Veterinary Microbiology 140 (2010) 131–135

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents

Maryam Muhammad [a,*], Lawal U. Muhammad [b], Abdul-Ganiyu Ambali [c], Aliyu U. Mani [c], Samuel Azard [a], Lisa Barco [d]

[a] *Bacterial Research Department, National Veterinary Research Institute, PMB 01, Vom, Plateau, Nigeria*
[b] *Veterinary Extension Research and Liaison Services, National Veterinary Research Institute, Vom, Nigeria*
[c] *Department of Veterinary Medicine, University of Maiduguri, Nigeria*
[d] *OIE Italian Reference Laboratory for Salmonella, Istituto Zooprofilatico Sperimentale delle Venezie, Padova, Italy*

ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form 29 June 2009
Accepted 3 July 2009

Keywords:
Salmonella
Antimicrobial susceptibility
Chicks
Hatchery
Nigeria

ABSTRACT

The prevalence of *Salmonella* associated mortality at hatching was investigated in three hatcheries in Jos, central Nigeria. Their susceptibility to antimicrobial agents was also evaluated. *S.* Kentucky and *S.* Hadar were isolated. While half of the isolates were from internal organs, 26.7% came from meconial swabs of dead-in-shell embryos, 17.8% from intestinal samples and 4.4% from egg shells. *S.* Hadar is known to colonise only the gut and is classified as non-invasive, but in this study 82% were obtained from internal organs which suggests that infections with this serotype may also cause invasive disease. Antimicrobial susceptibility tests showed a high prevalence of antimicrobial resistance in the study area with complete resistance to gentamycin, enrofloxacin, nalidixic acid, tetracycline and streptomycin and substantial resistance to triple sulphur and ciprofloxacin. Six multiple resistance profiles were recorded with a high level of multiple resistance to quinolones. Quinolone resistance has implications for veterinary and human therapy as their misuse in poultry could lead to the emergence of resistant animal and zoonotic pathogens.

1. Introduction

There has been variation in the occurrence of *Salmonella* serotypes in different countries at different times, with some serotypes becoming widespread in a geographical area for a given period of time and then declining in incidence. This behaviour has been attributed to microbial competition (Nogrady et al., 2003). Globalisation and increased international trade in animal food products have facilitated the introduction of new *Salmonella* serovars into importing countries (Uyttendaele et al., 1998). Epidemiological studies have demonstrated a variety of routes through which *Salmonella* can be disseminated within a poultry enterprise (Nayak et al., 2004). Poultry can become infected by horizontal transmission through infected litter, faeces, feed, water, dust, fluff insects, equipment, fomites, diseased chicks and rodents contaminated with *Salmonella* (Poppe, 2000). They can also be transmitted by other animals, wild birds and personnel. *Salmonella* may contaminate young chicks directly through ovarian transmission or penetrate the egg shell after the egg has been laid (Cox et al., 2000). Studies have also shown that when *Salmonella*-contaminated and *Salmonella*-free eggs were incubated together the horizontal transmission of *Salmonella* occurred during hatching (Cason et al., 1994; Cox et al., 2000). Once *Salmonella* traverses the membrane, it becomes very difficult to destroy or prevent further invasion of the egg and developing embryo (Cason et al., 1994).

Salmonellosis can be controlled by controlling the sources of contamination and transmission. To achieve

* Corresponding author. Tel.: +234 08033177427 (Mobile).
E-mail address: lawal54@yahoo.com (M. Muhammad).

0378-1135/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.vetmic.2009.07.009

this, it is necessary to isolate and serotype *Salmonella* species. Serotyping can be used to identify possible transmission pathways and apply intervention measures at critical points to reduce or eliminate the disease in poultry facilities.

Antimicrobial use in food animals is also increasingly coming under close scrutiny due to increasing levels of resistance to pathogens. The rapid spread of antimicrobial resistance is due to bacterial mutation, activation of resistance determinants and vertical or horizontal spread and selection pressure (Catrey et al., 2003). Problems associated with antimicrobial resistance include increase in morbidity, mortality and costs associated with disease. As with other microorganisms a resistance problem with *Salmonella* can occur in a specific geographical area or production site and may be limited to a specific serotype (Helmuth, 2000).

No data have been evaluated in recent years on the prevalence of *Salmonella* serotypes in poultry in Nigeria. The purpose of the present study was to determine the prevalence of *Salmonellae* associated with chick mortality in three commercial hatcheries and evaluate their antimicrobial susceptibility profiles.

2. Materials and methods

2.1. Sample collection

Ten repeated samplings were made from each of the three selected commercial hatcheries on the day of hatch between March and December, 2007. Samples comprised of meconial swabs of dead-in-shell embryos, dead chicks, sick chicks and egg shells. All samples were collected aseptically, placed into ice boxes, transported to the laboratory and processed immediately on arrival. Dead chicks were autopsied immediately and lungs, heart, liver and trachea harvested. Sick chicks were euthanized humanely using cervical dislocation and their internal organs harvested. On each occasion, 4 or 5 similar internal organs from dead chicks originating from the same hatchery were pooled together and those from euthanized chicks were also pooled together, while intestinal organs were treated separately.

2.2. Isolation and identification of Salmonella

Salmonella was isolated according to standard methods (Waltman et al., 1998). Samples (5 g each) were aseptically inoculated into 50 ml each of Rappaport–Vassiliades (Oxoid) broth for enrichment and incubated at 37 °C for 24 h. Intestinal organs were incubated at 40 °C for 24 h. A loopful of each broth culture was then inoculated simultaneously onto Salmonella–Shigella Agar (SSA) (Oxoid) and Brilliant Green Agar (BGA) (Oxoid). Meconial swabs from dead-in-shell embryos and egg shells (after pounding in sterile mortar) were also treated the same way and incubated at 37 °C for 24 h. The plates were examined for typical colonies of *Salmonella* i.e. transparent colonies with black centres on SSA and pink colonies surrounded by a red medium on BGA.

Presumptive *Salmonella* colonies were inoculated into triple sugar iron (TSI) agar slants. Tubes were incubated for 24 h at 37 °C. *Salmonella* produced alkaline slant over an acid butt reaction with hydrogen sulphide (H_2S) production. Each isolate was also tested for motility, *O*-nitrophenyl-β-galactopyranoside (ONPG) hydrolysis, lysine decarboxylation, urea hydrolysis and indole production. Isolates that did not hydrolyse ONPG, were negative for urea hydrolysis (urease production), were indole negative, and were motile and positive for lysine decarboxylation were considered *Salmonella*-positive. These were then freeze-dried and shipped to the Italian Reference Laboratory for *Salmonella* (Istituto Zooprofilatico Sperimentale delle Venezie) in Padova, Italy for serotyping.

2.3. Antimicrobial susceptibility tests

Antimicrobial susceptibility testing was determined using a modification of the Kirby–Bauer disk diffusion method. Each isolate was inoculated onto Muller–Hinton agar (Oxoid) and incubated at 37 °C for 24 h according to NCCLS (National Committee for Clinical Laboratory Standards, 2004). The antimicrobial agents tested and their corresponding concentrations were as follows: amoxycillin + clavulanic acid (20 + 10 μg), ampicillin (10 μg), cephalothin (30 μg), cefatoxime (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), colistine (10 μg), enrofloxacin (5 μg), gentamycin (10 μg), kanamycin 30 μg, nalidixic acid (30 μg), streptomycin (10 μg), sulphamethoxazole + trimethoprim (23.75 + 1.75 μg), tetracycline (30 μg), triple sulpha (0.25 μg). *E. coli* ATCC 25922 was used as reference strain.

3. Results

Of the 500 samples collected, 45 (9%) were positive for *Salmonella*. Two serotypes: *Salmonella* Kentucky and *Salmonella* Hadar were isolated. *Salmonella* Kentucky was the most prevalent serotype accounting for 34 (75.6%) of the isolates while 11 (24.4%) of the isolates were *S*. Hadar. A total of 19 (42%) of isolates came from H1, 24 (53.3%) were isolated from H2 and only 2 (4.4%) from H3. 23 isolates (51%) were from internal organs, 12 (26.7%) from meconial swabs of dead-in-shell embryos, 2 (4.4%) from egg shells and 8 (17.8%) from intestinal samples. *Salmonella* Kentucky was isolated from two hatcheries (H1 and H2) while *Salmonella* Hadar was isolated from all three hatcheries.

All 45 isolates tested against antimicrobial agents were resistant to tetracycline, enrofloxacin, nalidixic acid and streptomycin (Table 1). Thirty-six (80%) were resistant to triple sulpha, 35 (77.8%) to ciprofloxacin and gentamycin and only 1(2%) isolate each was resistant to sulphamethoxazole + trimethoprim and cephalothin. All 45 isolates were susceptible to colistine, cefatoxime, amoxycillin–clavulanic acid, ceftazidime and ampicillin and chloramphenicol (Table 1). Of 19 isolates from H1, 13 (68.4%) isolates of *S*. Kentucky were all resistant to gentamycin, nalidixic acid, tetracycline, streptomycin, triple sulphur, enrofloxacin and ciprofloxacin. On the other hand, 21 (87.5%) out of 24 *S*. Kentucky isolates from H2 were resistant to gentamycin, nalidixic acid, tetracycline, streptomycin, enrofloxacin and ciprofloxacin, while