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Assessment of *Salmonella* spp. presence among broilers of naked neck Label Rouge lineage in Northwest region of Paraná State, Brazil

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The presence of *Salmonella* serovars in poultry products is a worldwide public health concern. Moreover, salmonellosis is one of the most common foodborne diseases with approximately 10 million cases of human infection occurring per year worldwide. The aim of this study was to assess the presence of *Salmonella* spp. in broilers of the naked neck Label Rouge lineage by cloacal swab technique in a broiler house in the northwestern region of the state of Paraná, Brazil. Cloacal swab samples were collected from 100 broilers raised under the free-range farming system, and the detection of *Salmonella* spp. was carried out using biochemical and serologic techniques. No *Salmonella* spp. was detected on the poultry studied. This result may indicate the free-range farming system as a contributing factor to reduce the presence of foodborne pathogens in poultry production as compared to confinement and intensive farming system. More studies in this area are necessary in order to understand the real benefits of the free-range farming system in relation to foodborne pathogens.

Key words: Salmonellosis, free-range farming system, human health, poultry.

INTRODUCTION

Salmonellosis is one of the most common foodborne diseases with approximately 10 million cases of human infection occurring per year worldwide (Gopinath et al., 2012; Mercado et al., 2012). This disease is caused by different *Salmonella* spp. serovars, and is characterized by an acute onset of fever, abdominal pain, diarrhea, nausea and vomiting in humans. The most common source of foodborne salmonellosis to humans is the consumption of contaminated and inappropriately prepared broiler meat or chicken eggs (Ravel et al., 2009).

The presence of *Salmonella* spp. in poultry products is

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a worldwide public health concern since it causes asymptomatic infection in most poultry cases and does not cause high mortality in most situations. Studies have demonstrated that broilers on the slaughter line may be contaminated with these bacteria, representing risk to human health (Shinohara et al., 2008; Von Rückert et al., 2009; Mercado et al., 2012; Desin et al., 2013). In addition to public health problems, the presence of these bacteria in broilers also causes high economic losses due to a decrease in meat quality, an increase in drug costs and large expenditures in eradication and control strategies (Shinohara et al., 2008).

Currently, Brazil along with other countries is one of the largest broiler producers in the world. This elevated production requires rearing methods that result in products with high technical quality regarding food safety. Therefore, some producers have sought to use free-range farming system in broiler production (Savino et al., 2007; Sun et al., 2013; Sales et al., 2014).

This farming system consists of rearing the broilers in confinement until 28 days of life and after this period, the animals get access to the outdoors. This system allows obtaining better meat quality as compared to the confinement and intensive system, since it preserves the animal welfare, stimulates its immune system, decreases the stress level and reduces or eliminates the use of chemotherapy drugs to prevent infections or being used as growth promoters (Nazareno et al., 2009; Tong et al., 2014). The feed used in this system is preferably prepared with products from vegetable origin, according to the Brazilian Ministry of Agriculture, Livestock and Food Supply legislation (Nazareno et al., 2009; Tong et al., 2014).

The aim of this study was to assess the presence of *Salmonella* spp. isolates in broilers of naked neck Label Rouge lineage by using the cloacal swab technique on a broiler house in the northwestern region of the state of Paraná, Brazil.

MATERIALS AND METHODS

Study setting

The study was approved by the Ethics Committee and Research Involving Animal Experimentation of Paranaense University, Brazil, under protocol 26568/2013. From July 2013 to February 2014, cloacal swabs were collected from broilers of naked neck Label Rouge lineage in a broiler house located in the city of Umuarama, in the northwestern region of Paraná state, Brazil. All the cloacal swabs were collected from animals at 85 days of life raised in the four broiler houses during different periods, comprising four different flocks.

The animals studied were raised under the free-range farming system. The area used for poultry production had approximately 1700 m^2 divided into four different broiler houses, with approximately 1000 animals each. Each broiler house had an enclosed warehouse for water and ration supply, protection from predators, wind, cold and rain. A commercial feed was used to feed the animals during the first 28 days of life. After this period, the animals had access to the outdoor area and their feed was supplemented with banana, cassava flour and foliage.

Collection of biological samples

Fifty cloacal swabs were collected from broilers to detect the presence or absence of Salmonella spp. Each cloacal swab was sampled in two animals, totaling 100 broilers analyzed.

The number of animals studied was chosen using the formula by Barbetta (1999), taking into account a tolerable sampling error of 10% and considering a population of 4000 animals.

$n_o = 1/E_o^2$

$n = N.n_o/(N+n_o)$

Where: N= Population size = 4000; E_o = tolerable sampling error = 10%; n_o = first approximation of the sample = 100; n = birds sampled = 90,90 = 100.

After collection, swabs were stored in transport medium containing 1% buffered peptone water (BPW) and kept at 4°C until processing at the Laboratory of Veterinary Preventive Medicine and Public Health at Paranaense University (UNIPAR).

Microbiological and serological tests

The laboratory techniques used to detect *Salmonella* spp. were performed according to the recommendations of the Brazilian Ministry of Agriculture, Livestock and Supply (Ordinance 126 of 11/03/1995) with modifications.

The swabs were inoculated in sterile tubes containing buffered peptone water (BPW) for 24 h at 36°C. After this period, the bacterial growth was inoculated into both selective media Tetrathionate Broth (TT) and Rappaport-Vassiliadis (RVB) and incubated for 24 h at 36°C. The enriched samples were plated on xylose-lysine deoxycholate agar (XLD agar), brilliant green agar (BGA) and MacConkey agar and incubated overnight at 36°C. Each plate was evaluated for the presence or absence of lactose negative colonies.

Three suspicious colonies that were morphologically similar to *Salmonella* spp. from each plate were sub-cultured for biochemical examinations. Biochemical characteristics were examined on urea broth, triple sugar iron medium (TSI) and lysine iron agar (LIA). When typical *Salmonella* reactions were seen, they were analyzed by serological tests using somatic and flagellar polyvalent antisera (Probac®, Brazil) to confirm the presence of Salmonella (LeMinor, 1988). The positive control used in serological test was *Salmonella* Enteritidis fegotype.

RESULTS

Among the 50 cloacal swabs samples analyzed, 20 (40%) showed colonies with morphological characteristics of *Salmonella* spp. From these, only 6/20 (30%) isolates had the biochemical characteristics confirmed on TSI, LIA and urea broth (Table 1). All these six isolates were tested against *Salmonella* spp. somatic and flagellar polyvalent antisera, but no isolate was considered positive (Table 1).

DISCUSSION

The results indicated absence of *Salmonella* spp. strains in the cloacal swabs analyzed. Similar results were also found in confined and non-confined (free-range) broilers

Table 1. Isolation and characterization of *Salmonella* spp. in the cloacal swabs analyzed.

Test result	Morphological characteristics of culture media	Biochemical characteristics (TSI, LIA and urea broth)*	Serological tests (somatic and flagellar antigens)**
Positive strains	20/50 (40%)	6/20 (30%)	0/6 (0.0%)
Negative strains	30/50 (60%)	14/20 (70%)	6/6 (100%)

*Only strains with morphological characteristics of *Salmonella* spp. were characterized by biochemical tests. **Only strains identified as *Salmonella* spp. in the biochemical tests were submitted for serological tests.

house in other regions of Brazil (Moreira, 2002; Guimarães, 2006; Ravagnani et al., 2012; Pereira and Silva, 2005).

Guimarães (2006) in Brasilia (DF) evaluated 300 cloacal swabs collected from free-range raised broilers, but no *Salmonella* spp. strain was found. According to the author, the results found may indicate the absence or a very low prevalence of *Salmonella* spp. colonization, or the poultry from that broiler house were more resistant to *Salmonella* spp. asymptomatic colonization.

Similar results were found by Pereira and Silva (2005). These authors analyzed 44 cloacal swabs from nine properties in Uberlândia (MG), and found only one positive sample. The absence of *Salmonella* spp. was also observed in chicks and organ fragments from broilers in the states of Ceará and Paraná (Moreira, 2002; Ravagnani et al., 2012).

Despite these data, some studies have reported high prevalence of *Salmonella* spp. in broilers reared under confined and intensive system both in Brazil (Kanashiro et al., 2005; Ribeiro et al., 2007) and other countries (Limawongpranee et al., 1999; Siemon et al., 2007; Alali et al., 2010).

Kanashiro et al. (2005) found prevalence of 84% in broiler flocks and 57.5% in commercial breeders in the following Brazilian States: Bahia, Ceará, Goiás, Paraná, Mato Grosso, Mato Grosso do Sul, Santa Catarina and São Paulo.

Alali et al. (2010) compared the prevalence of Salmonella in organic and conventional broiler poultry farms in the same company in North Carolina and verified that the Salmonella prevalence in fecal samples were 5.6 and 38.8% in the organic and conventional farms, respectively. The authors concluded that the prevalence of fecal *Salmonella* spp. was lower in certified-organic birds than in conventionally raised birds.

Similar results were verified by Siemon et al. (2007). The authors reported that fecal *Salmonella* spp. prevalence in conventional poultry flocks (30%; 125/419) was significantly higher than in pasture flocks (16%; 83/512).

The results shown in this work may indicate the presence of an efficient biosecurity program in broiler production, which protect the animals from contamination (Tessari et al., 2003; Teixeira and Lima, 2008; Van Hoorebeke et al., 2011), reinforcing the evidence that the prevalence of *Salmonella* spp. in free-range raised poultry may be lower than that from poultry raised in feedlots (Guimarães, 2006). Commercial poultry are raised in small compartments where an infected animal can spread the bacteria very quickly, due to the high stocking rate per square meter. Furthermore, the excretas may contaminate the water, feed and litter, turning the broiler house into an environmental source of contamination for the animals.

Another fact to be considered is the use of large quantities of meat and bone meal for animal feed production by the commercial poultry industry. This feed is a rich source of amino acids, minerals and vitamins, but being an organic product, it is highly susceptible to *Salmonella* spp. contamination (Teixeira et al. 2003). The free-range rearing system uses vegetable-derived products in animal feed, which may explain the absence or no isolation of *Salmonella* spp. in this study, since the animals consumed cassava flour, banana and foliage as supplementary feeding.

On the other hand, Bailey and Cosby (2005) evaluated the *Salmonella* spp. prevalence in free-range and certified organic chicken in 135 processed free-range chicken carcasses from four different commercial free-range chicken producers and collected samples from 14 different lots for the presence of *Salmonella* spp. and verified that 9 (64%) from the 14 lots and 42 (31%) from the 135 carcasses were positive for *Salmonella* spp. The authors concluded that the consumers should not assume that free-range or organic conditions would have anything to do with the *Salmonella* spp. status of the chicken.

Furthermore, the age of broilers is considered as a factor that affects the susceptibility for *Salmonella* spp. colonization. Young broilers are more susceptible than adult ones (Bailey, 1987; Andreatt-Fillho et al., 2006). According to Andreatti-Filho (2006), a reduction in *Salmonella* spp. colonization susceptibility is observed with age increase due to intestinal microbiota development and strengthening of the immune system. In the present study, the cloacal swabs were collected from adult animals with 85 days of age.

The prevalence of *Salmonella* spp. in broilers may be influenced by parameters such as flock size, multi-aged placement of complexes or individual farms and a host of other variables (Van Hoorebeke et al., 2011). Therefore, the main form of controlling the presence of *Salmonella* spp. in poultry production is related to biosecurity measures

and vaccination, associated with the right use of antibiotics, prebiotics and probiotics. (Barrow, 2007; Van Immerseel et al., 2005; Picler et al., 2012).

Conclusion

According to the results obtained, *Salmonella* spp. was not isolated in broilers studied. This fact may reflect the correct management and application of biosecurity programs along with the free-range broiler farming system. However, it is important to emphasize that the *Salmonela* spp. isolation by conventional techniques requires a large number of viable cells, which may also explain the absence of positive results in this study. Further studies in this area, including molecular tests, are necessary to understand the real benefits of free-range farming system.

Conflict of interest

The author(s) have not declared any conflict of interests.

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