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Isolation and Antibigram of *Salmonella* spp. from Quails in a Farm from Kelantan, Malaysia

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Abstract

Salmonellosis is a major public health problem around the world affecting both animals and humans. A study was carried out to elucidate the prevalence of *Salmonella* spp. and antibiogram of the isolates in quails in a commercial farm located in Kelantan, Malaysia using cloacal swabs and standard isolation techniques for *Salmonella* species and the standard disk diffusion method for the antibiotic sensitivity tests. Ninety quails in two groups of 45 each, aged 3 weeks and 2 months, were sampled using sterile cotton swabs and transport media. The results showed that the prevalence of *Salmonella* spp. in the quails was 11.11% (CI= 6.19, 19.28) and all the isolates were resistant to ampicillin. There was no significant difference ($P>0.05$) between the prevalence of *Salmonella* spp. in birds aged 3 weeks compared with the birds aged 2 months using Chi square at 95% confidence level. The positive identification of *Salmonella* spp. in quails may have public health implications due to the rising outbreak of *Salmonella* spp. associated food poisoning cases. The resistance of the *Salmonella* spp. to ampicillin which is a common antibiotic in man and animals adds weight to the growing call for the prudent use of antibiotics in human and animal populations around the globe. Farms and food handlers should maintain strict hygiene to protect public health at all times.

Keywords: *Salmonella* spp., quails, antibiotics, prevalence, Malaysia.

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Introduction

Salmonellosis is a broad term used for infections caused by *Salmonella* species which according to the Kauffmann–White scheme, strains of *Salmonella* are classified into serovars on the basis of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) and currently over 2600 serovars are recognized (Hendriksen *et al.*, 2011; Gal-Mor *et al.*, 2014). Salmonellosis is considered one of the commonest food infections around the world and responsible for some 93.8 million cases of gastroenteritis and 155,000 deaths in humans around the globe every year in both developing and developed countries with about 80.3 million cases being foodborne (Kennedy *et al.*, 2004; Majowicz *et al.*, 2010; Crump and Heyderman, 2014; Rothrock *et al.*, 2015). Many infections are due to ingestion of food contaminated with *Salmonella* species which can be divided into two groups-typhoidal and nontyphoidal *Salmonella* serovars. Nontyphoidal serovars are more common, and usually cause self-limiting gastrointestinal diseases. They can infect a wide range of animals, and are zoonotic. Typhoidal serovars include *Salmonella typhi* and *Salmonella paratyphi* A, which are adapted to humans and do not occur in other animals (Gal-Mor *et al.*, 2014). The major source of human *Salmonella* infections are considered to be from food products of animal origin such as meat, eggs, egg products, milk and milk products which could serve as vehicles of transmission of infection that affects public health and food safety (Behraves *et al.*, 2014; Crump and Heyderman, 2014; McEntire *et al.*, 2014). There are two main kinds of systemic avian salmonellosis: the chicken-adapted *Salmonella* serovar gallinarum biovars pullorum and gallinarum are responsible for pullorum disease and fowl typhoid, respectively all over the world (Barrow and Freitas Neto, 2011).

In addition to these two clinically systemic salmonellosis, birds may also be infected by *Salmonella* paratyphoid serovars and other species of *Salmonella* and develop clinical disease or may become asymptomatic carriers and potential sources of human salmonellosis (Schoeni *et al.*, 1995; Gast *et al.*, 2013; Behraves *et al.*, 2014). The question

of what is the status of avian salmonellosis in quails and to what extent is it a problem in quail production today still needs to be answered, as literature reports on this subject are not conclusive (Kumar *et al.*, 2001; Sander *et al.*, 2001; McCrea *et al.*, 2006; Bacci *et al.*, 2012; Youssef and Mansour, 2014).

Just like the other domestic poultry industry, quail meat production has benefitted from genetic improvement, better feed efficiency and the use of modern housing facilities that allow rearing quails at high densities. However, some of these factors have also contributed to the entrance and dissemination of avian pathogens, such as *Salmonella* spp. (Burkholder *et al.*, 2008; Schulz *et al.*, 2011; De Vylder *et al.*, 2011). General biosecurity and hygiene measures adopted in poultry farms and processing plants have reduced, but not prevented the presence of *Salmonella* spp. But there is an increasing pressure to reduce contamination of food products especially at farms (Davies *et al.*, 2003).

Quail farming was introduced in Kelantan, Malaysia in the early 1990s for egg and meat production purposes. During the last decade, quail meat production has also become an alternative for the Malaysian poultry sector with increasing demand for quail meat and eggs. Due to special features, such as low initial capital investments, small size housing needs, easy management and fast financial returns, quail production has grown in Malaysia. But unfortunately the last Kelantan great flood of December 2014 to January 2015 had destroyed almost all the ten available quail farms in Kelantan, Malaysia leaving only three farms functioning.

Antibiotics which are very essential drugs for human and animal health are often used by veterinary surgeons and farmers on pets and farm animals for therapeutic and prophylactic treatment, and also to promote growth (Graham *et al.*, 2007). These routine practices are important factors in the emergence of antibiotic-resistant bacteria that subsequently can be transmitted from animals to humans through the food chain. Most antimicrobial-resistant *Salmonella* infections are acquired from eating contaminated foods of animal origin (Angulo *et al.*, 2000). During recent years

some bacteria have shown full or partial resistance to different antibiotics. This increasingly global phenomenon, called antimicrobial resistance, is a rising concern in both public and animal health (Holt *et al.*, 2007; Abo-amer and Shobrak, 2015). The mechanisms by which microorganisms exhibit resistance to antibiotics include drug modification or inactivation, development of new genes, alteration in metabolic pathway, alteration of target site and reduced drug accumulation (Andersson and Hughes, 2011; Blair *et al.*, 2014).

The quail industry is a rather new industry at Kelantan, Malaysia. There are not many studies published on *Salmonella spp.* in quails and their significance in public health. There has been no published report on the isolation of *Salmonella spp.* and antibiotic sensitivity tests in Kelantan quails. This study was carried out to isolate *Salmonella spp.* from the cloacal swabs of quails and to determine the antimicrobial susceptibility of the *Salmonella spp.*

Materials and Methods

Study Area and Sampling

This study was carried out in Kota Bharu, Kelantan state in the East coast of Peninsular Malaysia (06°10'N and 102°20'E). Out of three available quail farms in the state, one was identified as a commercial farm that serves the community located at Kota Bharu, the state capital that has a human population of 468, 438 (Khan *et al.*, 2014). This study was carried out on one of the few remaining quail farms at Kota Bharu Kelantan, Malaysia after the great flood, which was a commercial farm. Sample size was calculated using Epi Info version 7 (Dean *et al.*, 1990). Based on the population of quails on the farm and the calculated sample size, 90 samples were taken using sterile cotton swab: 45 samples from 3 weeks old birds and 45 samples from 2 month old birds. Each swab was placed in a bottle containing Buffered Peptone Water (BPW) (Oxoid) as pre-enrichment medium and placed straight inside an icebox with a temperature of 4 degree celcius. The samples were brought to the Bacteriology laboratory at Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The samples were processed within 3-4 hours.

Sample Preparation and Isolation

The bottles containing swabs in BPW were incubated aerobically at 37°C for 24 h. One ml of each pre-enriched culture were transferred into Tetrathionate (TT) Enrichment Broth (Oxoid) and incubated aerobically at 42°C for 48 hours. Then, two to three loopfuls of each enriched broth culture were streaked onto the surface of a selective medium, Xylose Lysine Tergitol 4 Agar (XLD) (Merck), then all plates were incubated at 37°C for 24 to 48 hours.

Identification

Typical *Salmonella* colonies are pink to red with black centre (or occasionally translucent red in the case of H₂S negative strains) on XLD agar. Ten colonies were picked and subcultured to obtain pure cultures on XLD agar at 37°C for 24 to 48 hours. Isolates were identified by colony morphology, Gram's stains and biochemical characterization including urease test and inoculation onto Triple Sugar Iron (TSI) agar (Oxoid) and Simon citrate test (Oxoid). This was to rule out other *Enterobacteriaceae*. These single colonies were then kept in nutrient agar as stock culture for further identification.

Polyvalent O Antisera Test

All of the presumptive colonies were tested using the polyvalent O antisera test to observe for agglutination of the bacteria sample that indicates positive for *Salmonella spp.*

Antimicrobial Sensitivity Test

Standard disk diffusion method using Mueller-Hinton agar (MHA) was carried out on all *Salmonella* isolates to test for their antimicrobial susceptibility patterns. The isolates were tested for resistance against ampicillin, fluoroquinolone, trimethoprim-sulfamethoxazole, third-generation cephalosporins and chloramphenicol, which are commonly used antibiotics against salmonellosis. Finally, the results were interpreted in accordance to the criteria set by the Clinical and Laboratory Standards Institute (CLSI, 2008).

Statistical Analysis

The data collected in this research was analysed using Microsoft Excel (Microsoft Corporation) and SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) using Chi square analysis at 95% confidence level for the two age categories of birds sampled.

Results

Salmonella spp were present in 10 of the 90 (11.11%) samples (Table 1). *Salmonella* spp was isolated from 7 out of 45 (15.56%) of the 3 week old chicks and 3 out of 45 (6.67%) of the 2 month

old birds. There was no statistical difference ($P>0.05$) between the prevalence of *Salmonella* spp. in the two age categories of 3 weeks old and 2 month old chicks using Chi square in SPSS version 22. Out of the 90 samples, 10 colonies were confirmed colonies of *Salmonella* spp. The XLD agar of the 10 colonies were red with black centers (Figure 1) and the polyvalent O antisera test (Figure 2) showed all 10 samples positive for *Salmonella* spp. The antibiotic sensitivity test (Figure 3) showed that all 10 samples were resistant to ampicillin only.

Table 1: Colony characteristics of isolates.

Colony description	Yellow, medium with black centers	Red, medium with black centers	Grey, mucoid, medium	Yellow, medium with scrambled egg appearance
Number of samples	40	10	7	33



Fig. 1: *Salmonella* spp on XLD agar.

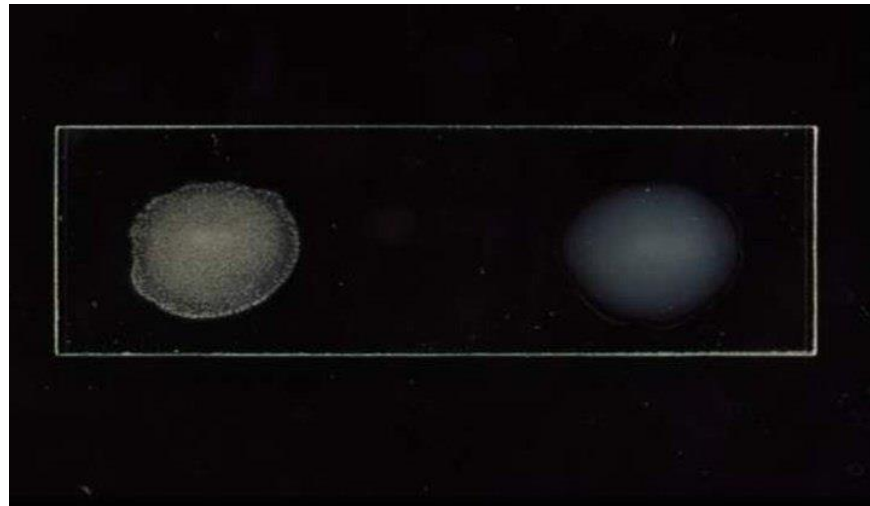


Fig. 2: Positive agglutination for polyvalent O antisera test (left) and negative (right).

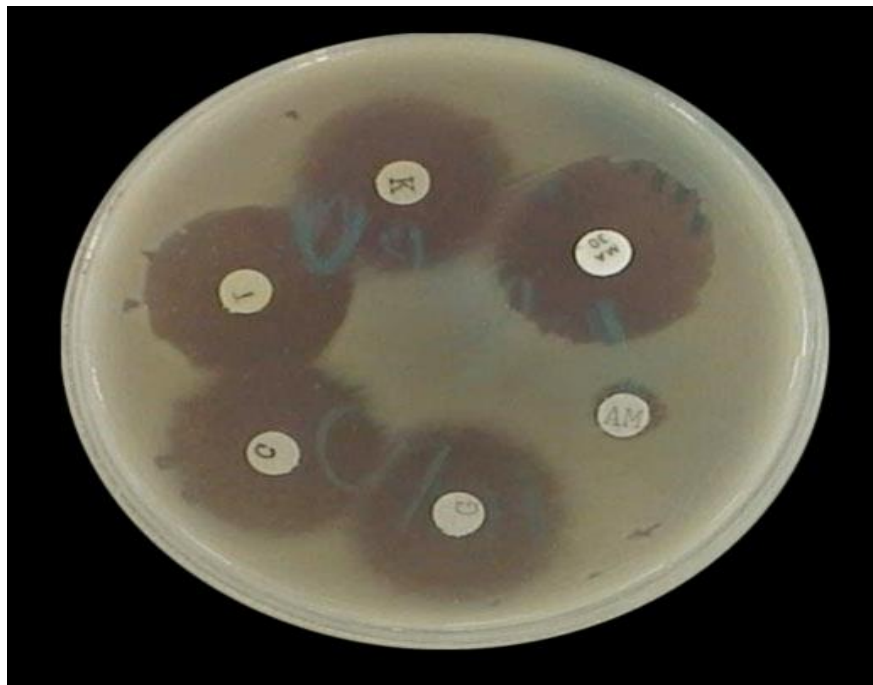


Fig. 3: Isolated *Salmonella* only resistant to ampicillin.

Discussion

The inability to identify the species of *Salmonella* isolated is a limiting factor for this study and therefore the findings should be interpreted with caution. It is known that a lot of serotypes of *Salmonella* affect humans and also animals but some are more pathogenic for humans and animals (Hendriksen *et al.*, 2011; Kennedy *et al.*, 2004) and some of these pathogenic species have been

reported in quails in some previous studies by other workers (Sander *et al.*, 2001; Bacci *et al.*, 2012). The high number of *Salmonella* negative cloacal swab samples may be associated with the sensitivity of this procedure for the identification of *Salmonella* spp. Some researchers have questioned the effectiveness of swabs for the detection of *Salmonella* in animals (Aho, 1992). Other methods such as rinsing the whole carcass or immersing it completely in sterile water or normal saline may

yield better results but are not feasible in life birds. The intermittent shedding of *Salmonella*, associated with the fact that it can be shed in small amounts in the feces may impair detection to an extent making cloacal swabs of low sensitivity and may lead to high false negatives results (Higgins *et al.*, 1982; Avaliaram-se *et al.*, 2007). However, our findings are much higher than the one in California, USA where no *Salmonella* was isolated from 3 flocks of farm quails (McCrea *et al.*, 2006). In another study in Italy there was also 0% prevalence for *Salmonella* in quails (Dipineto *et al.*, 2014). This shows that the prevalence in our study is relatively high and raises concern about the potential transmission of this infection to the human population during the course of handling or preparation because a commercial quail operation had been found to be dynamically contaminated with different zoonotic species of *Salmonellae* from the farm to the market in a United States commercial quail operation (Sander *et al.*, 2001). Food poisoning cases have increased by upto 100% in recent years in Kelantan, Malaysia with *Salmonella* spp implicated as one of the causes of the food poisoning outbreaks (Khaled *et al.*, 2011).

Age of birds does not play a determinant role in the prevalence of infection with Salmonellosis as birds and indeed animals of all ages are susceptible to infections (Feasey *et al.*, 2012; Setta *et al.*, 2012; Batista *et al.*, 2015). This can be seen by different reports around the world where salmonellosis was found to affect all age groups in animals as well as man but sometimes the severity and prevalence may show significant difference in some age groups (Gast and Beard, 1990; Holt and Porter, 1992; Tajbakhsh *et al.*, 2012; Uduak, 2015). Antimicrobial agents are medicines used to treat infections caused by bacteria in particular. They are essential drugs for animal and public health, but in recent years some bacteria, especially *Salmonella*, have demonstrated full or partial resistance to different antibiotics. This is a point of concern considering typhoidal and non-typhoidal salmonellosis is the most economically important foodborne disease (Uduak, 2015). In our study, the demonstration of resistance to ampicillin is of great concern because it is one of the commonly used antibiotics against salmonellosis in animals and

humans depending on the isolated species. In Korea, ampicillin resistance in hospital patients increased from 20% in 2009 to 36% in 2011 for non-typhoidal *Salmonella* spp. and the resistance rates varied significantly from 3% to 83% depending on hospital (Yong *et al.*, 2014).

The presence of antimicrobial resistance in the quail farm, which is a commercial farm that serves a whole state in Malaysia and beyond may have epidemiological and public health implications including the transfer of these resistance genes to *Salmonella* in humans and other animal populations (Uduak 2015; Wakawa *et al.*, 2015). With the ongoing discourse on antibiotic resistance that is increasing around the world, farmers and all food handlers from farm to fork must ensure that hygienic methods are employed in the whole process involved in the food distribution chain. The maintenance of strict hygiene on the farm and less use of antibiotics will ultimately reduce incidences of salmonellosis and antimicrobial resistance. This would lead to greater and better animal productivity and enhance public health.

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