
| Continuous variables | | | | | | | | |
| Farmer age | 270 | 252 | 93.30 | 1.04 | 0.14 | 1.05 | 0.98-1.12 | 0.14 |
| Farm age | 270 | 252 | 93.30 | 1.02 | 0.44 | / | / | / |
| Flock size | 270 | 252 | 93.30 | 1.00 | 0.35 | / | / | / |
| Categorical variables | | | | | | / | / | / |
| Location of farms | | | | | 0.00 | | | 0.00* |
| Nkong-ni | 160 | 155 | 96.90 | 10.34 | 0.00 | 10.01 | 2.54-39.44 | 0.00* |
| Dschang | 90 | 82 | 91.10 | 3.42 | 0.05 | 2.35 | 0.64-8.69 | 0.20 |
| Santchou | 20 | 15 | 75.00 | 1.00 | | 1.00 | – | |
| Farmer gender | | | | | 0.67 | | | |
| Male | 206 | 220 | 93.60 | 1.28 | | / | / | / |
| Female | 50 | 46 | 92.00 | 1.00 | | / | / | / |
| Training in animal breeding | | | | | 0.25 | | | 0.28 |
| Yes | 60 | 58 | 96.70 | 2.39 | | 2.39 | 0.47-12.01 | |
| No | 210 | 194 | 92.40 | 1.00 | | 1.00 | | |
| Water source | | | | | 0.84 | | | |
| Tap | 30 | 28 | 93.30 | 1.25 | 0.78 | / | / | / |
| Well | 100 | 95 | 95.00 | 0.78 | 0.36 | / | / | / |
| Spring | 30 | 28 | 93.30 | 0.36 | 0.78 | / | / | / |
| Drill | 110 | 101 | 91.80 | 1.00 | | / | / | / |

N = number of samples examined; n = number positive; (%) = prevalence in percentage.

  * = Significant p-value.

The location of farms was found to be significantly associated with *Salmonella* infection on farms, with the risk of transmission 10-fold higher in Nkong-ni compared with Santchou. This finding agrees with the result of another study in Korea where the prevalence of salmonellosis was shown to be significantly different among provinces [22]. This is probably related to the discrepancy in the veterinary care of farms among localities due to the fact that in some areas access to farms is very difficult. Another explanation is that most farmers are reluctant to seek for veterinary advice for various reasons. Indeed, the roads are bad in Nkong-ni, resulting in the enclosure and abandon of farms by the local veterinary health officials who dwell in towns rather than in the village. Also many farmers preferred to hide themselves from the veterinary officials for fear of taxation. As these farmers work in secret, they don't have access to advice and training workshops on good practices on the farm. In general, biosecurity on these farms is poor [29] though the level of biosecurity implementation is better for farmers located in towns (Dschang and Santchou) than for farms located in the village (Nkong-ni). Unexpectedly, the age of the farm was not identified as a risk factor since older infrastructures with time become difficult to clean adequately due to the wearing out of the building materials, taking into consideration the fact that the survival of *Salmonella* in empty poultry houses could extend a period of one year [30]. However in the current context of poor application of bio-security measures, even a newly built infrastructure can be infected since chicks themselves stand as reservoir of *Salmonella* acquired vertically from the infected breeder hens. A day-old chick is well likely to be the main source of contamination in new farms because there is no guaranty that breeder farms are *Salmonella*-free. Besides, breeder farm holders are not compelled or bound by any regulation to supply only healthy chicks on the market. A variety of risk factors have been linked to elevated environmental *Salmonella* prevalence, including larger flock size, greater flock age, and housing in older facilities elsewhere [31–33]. Though the environmental samples were not investigated in this study, potential risk factors such as flock size and age, and age of farm were investigated for any association with the cloacal *Salmonella* prevalence. The fact that none of these last factors was significant could be explained by the fact that *Salmonella* can be transmitted both vertically and horizontally. For instance, bird age and flock size would not be significant if transmission is mainly done vertically, from hens to chicks through the eggs.

The highly host adapted and invasive poultry isolates namely *S*. Gallinarium, and *S*. Pullorum, responsible for fowl typhoid and pullorum disease, were not identified in this study. However, this does not preclude their occurrence on poultry in Cameroon. Indeed clinical cases of pullorum disease have several times been recorded in broilers aged 20 to 25 days at the teaching and research farm of the University of Dschang (Kouam, unpublished data). The absence of these isolates in this study might be explained firstly by the fact that they mostly occur in chicks, since the animals sampled in this work were adults. Secondly, it is difficult for chicks chronically infected with *S*. Gallinarum and *S*. Pullorum to reach a mature age in commercial laying flocks, as the chicks with clinical signs of diseases are systemically removed from the flock to prevent disease spread and reduced productivity on the farm. Thirdly, *S*. Galinarum and *S*. Pullorum are overgrown by other bacteria in non- selective pre –enrichment broths such as buffered peptone water which was used in this study.

In conclusion, *Salmonella* occur with a high prevalence on laying hen farms, posing a threat to a sustainable poultry industry in the country. With the zoonotic isolates identified, it seems important to develop control strategies to address both animal and public health risk of infection. Localities with higher transmission risk need to be monitored to reduce the risk of transmission. Since the West Region of the country is the greatest poultry and egg production zone of the country, further studies need to be carried out to draw the epidemiological map of *Salmonella* infection in the Region. Typing of *Salmonella* isolates needs to be done in order to provide information on strain diversity and improve the epidemiological analysis of outbreaks.

**Ethical considerations**

This research did not involve experiments on animals proper. Cloacal swabs collected on animals were done humanely in accordance with all applicable international guidelines for the care of animals. Farmers accepted to participate in the study by granting oral informed consent.

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agencies in the public, commercial, or not-for-profit sectors.

## Declaration of Competing Interest

The authors declare that there are no competing interests regarding the publication of this paper

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