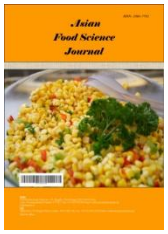

Asian Food Science Journal

20(7): 38-46, 2021; Article no.AFSJ.68866
ISSN: 2581-7752

Qualitative Risk Analysis of the Transmission of Highly Pathogenic Avian Influenza (HPAI) H5N1 through Manure Trade in Côte d'Ivoire

Tokpa Cyril[1], Acapovi-Yao Geneviève Lydie[2], Kallo Vessaly[1], Sevidzem Silas Lendzele[3*] and Bakou Serge[4]

[1]Direction des Services Vétérinaires (DSV), Abidjan, Côte d'Ivoire.
[2]UFR Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire.
[3]Laboratory of Vector Ecology (LEV), Department of Animal Biology, Institute of Research on Tropical Ecology (IRET), Libreville, Gabon.
[4]UFR Sciences de la Nature, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TC and AYGL designed the Study, wrote the protocol. Authors TC, KV, SSL and BS anchored the field study, gathered the Initial data and performed preliminary data analysis. While authors TC, AYGL and SSL managed the literature searches interpreted the data and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2021/v20i730319
Editor(s):
(1) Dr. Nelson Pérez Guerra, University of Vigo, Spain.
Reviewers:
(1) Kunwar Dhananjay Singh, Acharya Narendra Deva University of Agriculture and Technology, India.
(2) Bhujendra Soni, Indian Veterinary Research Institute, India.
(3) Balbir Singh Khadda, ICAR- KVK- Panchmahal, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68866

Original Research Article

Received 22 March 2021
Accepted 02 June 2021
Published 07 June 2021

ABSTRACT

The contamination with Highly Pathogenic Avian Influenza (HPAI) H5N1 viruses occurs via the digestive tract following the ingestion of water or food contaminated with droppings of asymptomatic carriers or sick birds. Regarding the local practice of the use of poultry manure as an agricultural fertilizer, this study focuses on the risk of spread of the HPAI through the manure trade pathway in Côte d'Ivoire. For this purpose, epidemiological data and 96 poultry droppings samples were collected from 18 farms. The droppings samples were tested using the real time polymerase chain reaction (RT-PCR). The qualitative risk assessment (QRA) took into account event patterns

**Corresponding author: Email: sevidzem.lendze@gmail.com;*

by integrating all the pathways involved in the spread of HPAI. From the diagnostic test, all the 96 samples tested negative. Further investigations revealed that 74% of the Agnibilékrou farms experienced HPAI outbreaks in the past two years. The main risk factors identified were the movement of people, animals and fomites from one infected area to another. Additionally, the duration of storage of droppings and the distance between stockpiles and farms were potential risk factors. The QRA identified two levels of risk: moderate to high (60%) and low to negligible (40%). The estimated high risk occurs when the dropping is fresh and is low after an optimal period of storage. It is therefore necessary to make storage systematic and mandatory as a measure of treatment before the adoption of other complex measures such as composting and industrial processing.

Keywords: Avian Influenza; manure; trade; transmission; Côte d'Ivoire.

1. INTRODUCTION

Poultry production is one of the lucrative livestock sector of Côte d'Ivoire, with estimated production of about 60 million in 2017 and an estimated turnover of 250 billion FCFA [1]. The poultry industry provides the population with nearly 50,000 tons of poultry meat and 1.6 billion table eggs, which corresponds to 2.2 kg of meat and 67 table eggs per capita per year [1]. This increase is mainly due to the development of intensive commercial poultry farming. Poultry meat is not subject to any religious prohibitions or taboos. Moreover, because of their relatively low prices compared to other animal products, poultry products are consumed by the entire population and constitute one of the safest ways to improve food security in terms of animal proteins [2].

In addition, this sector provides 250 thousand direct and indirect jobs [3]. It is also an important outlet for agricultural products and agro-industrial by-products [4].

Despite its high and rapid growth potential, the development of this sector remains quite fragile, due to health constraints. Indeed, the first notification of Highly Pathogenic Avian Influenza (HPAI) was made in May 2006, followed by a second incursion in 2015 thus constituting a growing threat to the poultry sector of Côte d'Ivoire. It has caused economic losses (80,000 mortalities and 97,371 slaughters) and represents a public health hazard [5].

Viral contamination occurs during close contacts, by air for low pathogenic avian influenza viruses (respiratory tract infections) and by digestive tract. For highly pathogenic avian influenza viruses, contamination occurs following ingestion of water or food contaminated by the droppings of asymptomatic or sick birds. Moreover, studies have shown that highly pathogenic viruses can survive for a long time in the environment, especially in water at low temperatures [6].

The avian influenza virus is not very resistant in the external environment. It can survive for up to 4 days at 22°C, 30 days at 0°C in contaminated water and 40 days in droppings. Thus, the uncontrolled use of poultry droppings as manure remains a risk for the spread of this deadly HPAI virus in the environment.

As forest resources for new plantations become increasingly scarce, producers are looking for alternative ways to intensify their existing plantations. Thus, since the year 2000, the use of fowl droppings has intensified in the western part of the country by cocoa producers. Breeders, mainly those in the Agnibilékrou area (east of the country), are meeting the demand [7]. However, this activity has been nuanced for the past 2-3 years with increasing problems of traceability, the transit of droppings from Ghana and the introduction of unreliable vendors in the marketing chain with poor quality fowl droppings.

The adoption of regulatory standards on the composition and quality of organic fertilizers and the implementation of measures for the composting of poultry manure before any use are therefore necessary to prevent the emergence and spread of a new HPAI epizootic. Composting is an "aerobic" decomposition of organic matter [8]. With this method, the manure is "hygienized" and can be used safely by animals, crops, and the environment [9].

However, very little work related to health risk analysis of poultry manure has been conducted. This lack of information triggered the conception of this study. The following objectives were fixed: 1) to improve our knowledge on the sanitary quality of poultry droppings via its trade in Côte

d'Ivoire; 2) to determine the level of contamination of poultry droppings by the HPAI virus through the manure distribution chain; 3) to determine the knowledge, attitudes and practices (KAPs) of stakeholders on the risk of HPAI spread through the manure distribution pathway.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in the departments of Agnibilékro and Soubré. These two areas were chosen because of their strong involvement in poultry farming (Fig. 1). The department of Agnibilékro is located in the east of the country in the Indénié-Djuablin region. The department's economy is flourishing, especially with the timber trade, coffee and cocoa cultivation, and the transboundary trade with Ghana. The department of Agnibilékro is the largest poultry production area in Côte d'Ivoire. The department produces on average 3,980,000 chickens of all species. It is bordered to the north by Koun-Fao, to the south by Abengourou, to the west by Daoukro and to the east by Ghana. In 2014, its population was 168,188. It covers an area of 1650 km^2.

The department of Soubré is located in the southwest of the country, 400 km from Abidjan. Soubré is the capital of the Nawa region. It is an administrative district rich in agricultural and forestry potential, making the region one of the most important economic poles of Côte d'Ivoire, but also one of the most coveted regions, sometimes leading to land conflicts. It is bordered to the north by the departments of Buyo and Issia; to the south by the departments of Méagui and Sassandra; to the east by the departments of Gagnoa and Gueyo and to the west by the departments of Taï and Tabou. According to the 2014 population census, the department of Soubré has 464554 inhabitants within an area of 4779 km^2.

2.2 Sample Collection and Screening

10 gm of fresh fowl droppings were collected from farms (n=18) and dry droppings collected from the droppings stockpiles ready to be used as agricultural fertilizer (14 sites). Two survey forms were prepared, one for the farmers and one for the collectors. Chain or sequential sampling was applied using the lists of farms and manure storage sites provided by the veterinary services of Agnibilékrou and Soubré. A total of 96 samples were collected, of which 3 samples per site were determined using the OpenEpi software with sensitivity of 95% 77pifor an expected prevalence of 50%. Of these 96 samples, 54 samples were collected from the farms and 42 from the manure stockpiles.

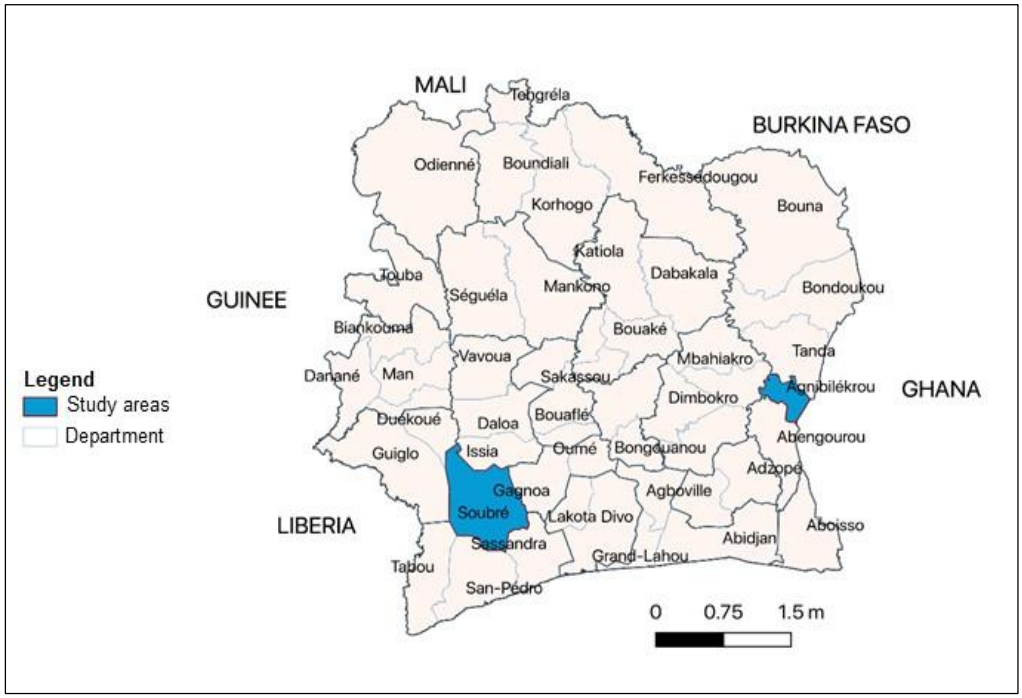

Fig.1. Map showing the study areas (in blue).

The samples were maintained in a cool chain and sent to the Laboratoire Central Vétérinaire de Bingerville (LCVB) for diagnosis using the RT-PCR. The detection of avian influenza virus type A in samples was performed in the following steps:

The first step consisted of realizing pools of samples. Each pool consisted of 3 specimens collected from the same site and ground together in 1X PBS. A total of 32 pools were generated. The virus RNA was extracted from the pools using the QiAmp viral RNA Mini kit, Qiagen, Germany according to the manufacturer's instructions. Finally, the M gene allowing the detection of influenza type A virus was amplified by the real-time PCR method according to the protocol of Spackman et al*.* [10]. Indeed, 5 µL of nucleic acid extract was added to 20 µL of reaction medium composed of 300 nM of each primer- M+25F (5'-AGATGAGTCTTCTAACCGAGGTCG-3') and M-124R (5'-TGCAAAAACATCTTCAAGTCTCTG-3'), 10 nM of Taqman FAM probe M+64 (5'-TCAGGCCCCCTCAAAGCCGA-3') using the QuantiTect RT-PCR One step multiplex kit (Qiagen, Germany). The following amplification programme was used: an initial reverse transcription step of 20 min at 50°C followed by an initial denaturation of 15 min at 95°C finally 40 cycles of 45 sec denaturation at 94°C and 45 sec elongation at 60°C. It should be noted that two positive controls (H5 and H9) were used. The RT-PCR was validated when positive controls were positive (presence of curve) and the negative controls negative (absence of curve).

2.3 Determination of the KAPs of the Target Population on the HPAI Virus

Epidemiological data were collected through a questionnaire in French administered to the target population (farmers, manure collectors and manure dealers) in the Agnibilékrou and Soubré areas. The questionnaire covered the history of HPAI in farms, the origin of manure, processing, traceability, use of manure and environmental conditions, etc.

2.4 Evaluation of the Risk of Spread of the HPAI Virus through the Manure Distribution Pathway

The approach used here was qualitative. It was conducted by combining the probabilities of the occurrence of the disease and its consequences. This approach was complemented by the risk estimation presented by Zepeda [11] (Table 1). This author suggested that each of the parameters should be analyzed using all available information, and that an evaluation of the probability of occurrence of each of them should be made separately to arrive at one of the following four assessments:

Negligible: The occurrence of the event would only be possible in exceptional circumstances,
Low: The occurrence of the event is low, but possible in certain circumstances,
Moderate: The occurrence of the event is clearly possible,
High: The probability of the event occurring is high.

2.5 Construction of Event Models or Pathways

The construction of event models is a preliminary step in the QRA that allows for the schematic representation of the different events that constitute the situation under analysis [12]. The construction of event-based models has been proposed:

- Contamination of farms by personnel and fomites (Event 1),
- Contamination of farms by manure collection trucks (Event 2),
- Contamination of farms by nearby manure stockpiles (Event 3),
- Contamination of farms by nearby land application (Event 4).

For each step, the elements that could be at the origin of the contamination of the farms and the dissemination of the virus were investigated and qualified. At the same time, the probability (P) of occurrence of the event was determined.

2.6 Determination of Risk Factors

The main risk factors identified were the status of the farm, movement from an infected to a non-infected area (building cleaning staff, visitors, roaming animals and manure collection trucks). In addition, the length of storage and the distance with farms are important risk factors.

2.7 Data Analysis

The data and laboratory results were recorded and analyzed using the Microsoft Office EXCEL spreadsheet version 2010.

2.8 Ethical Considerations

All stakeholders involved in the poultry manure value chain in these two localities were informed of the protocol and gave their informed consent. They will also be informed on the outcome of this project.

3. RESULTS

3.1 Detection of the HPAI Virus in Samples

All the 96 samples tested using the RT-PCR were negative (Fig. 2). The 4 curves represent the 4 positive controls (two positive extraction controls and two positive PCR controls).

3.2 The KAPs of Target Population on the HPAI Virus

3.2.1 Epidemiological situation in the Agnibilékro zone

From the present surveys it was noticed that 13 out of 18 or 74% of the farms in Agnibilékro experienced an epizootic of the HPAI virus from 2017 to 2018.

3.2.2 Biosecurity level

It was noticed that 14 farms or 79% did not have a fence and did not adopt adequate sanitary measures. In addition, out of the 18 farms surveyed, 3 or 16% practiced multiple bird species breeding (Fig. 3).

3.2.3 Origin and destination of manure

Of the 50,000 tons of manure transported to the west in 2019, 1,500 tons, or 23%, came from Ghana and transit through Agnibilékro where the rest were produced. The highest quantity (16,500 tons or 33%) of manure collected in the Agnibilékro area was transported to Duékoué (33%), followed by San Pédro and Soubré, each with 11,000 tons (22%) (Fig. 4).

3.3 Probability of Contamination and Spread of HPAI Virus through Manure Trade

Event 1: Contamination of farms by personnel and fomites

The cleaning of buildings to remove manure was conducted by a group of personnel known as loaders. The equipments used by them were not disinfected before being introduced to new farms. These equipments could be contaminated between farms. It should be noted that loaders may visit on average two farms per day. More than half (74%) of the farmers surveyed said they experienced at least one outbreak of the HPAI virus between 2017 and 2018. In most farms, there were several birds and sometimes even several species of birds and without much biosecurity measures. The risk of contamination is therefore very high.

Event 2: Contamination of farms by manure collection trucks

In 79% of the cases, manure collection trucks had access to the farms without being disinfected. They travel through several small farms that cannot fill a load on their own. Recall that 13 out of 18 or 74% of the farms surveyed experienced an outbreak between 2017 and 2018. The risk of contamination of farms by manure collection trucks is therefore high.

Event 3: Farm contamination via nearby manure stockpiles

The importation of manure from Ghana as well as those collected in the Agnibilékro area are piled near roads while waiting for collection trucks. These piles are kept for an average of 7 days before transportation. The mixing of manure in hermetic bags generates high heat that does not favor the survival of viruses in general and HPAI in particular. Therefore, the contamination of farms by manure after one week of storage is low. Once in the western cities, the transporters unload the bags of manure and distribute them to vendors. The latter do not have any warehouses, so storage is made on the roadside. It should be noted that the average period between collection and arrival of manure in the west is 14 days. The contamination of western farms by the manure originating from Agnibilékro is therefore low.

In Agnibilékro, the density of poultry production is very high. Poultry farms are concentrated on the roadsides around the town and surrounding villages along the main and secondary roads. Manure stockpiles from Agnibilékro and Ghana end up in farms. Biosecurity is required to avoid the high risk of contamination. In contrast to Agnibilékro, the density of poultry farms in western Côte d'Ivoire is very low. They are concentrated in peri-urban areas and far from major roads. The risk of contamination by the manure from Agnibilékro is therefore low. The

risk of contamination of farms by manure is linked to the presence of roaming village fowls and personnel. The lack of fencing in almost all farms (76%) and the poor enforcement of biosecurity measures guarantee the high transmission risk.

Table 1. Combined probabilities for QRA [1]

Probability of event 2	Probability of event 1			
	Negligible	**Low**	**Moderate**	**High**
Negligible	Negligible	Low	Low	Moderate
Low	Low	Low	Moderate	Moderate
Moderate	Low	Moderate	Moderate	High
High	Moderate	Moderate	High	High

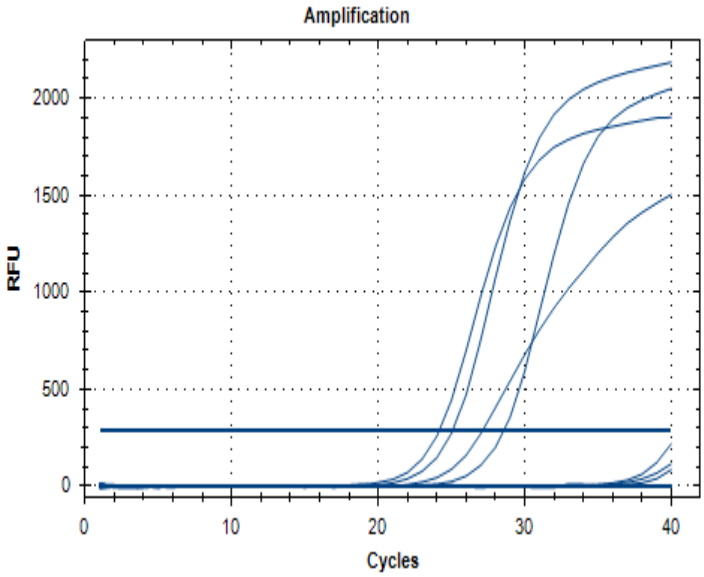

Fig. 2. Influenza A virus RT-PCR standard curve

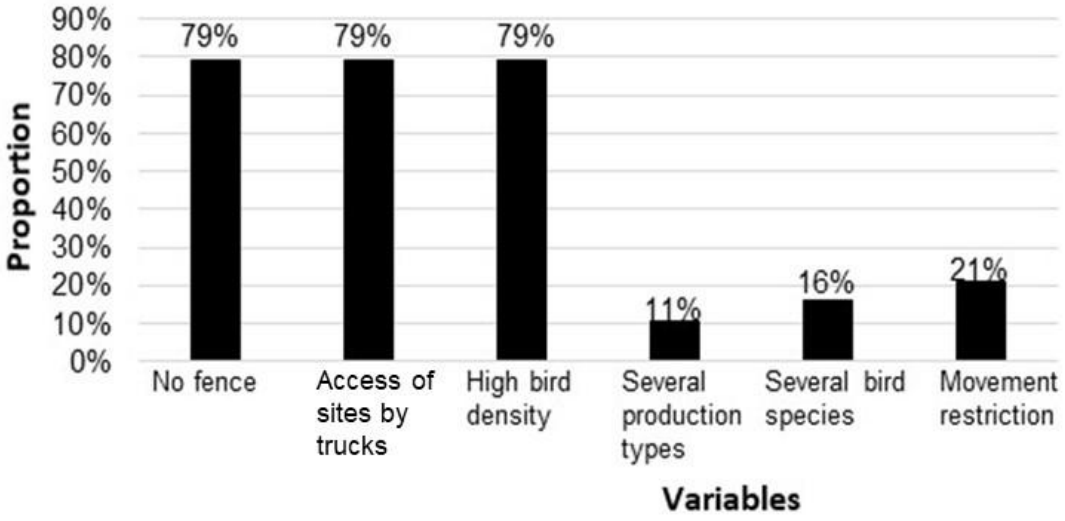

Fig. 3. Level of biosecurity in poultry farms of Agnibilékro in 2019

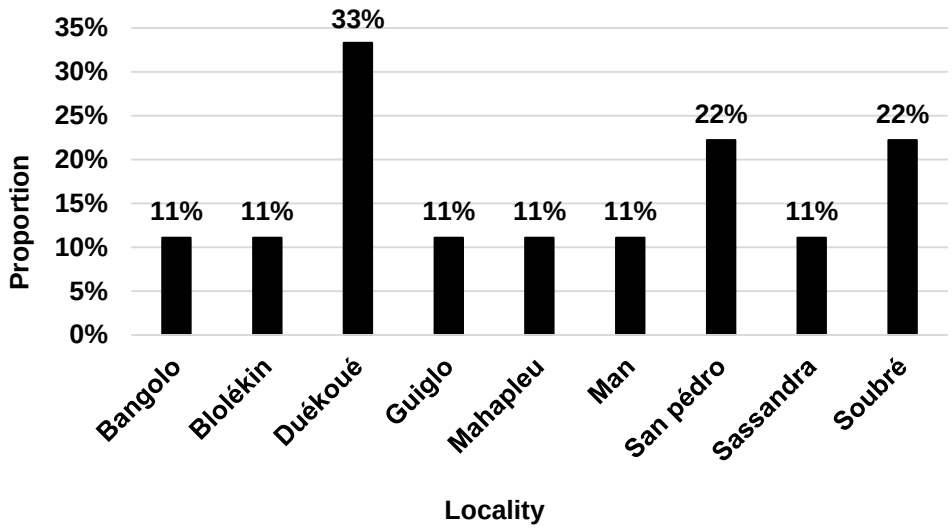

Fig. 4. Proportion of manure demand by different cities of Côte d'Ivoire in 2019

Event 4: Contamination of farms by nearby fields

Cocoa farms are located in remote areas and are difficult to access. Once manure trucks arrive in the western cities, the transporters unload the bags where either the resellers supply the producers, or the producers come to buy directly. Since the farms are concentrated in peri-urban areas, contamination is negligible.

3.4 Risk Estimation

The estimated risk is presented in Table 2.

The different stages of manure distribution contribute to 60% of the HPAI virus spread. Moreover, the risk is considered "high" when the droppings are fresh and becomes low after one week of storage. Therefore, the risk is high in the production areas and becomes low in the application sites.

From the Table 2, Table 3 was constructed, representing the different levels of risk established with their proportions as follows:

4. DISCUSSION

All the samples collected were negative for the type A avian influenza virus. According to De Benedictis et al. [13], the survival of this virus in manure probably depends on the strain of the virus, the type of manure and the temperature. From the present study, it was found that a fence was present in only 4 or 21% of the farms surveyed. The lack of information by farmers on the importance of fencing and the lack of financial means could explain this observation. This proportion is higher than that reported by Dosso [14], who reported that only 6% of farms in the department of Agnibilékro had a fence. It appears from this study that it is during the cleaning of farm buildings and the loading of manure that the risk of contamination of farms is high. This is due to the use of loaders that visits several farms without disinfecting themselves as well as their working tools. In addition, the absence of sanitary measures and the poor application of biosecurity measures elevates this transmission risk. The survey of Rozier [15] revealed that clothings easily picks up microbes and releases them in the same way. The manure collection trucks visits several small farms that cannot fill a load by themselves. According to Augustin et al. [16], the transmission of HPAI is primarily via direct contact, but also indirectly through passive transport by people or objects coming from infected areas.

The storage of manure near farms is not without the risk of contamination of these farms with the HPAI virus. This contamination is favored by the presence of animals in general and in particular by village fowls roaming in the locality. Viral contamination occurs during close contact, by air and through the digestive tract following ingestion of water or food contaminated by the excretions of asymptomatic or sick birds [6]. The HPAI virus can persist for 7 days at 20°C [17]. It can persist for 4 days at 22°C and 40 days in manure [18].

Table 2. Estimated risk of HPAI virus transmission to farms through the manure trade pathway

Events (pathways)	Probability of contamination by personnel and fomites	Probability of contamination by collection trucks	Probability of contamination by nearby manure stockpiles	likelihood of contamination of farms by manure application	Consequences	Estimated risk
1	Negligible	Negligible	Negligible	Negligible	High	Negligible
2	Negligible	Low	Negligible	Low	High	Low
3	Moderate	Low	High	Moderate	High	High
4	High	High	Low	High	High	High
5	Negligible		High	High	High	High

Table 3. Summary of the different levels of risk with their respective proportion by scenario

Level of estimated risk	Number of scenarios	Percentage
Moderate to High	3	60%
Negligible to Low	2	40%
Total	5	100%

Furthermore, storage is not actually a treatment option, but it is essential [19]. The influence of storage on pathogen inactivation is related to both the nature of the stored manure, the temperature, and the duration of storage. Storage seems to provide room for the rapid inactivation of microorganisms during the first weeks [20]. The distance between the cocoa plantations and the farms does not suggest that they are contaminated by the manure. Indeed, cocoa plantations are found in remote settings and are difficult to access, while the poultry farms are located in peri-urban areas.

The consequences of HPAI are sanitary and economic. At the sanitary level, the persistence of the low pathogenic strain allows for mutation into a high pathogenic strain. The economic consequences of this disease are mainly financial losses in the production system, permanent abandonment of the activity, loss of employment, reduction of income in family farms and the very high costs of control measures.

5. CONCLUSION

In conclusion, the prevalence of the type A HPAI virus in fresh and dry fowl droppings samples was 0%. The main risk factors identified were the status of the farms (infected/non-infected), the movement from an infected to a non-infected farm (loaders, visitors, roaming animals, droppings trucks). In addition, the duration of storage and the distance of the stockpiles from the farms were also considered in the QRA. From this analysis, two levels of risk were determined-moderate to high (60%) and low to negligible (40%). Furthermore, the different transmission pathways analysis showed that the risk is considered high when the manure is fresh and low after one week of storage. Indicating that the risk is high in production areas and low in application sites. It is therefore necessary to regulate the use of manure and its trade by making storage systematic and mandatory as a treatment measure before adopting other complex measures such as composting and industrial treatment. Also, manure coming from neighboring countries should follow stringent biosecurity measures before being introduced in Côte d'Ivoire.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. JNA (Ivorian Poultry Day). Second edition of the Avi-Invest forum ; 2018 (Page consulted on the 30/12/2018).
2. Bakayoko KV. Strengthening avian influenza control measures in: Seminar on biosecurity in live poultry farms and

markets, Grand Bassam, Ivory Coast. 2007;3.
3. SARA. The Ivorian poultry sector calls on French investors. [Online] Internet access ; 2017. Available: https://www.afrique-agriculture.org/articles/lessentiel/la-filiere-avicole-ivoirienne-fait-appel-aux-investisseurs-francais; 2017 (Page consulted on the 30/12 / 2018).
4. IPRAVI. General presentation of Ivorian poultry farming ; 2013 (Page consulted on the 22/12/2018)
5. CIRAD. Analysis and risk mapping of highly pathogenic avian influenza along the poultry value chain in Côte d'Ivoire. 2018;5.
6. INRS. Avian influenza and the threat of a pandemic: a new issue in occupational health, NRS. 2006; 4p.
7. CIRAD. The production and distribution practices of "chicken manure" in the Ivory Coast. 2017; 13.
8. GIVC Bretagne. Poultry manure composting specifications - Report. 2002;11.
9. IF2O. Practical Guide. Manufacture of organic fertilizer on the farm, IF2O. 2013;4.
10. Spackman E, Senne DA, Myers TJ. Development of a real-time reverse transcriptase PCR assay for type Ainfluenza virus and the avian H5 and H7 hemagglutinin subtypes. Journal of Clinical Microbiology. 2002;40(9):3256-3260.
11. Zepeda SC. Method for assessing zoosanitary risks during international trade. In Seminar on animal health safety in the Caribbean (ed. OIE). 1998;2- 17.
12. OIE. Handbook on import Risk Analysis for Animals and Animal Products, Volume 1: Introduction and qualitative risk analysis. Paris: OIE ; 2004.
13. De Benedictis P, Beato MS, Capua I. Inactivation of avian influenza viruses by chemical agents and physical conditions: a review. Zoonoses and Public Health. 2007; 54 (2): 51-68.
14. Dosso S. Analysis of poultry practices and the use of antibiotics in modern poultry farming in the department of Agnibilékrou (Ivory Coast). Thesis in Veterinary Medicine, Dakar. 2014;13:152.
15. Rozier J. Understanding and practicing kitchen hygiene. Milan: Imp. Maury. 1990;200.
16. Augustin JC, Carlier. Highly Pathogenic Avian Influenza: Risk linked to the consumption of foodstuffs - Communication, Avian Conference - Veterinary news. Chair of medical pathology of livestock and farmyard animals, ENVA-France ; 2007.
17. Delvallee T. Avian influenza: news and transmission in humans, [Research report] Institute of Scientific and Technical Information (INIST-CNRS). 2006;78.
18. Schmitz A, Pertusa M, Le Bouquin S, Rousset N, Ogor K, LeBras MO, Martenot C, Daniel P, Belen Cepeda Hontecillas A, Scoizec A, Morin H, Massin P, Grasland B, Niqueux E, Eterradossi N. Natural and experimental persistence of highly pathogenic H5 influenza viruses in slurry of domestic ducks, with or without lime treatment. Applied Environmental Microbiology. 2020;86:e02288-20. Available: https://doi.org/10.1128/AEM.02288-20.
19. Lekefack JP. Assessment of the management of laying hen droppings: case of the large farm of the gic aecam de mendong. Master's thesis University of Yaoundé I, Faculty of Sciences. 2015; 65.
20. Deleryl. Scientific basis for the assessment of health risks relating to pathogens, ADEME Conventions no. 03 75 C 0093 and 06 75 C 007. 2007;130.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68866