



RESEARCH ARTICLE

SCREENING OF ANTIBIOTICS RESIDUES IN THE EGGS CONSUMED IN BURKINA FASO

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ABSTRACT

This study was conducted to highlight antibiotics' residues in eggs from laying hens consumed in Burkina Faso. 400 eggs from four areas of Burkina Faso were collected. The presence of antibiotics residues has been put in evidence by a microbiological method using *Geobacillus stearothermophilus*, *Bacillus subtilis* and *Escherichia coli* as test microorganisms. It appears from this study that 41.75% of eggs laying hens consumed in Burkina Faso contain antibiotics' residues. Evolution in the prevalence of antibiotics residues in eggs ranged from 39% to 46% from one zone to another. This study reveals a strong presence of antibiotic residues in the yolk than in the albumen of eggs. The presence of antibiotics residues in table eggs in Burkina Faso is a danger to the health of consumers.

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INTRODUCTION

Livestock of Burkina Faso, is characterized firstly by the existence of an important and diverse animals stock and, secondly, by an operating system dominance extensive but well suited to the seasonal variability and inter-annual pastoral resources (MRA, 2011). However more intensive operating systems are developed around large cities for growth sectors such as milk, modern poultry farming with laying hens and chicken of flesh (MRA, 2011). Breeding is the third sector that contributes more to export after gold and cotton and represents approximately 15% of export earnings. In the national herd chickens occupy the first rank in terms of numbers with 30, 888,112 heads (MRA, 2012). National production of eggs of local strains and laying hens is respectively of 675 million and 10 million per year (Némaoua and Ramdé, 2008; MRA, 2012). The intensification of animal production in recent decades has been facilitated by the use of veterinary medicines, in modern breeding (Morétain, 2005 and Tatsadjieu et al., 2009).

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These drugs are used either as a curative treatment applied individually or collectively in animals with microbial diseases, either as a preventive treatment to prevent the onset of certain diseases or, in extreme cases, to overcome the hygiene shortcomings in poultry farming (Sanders, 2005). These antimicrobials drugs are also used as food additives or growth promoter (Dibner et al., 2005). The misuse of these antibiotics by farmers and veterinarians as well as non-compliance with waiting periods after treating animals lead to the presence of antibiotic residues in food of animal origin (Aning, 2007). The bad practice of bovine antibio-therapy in Burkina Faso has been highlighted and the prevalences of antimicrobial residues were 31% and 51.72% respectively in meat and raw milk (Samandoulougou et al., 2015; and Bagre et al., 2015). Also these antimicrobial residues in food of animal origin can cause allergies, poisoning (Kabir et al., 2004), modification of the intestinal flora (Baquero and Garau, 2010), and bacterial resistance to antibiotics (Persoons, 2011) among consumers. In most West African countries, Biagui, (2002) has reported of misuse of antibiotics by farmers. Also Samandoulougou et al. (2016) have reported bad antibiotic practices especially in laying hens in Burkina Faso. Those bad practices can encourage the presence of antibiotic residues in eggs of laying hens.

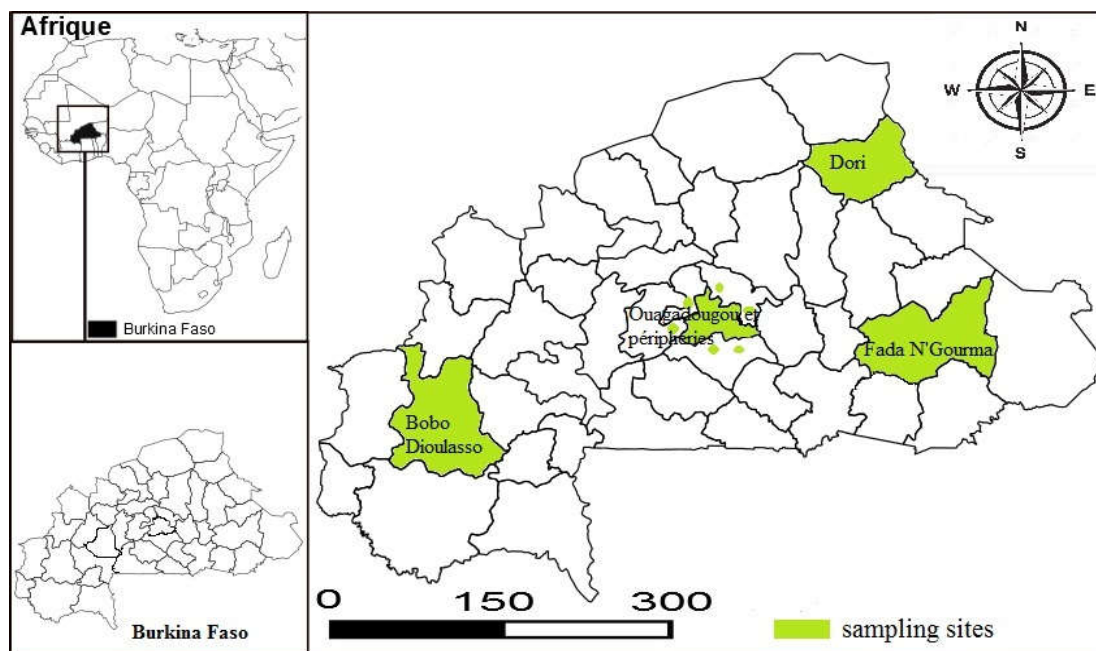
The objective of this study is to put in evidence antimicrobial residues in eggs from laying hens consumed in Burkina Faso.

MATERIALS AND METHODS

Period and study site: The study took place from January to september 2016, in four areas of Burkina Faso. It is the Ouagadougou area and peripheral rural communes (Komki-Ipala, Koubri, Loubila, Pabré Saaba and Tangin Dassouri), the area of Bobo Dioulasso, the area Fada N'gourma and Dori area (Figure 1).

Firstly 5ml of vitellus (egg yolk) and the albumen (egg white) of each sample was taken and mixed with 10 ml of phosphate buffer pH 6 ± 0.01 then homogenized. Secondly the yolk was centrifuged at 5000 tr / min for 15 min and the supernatant was recovered for analysis. Thirdly supernatant of yolk and albumen were placed in a water bath at 75°C for 15 min to inactivate lysozyme.

Antibiotic residue testing: The presence of antibiotic residues was detected in the different samples following the methods described by Ben-Mahdi and Ouslimani (2009) based on the inhibition' growth, of the tests bacteria.



SOURCE : BNDT

Figure 1. Sampling sites

Table 1. Proportion of eggs tested positive for antibiotic residues

Area of origin of eggs	Positives samples		
	<i>Geobacillus stearothermophilus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>
Ouagadougou (n=100)	46 (46%) [3-11]	21 (21%) [3-7]	7 (7%) [3-5]
Bobo Dioulasso (n=100)	42 (42%) [3-9]	19 (19%) [3-8]	1 (1%) [3-5]
Fada N'gourma (n=100)	40 (40%) [3-9]	21 (21%) [3-7]	0 (0%) [0]
Dori (n= 100)	39 (39%) [3-9]	18 (18%) [3-7]	0 (0%) [0]
Total	167 (41,75%) [3-11]	79 (19,75%) [3-8]	8 (2%) [3-5]

n = number of samples of eggs; () = Percentage of positive; [] = Diameter minimum and maximum inhibition measured in mm.

Table 2. Proportion of albumen and yolk tested positive for antibiotic residues

Positives samples	
N=100	<i>Geobacillus stearothermophilus</i>
Albumen	20 (18 %) [3-6]
yolk	36 (36 %) [3-9]

N = number of samples of eggs; () = Percentage of positive; [] = Diameter minimum and maximum inhibition measured in mm.

Sampling: The eggs of laying hens (n = 400) were collected on farms and / or in shops belonging to the farms visited. A total of 400 eggs were collected (100 eggs per region), labeled and identified according to the number of the farm and the area. These samples were sent to Food Microbiology Laboratory of the National School of Animal Husbandry and Animal Health (ENESA) of Burkina Faso for analysis.

Sample preparation: The sample preparation was done as described by Diop (1991).

This method is applied in the European Community since 1 January 2003 (European Commission Decision 96/23 / EC) (EC, 2003).

Preparation of culture medium: The medium Mueller Hinton (MH) (Liofilchem, Italy) is prepared according to the manufacturer's instructions, autoclaved, cooled to $45-47^{\circ} \text{C}$ and cast in Petri dishes for seeding.

Inoculation of the culture media: Three reference strains were used according to their sensitivity to antimicrobials' families. It is *Geobacillus stearothermophilus* ATCC 10149, sensitive to most of the antibiotics and very sensitive to penicillin and tetracyclines (Pikkemaat, 2009). *Bacillus subtilis* ATCC 6633, sensitive to the macrolide and aminisides (Dey *et al.*, 2005). *Escherichia coli* ATCC 128, sensitive to quinolones and fluoroquinolones (Okerman *et al.*, 2001). Well separated colonies from pure culture of these microorganisms tests obtained by culture on Petri dishes, were separately suspended in physiological saline (NaCl 9 g / L of water) and then adjusted to the optical density (OD) 0.08 -0.1 evaluation by spectrophotometer at 625 nm (equivalent to McFarland standard 0.5). This suspension was used to inoculate boxes.

Deposit of samples and incubation: Fifty (50) ml of each prepared sample is used to impregnate the disks of 6 mm diameter of Wattman paper sterile. Soaked disks of samples are deposited on already inoculated Petri boxes. The Petri boxes were then incubated at 55 ° C, 30°C and 30 ° C respectively for *Geobacillus stearothermophilus*, *Bacillus subtilis*, and *Escherichia coli*.

Data analysis: The data were analyzed with ANOVA variables with the XLSTAT software, version 7.5.2. Means were compared using the Student t-test at the significance level (5%).

RESULTS

Table I shows that there are antimicrobial residues in eggs of laying hens consumed in many parts of Burkina Faso. The prevalence of antibiotic residues in eggs slightly varies from one zone to another. So it is 46%, 42%, 40%, 39% for respectively eggs from the Ouagadougou area, Bobo Dioulasso, Fada N'gourma and Dori. Also 19.75% and 2% positive samples were obtained by using respectively *Bacillus subtilis* and *Escherichia coli* as test microorganisms. Table II shows an unequal distribution of antibiotic residues in various parts of the egg. Thus in 100 eggs, 36% of yolk contained residues of antibiotics and only 18% of albumen contained antibiotic residues.

DISCUSSION

The high prevalence's of antibiotics residues in eggs has been found in those from Ouagadougou (46%) and low prevalence has been found in samples of Dori (39%). On average 41.75% of the eggs of laying hens in Burkina Faso contained antibiotic residues. The presence of antibiotics residues in eggs is due to the excessive use of antibiotics in breeding of laying hens, combined of the no respect of waiting times. *Geobacillus stearothermophilus* used as test organism is sensitive to most of the antibiotics but especially to beta-lactams, sulfonamides and tetracyclines. Thus the results found using this microorganism does not permit to conclude on the family of antibiotics found. As against the results found using *Bacillus subtilis* and *Escherichia coli* as tests' microorganisms suggest the presence of antibiotic residues respectively belonging to aminoglycoside, macrolides and quinolone and fluoroquinolones. Indeed *Bacillus subtilis* is sensitive to antibiotics of aminoglycoside and macrolide then that *Escherichia coli* is sensitive to quinolones and fluoroquinolones.

This Table I shows that 19.75% of the eggs containing residues of antibiotics may belong to the family of aminoglycoside and macrolide and 2% of eggs containing antibiotic residues may belong to the family of quinolones and fluoroquinolones. The 41.75% of eggs containing antimicrobial residues were already in the marketing chain, which is a risk to consumer health. The low variation of prevalence (39% to 46%) of antibiotic residues in eggs from one zone to another in Burkina Faso is mainly due to the similarity of antibiotic therapy practices in laying hens which are exotic breeds. The results found in this study are higher than those found in Dakar, Senegal, which reported that 33% of eggs taken from 6 farms contain antimicrobial residues (Niyibizi, 2012). This difference could be due to the higher sampling size in this study than their study. However, the results reported in this study are lower than in Sirdar (2010) in Sudan, which found on 175 farms in April June and August of the same year, respectively the prevalence of antimicrobial residues of 61.1%, 60.2% and 68.7%.

Similarly Abiola *et al.* (2005) obtained results (54% of positive samples) superior to ours in their work on antimicrobial residues in the liver and gizzard of broilers in the regions of Dakar and Thies (Senegal). The high prevalence of antimicrobial residues in eggs can be explained by the addition of antimicrobials in food and / or in water consumed by laying hens and coupled of non-compliance with waiting periods. Samandoulougou *et al.* (2016) found this common practice among breeders of laying hens. Also Ezen-duka *et al.* (2011) reported a presence of antibiotics in feed for laying hens sold in markets. The 19.75% positives found in this study is higher than the 6.8% found positive in Ghana Donkor *et al.* (2010) in a sample of 220 eggs using the test *Bacillus subtilis*. The results in Table II show that a same egg can have his albumen negative to the screening test and its positive yolk. This study relates more of antibiotics residues in the yolk than in the albumen. These results corroborate those of Roudaut, (1997) that reported a wide disparity in the distribution of antibiotics between the albumen and yolk. This disparity can be explained in part by the very different composition of the two components of the egg, by a difference in training places (ovarian for yolk and oviduct for albumen) and time taken to build the component of each part of egg. The selective transfer of antibiotics is dependent on the physicochemical properties of the antibiotic (percentage of dissociation, and lipophilic ability to bind to proteins). Also the influence of the route of administration, the possibility of diffusion of an active principle between the albumen and yolk are elements that can explain the distribution of antibiotic residues in the albumen and yolk (Roudaut, 1997). The use of antibiotics is essential in poultry farming. However, the presence of residues in eggs is a public health problem in that it can present risks of direct toxicity, allergy, cancer, pathology related to the modification of the intestinal flora, and development of resistant bacteria. In Burkina Faso there is a mechanism of registration and sale of veterinary drugs, but the strong presence of veterinary drugs (illicit) of the street in urban areas and especially in the villages makes it ineffective control system and exposes people to potential risks (Samandoulougou *et al.*, 2016).

Conclusion

This study enabled the detection of antibiotic residues in eggs from four areas of Burkina Faso. It appears from this study that 41.75% eggs consumed in Burkina Faso contain antibiotic residues.

The low prevalence of antibiotic residues in eggs has been found in those from the Dori area and the high prevalence of antibiotic residues in eggs concerned those from Ouagadougou. Next the sensitivity of the microorganisms utilized, it is possible that the antibiotics residues found belong to the family of beta-lactam antibiotics, tetracyclines, sulfonamides, macrolides, aminoglycosides, quinolones and fluoroquinolones. This study reports a disparity in distribution antibiotic residues in the albumen and the vitellus of the eggs. It relates more of antibiotic residues in the yolk than in the albumen of eggs. The use of high performance liquid chromatography would be necessary for to identify and quantify the antibiotic residues in the eggs consumed in Burkina Faso

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