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POTENTIAL RISK OF LITTER USED IN POULTRY FARMING IN THE DISTRICT OF ABIDJAN ON THE HEALTH OF CHICKENS

Patrick-Roch Kevin N'DEPO[1], *Eric Essoh AKPA[1], Lamine SAMAGACI[1], Christelle Suzanne DJOMAN[1], Bernadette Gblossi GOUALIE[1]
[1]*Laboratory of Biotechnology, Agriculture and Biologic resources valorization, Faculty of Biosciences, Felix Houphouet-Boigny University, Abidjan, Cote d'Ivoire,* akpae@yahoo.fr
*Corresponding author
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Abstract: *Litter is an important constituent of poultry production, which can influence animal welfare, flock health, food safety, environmental impacts and production efficiency. However, the presence of the pathogenic microorganisms in the litter constitutes a potential health risk for chickens. The purpose of this study is to highlight pathogenic strains from litter of poultry farming in Abidjan. This study was conducted in five municipalities of Abidjan District, Côte d'Ivoire. A total of 360 poultry litter samples was collected and transported to laboratory for physicochemical and microbiological analysis. Molecular identification of avian pathogenic Escherichia coli (APEC)was carried out by the detection of two virulence genes,iss and iucD. Results showed litter pH and water content ranging from 8.12 to 8.65 and 14.93 to 39.16% respectively. Analysis of these samples showed presence of Escherichia coli and Salmonella at 100%, and Aspergillus at 44.44%, 16.60% and 22.20% in Anyama, Bingerville and Yopougon respectively. In Songon and Port-Bouët areas, Escherichia coli was found at 100% and 95.24%, Salmonella at 71.43% and 95.24% and Aspergillus at 15.87% and 4.76% respectively. Of 48 isolates tested for APEC, 8 were positive for iss gene (16.67%) and 5 positives for iucD gene (10.42%). Of the 13 isolates tested positive, 3 (6.25%) were found to be positive for both genes. Therefore, litter would be responsible for the proliferation of potentially pathogenic germs that cause avian diseases. Litter waste could pose a pathogenic risk for the environment and animal health.*

Keywords: *litter, poultry farming, pathogens, diseases and Abidjan*

1. Introduction

Poultry farming is a very important sector for food security in Côte d'Ivoire. Indeed, the sector generates 250,000 direct and indirect jobs and generates 350 billion CFA francs income [1]. Despite the importance of poultry farming, the sector faced with several problems including quality of the litter [2].
Litter or bedding can be obtained from organic materials like wood (shavings, sawdust, pellets or chips), plants (rice husk/ hulls, sugarcane bagasse, wheat straw, soybean residue, corn cobs and silage) or from inorganic materials like sand and clay [3,4,5,6]. Every bedding type has its own advantages and disadvantages regarding, availability, cost, absorbency, bulk density, comfort to birds, nutrient value, insulation, reusability, chemical and microbiological hazards to human, animal and environmental health [7,8,9]. Litter material is an important constituent of poultry production, which can influence animal welfare, flock health, food safety, environmental impacts and production efficiency [10]. In general, bedding is chosen according to its absorption capacity, comfort, cost and availability in different regions of the world [11,12,13].
During chicken breeding, at the start of the batch, the litter has an ideal composition. It is dry, aerated and balanced, but its quality can be degraded by many factors such as temperature, ventilation, animal density,

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chicken feed, water from drinkers and animal excrement. All these factors contribute to the moistening or deterioration of the litter. Damp litter favors the proliferation of pathogenic germs, in particular virus, parasite, fungi and bacteria [14,15].The presence of these pathogenic microorganisms in the litter constitutes a potential health risk for chickens. In fact, after their use in poultry farming, the litter are directly dumped into the environment and used as fertilizer without prior treatment [2].

To assess the health risk of litter, its contamination by potential pathogenic strains must be known in order to implement effective control methods to help prevent their proliferation. Such information doesn't exist in Côte d'Ivoire, especially in the District of Abidjan, which concentrates the maximum number of poultry farms [16]. Therefore, the purpose of this study is to highlight pathogenic strains of poultry litter from Abidjan to alert on the health risk of chicken and environment.

2. Material and methods

2.1. Study area and sampling

Litter samples were collected in five municipalities (Yopougon, Songon, Bingerville, Port-Bouët and Anyama) in the District of Abidjan. These cities concentrate area of high poultry production in Côte d'Ivoire [16]. In 2021, this country had a population of 29,389,150 inhabitants, consisting of 14,044,160 women (47.8%) and 15,344,990 men (52.2%), with an annual intercensal growth rate of 2.9% observed between 1998 and 2021. Abidjan, the economic capital of Côte d'Ivoire is a cosmopolitan city in sub-Saharan Africa and the second most-populated city in West Africa with a total of 5,616,633 inhabitants. The Autonomous District of Abidjan, has the highest concentration of inhabitants, with 2,994 inhabitants per square kilometer [17]. Indeed, a visit to the different farms in each locality by municipalities allowed us to select the farms with around 1000 chickens [18,19]. All visited poultry farms used only wood shavings from sawmills in Abidjan as bedding **(Figure 1)**. No other type of bedding was used in the visited farms. Wood shavings are pieces of wood made by cutting or planning wood; they come from hardwood or softwood. They are very easy to clean and clump together easily when wet, and also help to retain moisture. They are easily accessible and effective in keeping chickens clean and comfortable. A total of 360 litter samples were collected with a distribution of 72 samples of 200 g each per farm visited according to WHO [20] report:

$$N = Z^2 P\,(1-P)/e^2 \qquad (1)$$

(N: sample size, Z: constant resulting from the normal distribution, P: expected prevalence of *Salmonella*, *Escherichia coli* and *Aspergillus* strains in poultry litter during this study, e: represents the chosen margin of error (5%), (Z = 1.96, P = 0.5)).

Fig. 1. Photography of the wood shaving used in the poultry farms of Abidjan

Moreover, the 200 g of litter sample were collected at different areas (feeders, drinkers) in the henhouses, using sterile spoons and stomachers. Collected samples were placed immediately in a cooler and transported to the laboratory for analysis.

2.2. Physicochemical and microbiological analysis of litter

For physicochemical characterization, water content is defined as the quantity of water in a sample that can be evaluated after removal under given experimental conditions. The method used is that proposed by AOAC,

based on the principle of dehydrating samples by oven drying until a constant weight is obtained [21]. A mass of 5 g (P0) of each litter sample is put evenly in a porcelain box, the ensemble is weighted (P1) and placed in an oven at 105°C for 24 hours. After cooling inadesiccator, the mass of the ensemble (P2)is taken a second time. Moisture content (H)is determined by using the following formula:

$$H = \frac{P1-P2}{P_0} \times 100 \qquad (2)$$

Dry matter (DM) content, is deduced from the moisture content using the following relationship:

$$DM=100-H \qquad (3)$$

pH is measured using a pH meter, which gives the electromotive force of a solution by dipping its electrode into the solution. According to AFNOR method used in this work, five (5) grams litter sample are mixed in 25 mL of distilled water. By soaking the glass electrode of ROUCAIRE brand pH meter (previously calibrated) in 5 mL of the supernatant under stirring, the pH is automatically read on the display. [22].

For microbiological analysis, twenty-five (25) grams of litter sample were added to 225 mL BPW (Buffered Peptone Water), and the mixture was incubated at 37°C for 24 hours in the EHRET BK 4029 incubator (Burladingen, Germany). The resulting stock solution was used to isolate *Salmonella* sp, *Escherichia coli* and *Aspergillus* sp.

Using the ISO method to isolate *Salmonella* sp.,0.1 mL stock solution was addedto10 mL of Rappaport-Vassiliadis broth (Biokar Diagnostics, Beauvais, France) and incubated at 42°C during 24 h. Then, inoculation was carried out using the streak-depletion method with a Pasteur pipette on Hektoen agar into Petri dishes. The culture was incubated at 37°C for 24 h and characteristic colorless *Salmonella* colonies with or without black centers are transferred to Hektoen agar for biochemical identification; notably Gram staining, oxidase test and Le Minor's reduced carriage [23, 24].

ISO method was also performed for *Escherichia coli* (*E. coli*) isolation. A volume of 100 µL stock solution was spread on a solid surface of TBX (Tryptone Bile X-glucoronide) agar (Conda, Madrid, Spain) and incubated at 37°C during 24 h. After incubation, a typical colony of *E. coli* (blue colony on TBX agar) was selected and identified using some biochemical tests which included Gram staining, tests for oxidase, methyl red, Voges Proskauer reactions, indole, citrate, catalase, urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation [25].

The method used for isolation and biochemical identification of *Aspergillus* is based on ISO standard and the agar used was OGA (Oxytetracycline-Glucose-Yeast Extract Agar). This medium, previously poured into sterile Petri dishes, is streaked with the stock solution followed by incubation at 30°C in MELAG oven (Berlin, Germany) for 3 days [26]. After macroscopic observation, various colonies characteristic of *Aspergillus*(gray colonies with a white velvety border, black colonies with a white velvety border, green velvety colonies, powdery green-gray colonies with a yellow border and center)were selected for microscopic observation. Microscopic study of the mycelium was based on absence or presence of septa, color of mycelial filaments, branching mode of septa, thallospore differentiation[27].

2.3. Molecular characterization of APEC strain

Molecular identification of avian pathogenic *Escherichia coli*(APEC) in *E. coli* isolates was performed by detection of two virulence genes including *iss*and *iuc*D (**Table 1**) [28]. Polymerase chain reaction (PCR) was performed in a final volume of 45 µL (0.6 µL of each dNTP (10 mM), 3 µL of $MgCl_2$ (25

mM), 10 µL of Buffer 5X DNA Taq polymerase, 0.2 µL of Taq polymerase (Promega, WI USA), 1.4 µL of each primer (100 µM) and 28,4 µL of water) using a thermal cycler (Gene Amp PCR system type 9700, Applied Biosystems, Villebon-sur-yvette, France) and the following program: an initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and polymerization at 72°C for 1min. A final extension was performed at 72°C for 7 min. For visualization of PCR products, 15 µL samples of the reaction mixtures were analyzed by gel electrophoresis in a 1,5% agarose, dissolved in 1 X TBE (8.9 M Tris, 8.9 M boric acid, 0.2 M EDTA), for 90 min at 90 V. The gel was stained with safe SYBR green and photographed under UV exposure.

Table 1.
Increased serum survival (*iss*) and involved in aerobactin synthesis *(iuc*D) primer sequences used in PCR

Gene	Primer sequence	Amplicon size (bp)
iss	F 5'-3' ATCACATAGGATTCTGCCG 5'-3' R 5'-3' CAGCGGAGTATAGATGCCA 5'-3'	309
*iuc*D	F 5'-3' ACAAAAAGTTCTATCGCTTCC 5'-3' R 5'-3' CCTGATCCAGATGATGCTC 5'-3'	714

iss: increased serum survival; iucD: involved in aerobactin synthesis

3. Results and discussion

3.1. pH and water content of poultry litter

All litter samples from the main chicken production areas in Abidjan gave mean pH values ranging from 8.12±0.230 to 8.65±0. 100.Their water content ranged from 14.93±0.079 to 39.16±0.277% (**Table 2**). The results show that wood shavings in breeding are affected by various factors, in particular the presence of water, animal density and animal droppings in the breeding building. These factors modify the composition of the bedding and can make it very unpleasant for the animals. Such pH and water content values are generally found by several authors who have studied litters from poultry farms [29,30]. However, pH and water contents obtained in our study are largely lower than those obtained in other studies where pH ranged between 9.17 and 9.32, and water content between 55 and 60% [31]. Indeed, the ideal moisture maximum content of litter is between 25% and 30%. If moisture content rises to 40%, for example, the litter becomes damp and clumps together; this is favorable to development of certain pathogenic germs [32]. Regarding pH values obtained in our study, all analyzed litter samples are favorable to the proliferation of pathogenic germs. Indeed, litter pH found between 7.8 and 8.8, constitutes good environment to the development of pathogenic germs [33].

Table 2.
pH and water content of poultry litter samples from different areas of Abidjan

Origin of Samples	pH	Water content (%)
Songon	8.25±0.050	14.93±0.079
Port-Bouët	8.3±0.278	27.5±0.500
Anyama	8.12±0.230	39.16±0.277
Bingerville	8.16±0.034	35.1±0.000
Yopougon	8.65±0.100	34.8±0.529

3.2. Potential pathogenic microorganisms found in poultry litter from different areas of Abidjan

A total of 360 litter samples from 5 zones of the District of Abidjan were analyzed. Results showed prevalence rate of 95.24 to 100% of *Salmonella* sp and *Escherichia coli* for all areas studied except Songon which had a prevalence rate of 71.43% for *Salmonella sp.* According to *Aspergillus* sp prevalence, they were above 20% for Ayanma and Yopougon while below 20% for the 3 other municipalities with the lowest prevalence obtained for samples from Port-Bouët **(Figure 2)**. These potential pathogen germs (*Salmonella* sp*, Escherichia coli* and *Aspergillus* sp) isolated in different parts of Abidjan were also isolated from most of the litter from various origin [34,35]. High prevalence rate of *Salmonella* sp and *Escherichia coli* in litter from Abidjan constitutes a real risk for health safety of chickens and environment. Indeed, after use on farms, the litter containing these potential pathogen germs is directly dumped into the environment without prior treatment or is used as fertilizers for growing market gardens in Abidjan [2]. Furthermore, the threat to human and chickens' health is real because of the presence of these potential pathogen germs**.** *Salmonella* bacteria are one of the most frequently isolated food-borne pathogens associated with human and chickens' diseases. It is responsible for infectious diarrhea among humans and comes from several origins in Côte d'Ivoire [36,37,38]. *Salmonella* bacteria are commonly found in the gastrointestinal tract of chickens and on finished retail poultry products [39,40]. As for *Escherichia coli*, it is also present in the intestinal tract of birds and mammals and is widely spread in the environment via feces [41]. It is responsible for diverse infections in poultry farms [42,43]. In addition, molds of the genus *Aspergillus* sp are responsible for aspergillosis in poultry farms. These organisms are common soil saprophytes that thrive on organic matter in a warm (>25°C) and humid environment [44,45]. Studies carried out in countries such as Cameroon, Nigeria and Egypt have shown presence of these germs on chicken farms [46,47,48]. Similar studies carried out on chicken farms in Senegal, Morocco and Chadhave shown the presence of *Salmonella* and *Escherichia coli* [49,50,51]; other works in China on samples from sick chickens also revealed the presence of *Escherichia coli* [52]. Taking into account these microbiological analyses in our study, litter, which is the environment that collects all kinds of waste from the birds in a farm, is a source of contamination by potentially pathogenic microorganisms. This has been shown by previous work in India, Serbia, France and the USA [53,54,55].

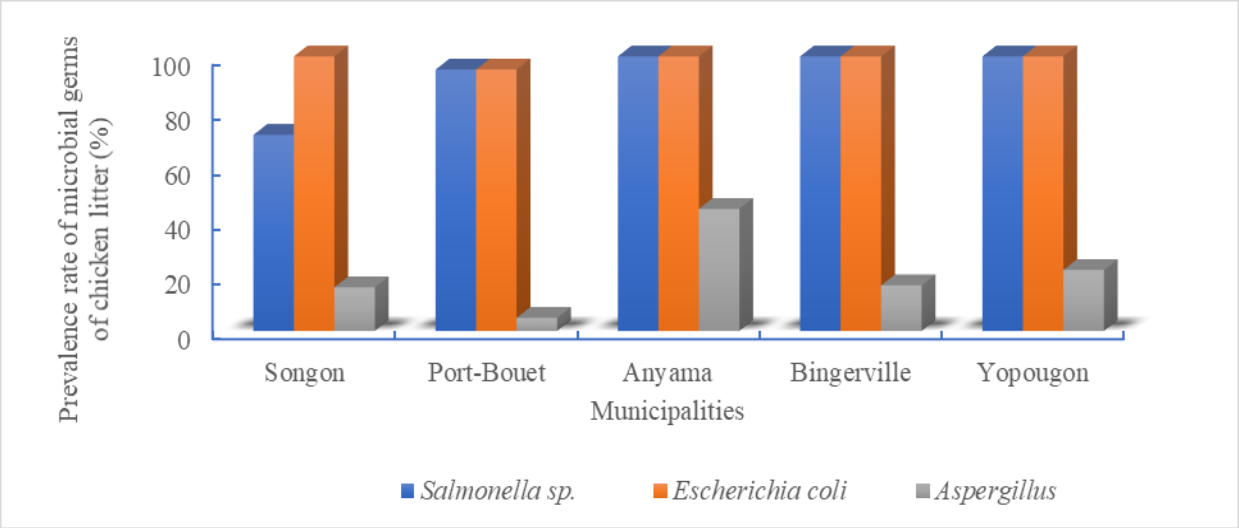

Fig.2. Prevalence rate of potential pathogen germs isolated from some areas of Abidjan

3.3. Prevalence of APEC-specific virulence genes in *Escherichia coli*

The identification of *Escherichia coli* (APEC) is based on the detection of specific markers involved in their pathogenicity, notably *iss* gene (309 bp) and *iuc*D gene (714 bp) **(Figure 3)**. Out of the 48 isolates tested, 8 were positive for the *iss* gene (16.67%) and 5 positive for *iuc*D (10.42%). Of the 13 isolates tested positive, 3 (6.25%) were found to be positive for both genes **(Table 3)**. Prevalence of APEC in our study is low compared to that obtained by other researchers who worked on poultry feed in Abidjan [56]. This would predict the hypothesis according to which the contamination of the litter by APEC would come from the poultry feed on one hand, and through their feces on the other hand.In countries such as India, Serbia and the USA, APECs have been identified by the presence of a single type of *iss* virulence gene (increased serum survival) carried by these strains in matrices like litter, healthy chicken feces and chicken farms [57,58,59]. Other studies carried out in Germany and Bangladesh have shown presence of APEC by both *iss* and *iuc*D (involved in aerobactin synthesis) genes isolated from chickens [28,60]. However, the prevalence rates obtained by these researchers were higher than those found in our study for poultry litter. Although this lower prevalence rate in our study, presence of APEC in poultry litter in Abidjan represents potential health risk for both animals and human.

Table 3.

Prevalence of virulence genes in *Escherichia coli* isolates

Virulence genes	Number of isolatestested	Number of positive isolates	Prevalence (%)
iss	48	8	16.67
***iuc*D**	48	5	10.42
***iss* and *iuc*D**	48	3	6.25

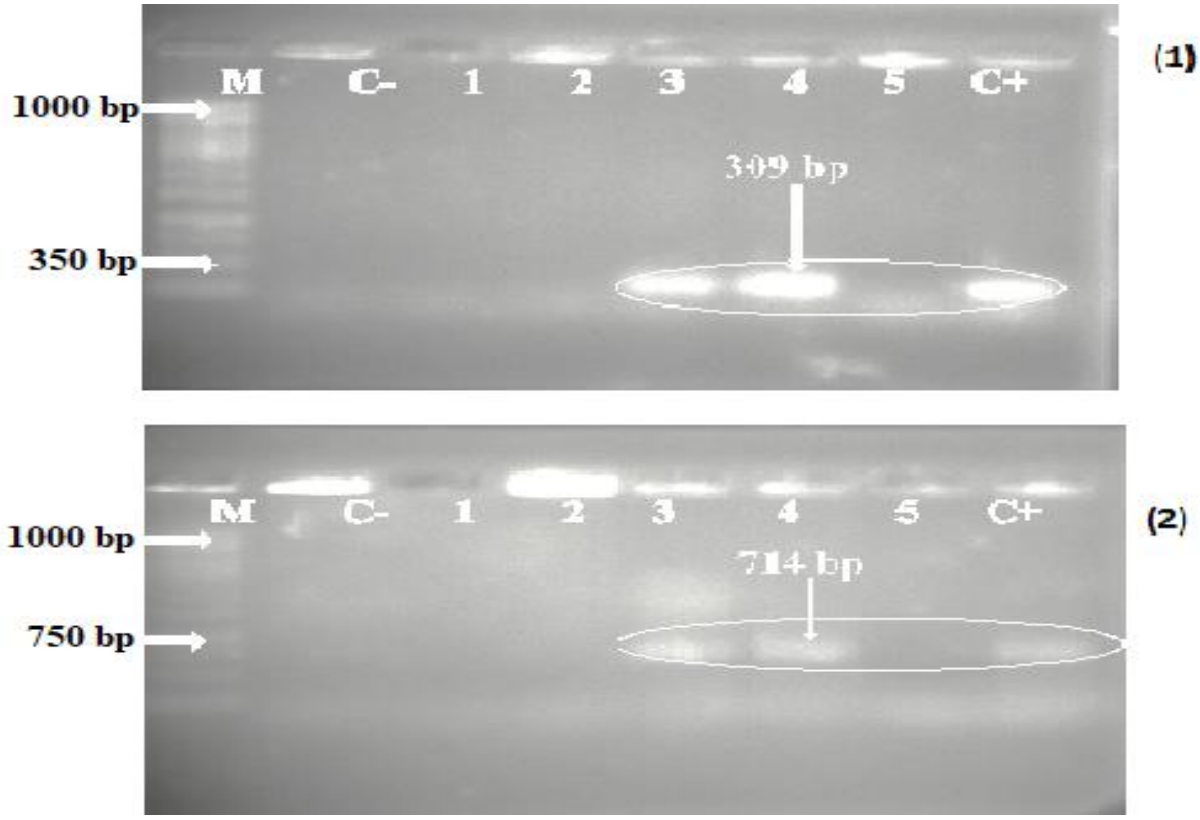

Fig. 3. Electrophoretic profile of APEC strains after PCR amplification in the presence of *iss* and *iuc*D genes

(1) Analysis of *iss* gene*; **(2)*** Analysis of*iuc*D gene
Lines 1 to 5: Presence or absence of iss and iucD genes in Escherichia coli; C-: negative control; C+: positive control; M: Molecular weight marker

4. Conclusion

pH and water content of poultry litter are the key parameters that facilitate multiplication of germs present in this matrix. Microbiological analysis revealed that litter samples from the five major zones of chickens breeding in Abidjan were contaminated. Prevalence rates varied from 71.43 to 100% for *Salmonella*, from 95.24 to 100% for *Escherichia coli* and from 4.76 to 44.44% for *Aspergillus* depending on the study area. From the above, poultry litter used in chicken farms in the Abidjan District would contribute to the proliferation of potentially pathogenic germs that cause avian diseases. This represents a great economic loss for farmers and the Ivorian government. Litter waste that is thrown in the environment or used in other domains could be a pathogenic risk for animal health and environment. We recommend farmers to observe good practice of hygiene rules, good follow-up from start to finish of the breeding and have some notions in poultry farming before launching the activity.

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