First isolation of Salmonella Duisburg from quail flock

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SUMMARY

The first isolation of *Salmonella enterica* subsp. *enterica* serovar Duisburg (*S.* Duisburg) (4,12,[27]:d:e,n,z_{15}) from quails was presented in this case report. Internal organs and ileocecal parts of intestines were collected from quails at 20-day old age in the flock (total of 150 quails) located in South Marmara region of Turkey. Isolation was performed according to International Organization for Standardization Method 6579. Regarding the identification of *Salmonella*-suspected colonies, API 20E test strips and Phoenix 100 ID/AST system were used. Serotyping of the isolate was undertaken using the slide serum agglutination test. Minimum inhibitory concentration results showed that *Salmonella* isolate was susceptible to all the tested antimicrobials. Although the prominent species is chicken in poultry, quail breeding increases its importance and extensiveness. Therefore this study may be useful not only for current antibiotic practices in quail breeding but also for further studies on avian microbiology.

KEY WORDS

Quail, *Salmonella*, serotyping, *S.* Duisburg, antibiotic susceptibility.

INTRODUCTION

Salmonella serotypes are significant bacterial pathogens for poultry worldwide. Vertical (from breeder stocks to their chicks via eggs) and lateral transmission (from environment contaminated with faeces from infected chickens to healthy chickens) occur in *Salmonella* infections.

Moreover, the infection has a high transmission capability among individuals with respect to feed, drinking water or environmental sources. Therefore prevention strategies including good monitoring and screening programs are essential in the flock to preclude economic losses[1].

Salmonella genus has more than 2500 serotypes and has a wide host range which comprises several animal species within mammals, birds and reptiles[1]. A number of studies in Turkey have shown that *Salmonella* infections are endemic in many parts of the country and *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) and *S.* Infantis are the most common serotypes[2,3]. Observations of the remaining serotypes are rare, such as *S.* Thompson, *S.* Agona and *S.* Duisburg. Worldwide, *S.* Duisburg was isolated from cattle egrets[4], chicken carcasses[5], turkey[6], badger, cattle, sheep, pig[7], lizard[8], and drinking water[9]. However, no information about this pathogen is available in quails. Hence, to the best of authors' knowledge, this is the first report indicating the presence of the *S.* Duisburg from ileocecal part of the intestines in quail flock.

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CASE PRESENTATION

Samples were collected from egg-laying Japanese quail (*Coturnix coturnix japonica*) flock (a total of 150 quails) that suffered from diarrhoea. The flock was located in Bursa, Turkey and the birds were housed in cages with dimensions of 100x45x20 cm. Each cage consists of 8-10 quails which were fed with the same commercial diet. The birds were provided with 16 h of light in a ventilated room at approximately 22-24 °C ambient temperature. No vaccinal program was applied. In the flock, the mortality rate was 20%. Four quails at 20-day old age were necropsied and samples from lung, liver, kidney, heart and ileocecal part of intestines were collected for culture method. Ileocecal parts of intestines were pooled into one sample.

Concerning necropsy findings, there were no lesions in the internal organs including lung, liver, kidney, and heart. However, there were signs for enteritis such as gross lesions and swelling in intestines. In order to investigate the presence of bacteria that could be the potential cause of clinical signs, initially, internal organs were examined by standard bacteriological method. In this context samples were inoculated on two 5% blood agars (GBL, Turkey), a MacConkey (MC; Merck, Darmstadt, Germany) agar and a Mycoplasma agar (Oxoid, CM0401, England). One blood agar and Mycoplasma agar were incubated at 37 °C in microaerophilic atmosphere while the others were incubated in an aerobic atmosphere. Results revealed that there was no *Salmonella* agent in internal organs nor was there any other bacteria.

Isolation and identification of *Salmonella* from ileocecal parts of intestines were performed according to International Organization for Standardization Method 6579[10]. Briefly, 1 g of the sample was pre-enriched in 9 ml of buffered peptone wa-

ter (BPW; Merck, Darmstadt, Germany) and incubated at 37 °C for 18 h. After incubation, 0.1 ml of pre-enrichment broth culture was transferred into modified semi-solid Rappaport Vassiliadis agar (MSRV; Oxoid, CM1112) and incubated at 41.5 °C for 24 h. Afterwards, a loop, full of MSRV culture, was streaked separately onto the surface of Xylose Lysine Desoxycholate agar (XLD; Oxoid, CM0469) and MC agar. They were incubated overnight at 37 °C. Following incubation, colonies were analysed to identify typical morphology of *Salmonella*. Typical *Salmonella* colonies are pink-edged and black centred on XLD agar, while, pale translucent on MC agar. Identification of suspected *Salmonella* colonies was performed by biochemical tests according to their urease activity (Urea Agar Base, Oxoid, CM0053), triple sugar utilization and hydrogen sulphide (H_2S) formation (Triple Sugar Iron Agar, Oxoid, CM0277), and lysine decarboxylase activity (Lysine Iron Agar, Oxoid, CM0381). Final identification was performed using both API 20E test strips (bioMerieux, Marcy L'Etoile, France) and Phoenix 100 ID/AST system (Becton Dickinson Co., Sparks, Md.). The NMIC/ID-433 panel was processed in a Phoenix 100 ID/AST system according to the manufacturer's directions. The existence of *Salmonella* spp. was substantiated by the above-mentioned test protocols. Minimum inhibitory concentration (MIC) results were evaluated by using Phoenix AST panel for amikacin (8-32 µg/mL), gentamicin (2-8 µg/mL), ertapenem (0.25-1 µg/mL), imipenem (0.25-8 µg/mL), meropenem (0.125-8 µg/mL), cefazolin (4-32 µg/mL), cefuroxime (4-16 µg/mL), ceftazidime (1-8 µg/mL), ceftriaxone (1-4 µg/mL), cefepime (1-8 µg/mL), ceftolozane-tazobactam (1/4-4/4 µg/mL), ampicillin (4-16 µg/mL), amoxicillin-clavulanate (2/2-16/2 µg/mL), ampicillin-sulbactam (1/8-8/8 µg/mL), piperacillin-tazobactam (4/4-16/4 µg/mL), colistin (1-4 µg/mL), trimethoprim-sulphamethoxazole (2/38-8/152 µg/mL), ciprofloxacin (0.0625-1 µg/mL), levofloxacin (0.5-2 µg/mL), and tigecycline (0.5-2 µg/mL). Isolate, identified as *Salmonella*, was susceptible to all the tested antimicrobials.

Salmonella isolate was serotyped according to the Kauffmann-White-Le Minor Scheme[11]. Identification of the O and H group antigens was performed by slide agglutination tests by means of O4, O12, O27, Hd antisera (Statens Serum Institute, Copenhagen, Denmark) and He, Hz_{15} antisera (Denka Seiken Co., Ltd. Tokyo, Japan). Thus, the isolate was serotyped as Duisburg with the antigenic formula 4,12,[27]:d:e,n,z_{15}.

DISCUSSION

Salmonellosis is one of the major bacterial diseases in poultry industry and it frequently involves in outbreaks and cases of foodborne diseases which cause millions of human infections and even deaths[12]. The consumption of contaminated poultry products has been reported to be the most important reason for *Salmonella* infections in humans[3]. Therefore, studies on salmonellosis should be carefully considered not only for poultry industry but also the humans' health. It is clear that the main component of poultry is chicken but other species, such as quail, turkey, duck, geese, and etc., are significant sources of both meat and egg production worldwide. These species have also been reported to be the potential carriers of *Salmonella* spp. which serve as the sources of exposure or infection for humans. However in the literature there is limited information about *Salmonella* infections in quail. In this study *S.* Duisburg was firstly identified in quails. This serotype, was first isolated by Korell and Seeliger[13] from a child with diarrhoea in 1953. Although *S.* Duisburg is not a common serotype in poultry, there are few studies indicating the presence of *S.* Duisburg observed in salmonellosis cases[6,14].

In the present study *S.* Duisburg was isolated from ileocecal part of the intestines in quails. It is important to note that the knowledge on the existence of *Salmonella* serovars in quail is rather limited compared to prominent avian species such as chicken and turkey. In this context, there are some studies reporting *Salmonella* spp. in quails but the determination of serovars was not performed in these studies. For instance, in the study by Omoshaba et al.[15], four hundred cloacal swabs of quail birds were collected in Nigeria, and in all, *Salmonella* was isolated from 14 (3.5%) cloacal swabs. In addition Palanisamy and Bamaiyi[16] indicated that the prevelance of *Salmonella* spp. was 11.11% in the three quail farms located in Malaysia. Jahan et al.[17] suggested that the overall prevalence of *Salmonella* spp. in quails was found to be 13.33%. On the other hand, McCrea et al.[18] performed a detailed analysis to investigate the existence of *Salmonella* spp. and *Campylobacter* spp. among the various avian species including squab, quail, guinea fowl, duck, poussin (young chicken), and free-range broiler chickens. However, their results showed that there were no *Salmonella* isolates in the samples obtained from guinea fowl and quail flocks. Similarly, Dipineto et al.[19] reported that there was no detection of *Salmonella* spp. in common quails cloacal swab samples.

In the literature, there are several studies on the isolation of *Salmonella* serovars including *S.* Virchow, *S.* Meleagridis, *S.* Typhimurium in Japanese quails[20], *S.* Typhimurium, *S.* Typhimurium variant Copenhagen, and *S.* Hadar in live commercial quails and carcasses, and *S.* Paratyphi in the flock environment[21,22]. Moreover, Boroomand et al.[23] investigated Enterobacteriaceae responsible for early mortality in Japanese quail chicks and the results showed that 78% of them were infected with *Escherichia coli (E. coli)*, *S.* Ruzizi, *S.* Typhimurium. These authors reported that *E. coli* and *Salmonella* spp. are the major causes of early mortality in newly-hatched Japanese quail chicks.

In this study antimicrobial susceptibility was evaluated by MIC analysis and the results revealed that *S.* Duisburg was 100% susceptible to all tested antimicrobials including amikacin, gentamicin, ertapenem, imipenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, colistin, trimethoprim-sulphamethoxazole, ciprofloxacin, levofloxacin, and tigecycline. Unlike our results, many studies showed the presence of multidrug resistant *Salmonella* in quails. In this respect, Omoshaba et al.[15] reported that the *Salmonella* isolates exhibited variable rates of resistance to antimicrobials, as follows: ampicillin (100%), tetracycline (100%), doxycycline (100%), sulphamethoxazole (92.9%), nalidixic acid (85.8%), ceftazidime (78.6%), neomycin (64.3%), streptomycin (50%) and gentamicin (28.6%). However all the isolates were susceptible to ciprofloxacin. In a similar vein, Jahan et al.[17] suggested that all *Salmonella* isolates were found to be multidrug resistant (100% resistant to erythromycin and tetracycline and 90% to colistin sulphate). On the other hand Palanisamy and Bamaiyi[16] found that *Salmonella* isolates were resistant to ampicillin only. Antibiotic resistance, which can affect the severity of salmonellosis, makes the infections much more difficult to treat be-

cause it reduces the number of effective antibiotics and causes delayed or inadequate therapies[5,14]. The resistance formation can be prevented by prudent use of antibiotics supported by antibiogram tests before drug administration or avoiding incorrect use of antibiotics in livestock as well as poultry. The 100% susceptibility to tested antibiotics observed in this study may be a result of limited utilization of the mentioned antimicrobials in quails.

Consequently, the present study is the first report of *S.* Duisburg in quail. Further detailed epidemiological and molecular studies are essential on the determination of observation frequency of this serovar in various avian species such as quails.

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