

Potential Role of Migratory Quail in Spreading of Some Zoonotic Pathogens in Egypt

¹Ahmed I. Youssef and ²Dalia H. Mansour

¹Department of Animal Hygiene and Zoonosis (Division of Zoonosis),

²Department of Poultry and Rabbit Medicine,

Faculty of Veterinary Medicine, Suez Canal University, 41522, Ismailia, Egypt

Article history

Received: 27-05-2014

Revised: 10-07-2014

Accepted: 28-11-2014

Corresponding Author:

Dalia H. Mansour

Department of Poultry and
Rabbit Medicine, Faculty of
Veterinary

Medicine, Suez Canal

University, 41522, Ismailia,

Egypt

Email: Dalia97.mh@gmail.com

Abstract: This study aimed to illustrate the potential role of wild migratory quails in the epidemiology of some zoonotic pathogens in Egypt. Ninety four apparently healthy migratory quails were collected from public markets after being trapped during hunting season from the North Sinai. Oral, cloaca and organs (liver, lung and heart) swabs were collected for bacteriological and viral examination. Bacteriological examination revealed high percentage of lactose fermenter bacteria (48.9%) including *E. coli* spp. (37.2%) and *Salmonella* spp. (24.4%). Three out of 12 (25%) of *Salmonella* spp. Isolates were *S. enterica* subspecies *typhimurium*. Other bacteria isolation rate was differed such as *Staphylococcus aureus* spp. (19.1%), *Proteus* spp. (7.4%), *Listeria* spp. (4.2%) and *Klebsiella* spp. (3.1%). *E. coli* spp. and *Salmonella* spp. isolates were further examined by API20 and PCR techniques. Antibiogram testing against 32 *E. coli* spp. and 12 *Salmonella* spp. isolates, revealed multi-drug resistance pattern and high sensitivity to enrofloxacin, nitrofurantoin and ofloxacin. Parasitological examination revealed that 35 (37.2%) quails were infected with chewing lice (*Phthiraptera* spp.). Intestinal infection with *Heterakis* spp. and/or *Ascarida* spp. Was detected in 27 (28.7%) quails. All quails were negative for avian influenza virus by direct detection by Rt-PCR for oral and cloaca swabs followed by inoculation in embryonated chicken eggs. In conclusion, the migratory quails might play a potential role in dispersion of zoonosis in Egypt.

Keywords: Quails, Migratory Birds, Bacteria, Parasites, Viruses, Zoonosis

Introduction

Migratory quail, known as common quail (*Coturnix coturnix*), is one of the most migratory birds migrates from Europe to Egypt during autumn. During the hunting season, many thousands of these birds are caught in hunter nets on arrival to Mediterranean shore of the North Sinai, Egypt. As other migratory birds, migratory quail act as possible (biological and/or mechanical) vectors playing role in the ecology and circulation of some zoonotic pathogen threatening human health and domestic animals (Abulreesh *et al.*, 2007; Benskin *et al.*, 2009). These zoonotic pathogens cause losses of efficient production and quality of food of animal origin (Acha and Szyfres, 1987; Stohr and Melsin, 1997; Kahn, 2006). Migratory quails pose a risk of transmission of many zoonotic diseases to hunters or consumers who handle or eat these birds by either direct or indirect contact (Smith, 1999). Moreover, wild birds

encourage new health problems in wildlife population to emerge, as well as novel reservoir of zoonotic disease could be transmitted and emerge from one continent to another by migratory birds (Cole *et al.*, 2005).

Escherichia coli and *Salmonella* are considered as the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry. Other zoonotic pathogens such as *Staphylococcus* spp. and *Proteus* spp. are considered the most important bacterial pathogen isolated from migratory quails (Mohamed *et al.*, 2001; Effat and Moursi, 2005). Moreover, the development of antimicrobial resistant strains of zoonotic bacteria constitutes a public health risk, increasing risk of treatment failures (Middleton and Ambrose, 2005).

Avian influenza AI viruses constitute global health concern. Susceptibility to AI Viruses (AIV) varies deeply among wild bird and poultry species, as well as their possible role as vectors, intermediate hosts or reservoirs. Quails could play a key role in AIV

epidemiology (Bertran *et al.*, 2011). It is proposed that quails may generate new re-assortant influenza viruses (Thontiravong *et al.*, 2012).

This study aimed to evaluate the occurrence of some zoonotic pathogens which could be transmitted by wild quails in Egypt as well as studying the antibiotic resistance of some isolated bacterial strains.

Material and Methods

Bird Collection

Ninety four apparently healthy live migratory quails were collected from the public markets in two different geographic locations namely Ber-Elabd and Romana (Fig. 1) across the shore of Mediterranean sea in the North of Sinai, Egypt during hunting season (5th and 20th September and 5th October 2012). All birds were live trapped through nets before being sold out in the markets. Quails were delivered quickly to the laboratory.

Sample Collection

After being delivered to the laboratory, quails were kept under observation for 24 h for clinical examination. Oral and cloaca swabs were collected in two sets tubes, the first contained 10 mL buffered peptone water for bacterial isolation and the second contained 10 mL of Phosphate-Buffered Saline (PBS) solution with antibiotic

for viral isolation. Swabs were collected from organs (heart, liver and lung) under aseptic conditions. Intestines were directly opened during postmortem examination and were examined for presence of adult helminthes.

Bacteriological Examination

Isolation and Identification of Bacteria

Swabs pre-enrichment was performed in buffered peptone water (LabM) at a temperature of 37°C for 18-24 h followed by plating onto MacConkeyagar (OXOID, Hampshire, UK) and incubated at 37°C for 24 h. The lactose fermenting colonies suspected to *E. coli* were re-inoculated to Eosin Methylene Blue (EMB) agar. Colonies producing metallic sheen were suspected to *E. coli* strains.

For *Salmonella* spp. isolation, pre-enrichment was followed by selective enrichment in Rappaport-Vassiliadis (OXOID, Hampshire, UK) at 42°C for 24 h followed by plating on Xylose, Lysine and Desoxycholate (XLD) (LabM) and incubation at 42°C for 24 h. Suspected *E. coli*, *Salmonella*, *Proteus* spp. and *Keilbseilla* spp. were biochemically identified according to Edwards and Ewing (1972)? Furthermore, suspected *E. coli*, *Salmonella* isolates were confirmed using the API 20 test (bioMe'rieux, France). Multiplex PCR was used for identifying *S. Enteric serovars typhimurium and enteritidis*.



Fig. 1. Map of Egypt showing the areas of sample collection referred as two red dots

Isolation and Identification of *S. Aureus*

Pre-enrichment swabs were plated onto selective Mannitol salt agar (LabM) and cultured at 37°C for 24-48 h. Colonies were identified according to Kloos and Bannerman (1999). *S. aureus* strain, Designation; NCTC7447/ATCC ®6538P was used as control positive.

L. monocytogenes was isolated by pre-enrichment broth incubated at 30°C for 48 h for followed by plating on Listeria Isolation Media, Oxford (ISO) with selective additives (LabM). Grey colonies with black halo were identified as *L. monocytogenes* after incubation at 37°C for 48 h.

DNA Extraction and PCR Amplifications

Bacterial DNA was extracted from cultured broth by centrifugation at 4°C at 3,000×g for 10 min. The pellet was washed twice with PBS and the cells were re-suspended in 800 µL of sterile distilled water and boiled for 10 min. The resulting solution was centrifuged at 3,000×g for 10 min and the supernatant was used as the DNA template. Amplification reactions were carried out with 5 µL of boiled bacterial suspensions, 5 µL of 5X Taq Master Mix/high yield (Jena Bioscience, GMBH, Germany) and two pairs of each primer 50 pmol. Distilled water was added to bring the final volume to 25 µL. *E. coli* spp. isolates; general primers were used in PCR amplification of 670 pb segment of Glutamate Decarboxylase (GAD) *gadA/B* gene according to McDaniels *et al.* (1996). Positive microbiological *Salmonella* were confirmed and serotyped as *typhimurium* and *enteritidis* by using multiplex PCR using three sets of primers were used for PCR as described previously by Alvarez *et al.* (2004). *S. aureus* isolates were confirmed by amplification of *16SrRNA* gene according to Monday and Bohatch (1999) that generated a 228-bp amplicon during the amplification process. PCR conditions were started by initial denaturation step of 2 min at 95°C, followed by 30 cycles of 95°C for 30 min, 57-60°C for 30 s and 72°C for 30 sec. The reaction was terminated with 10 min incubation at 72°C. The PCR reaction products were subjected to electrophoresis in a 1.5% agarose gel, stained with ethidium bromide and visualized and photographed under UV light. Products of the appropriate sizes were identified by comparisons with a 100 bp DNA ladder (Gibco). In each PCR run, a non-template control was included to detect possible external DNA contamination and control positive were used for confirmation.

Antimicrobial Susceptibility Testing

Susceptibility of the isolates to 13 types of antibiotics was performed using the standard Kirby-Bauer method. The isolates were inoculated in nutrient broth at 30°C. Commercially available discs (Oxoid, UK) containing Amoxicillin (AMC) (20+10), Cefotaxime (CTX)(30) and

Ciprofloxacin (CIP)(5), clavulanic, clindamycin (DA)(2), colistin (10), Erythromycin (E)(15), gentamycin (CN)(10), Oxytetracycline (T)(30), Sulbactin-Ampicillin (SAM) (10+10), Trimethoprim Sulphamethoxazole (SXT)(1.25+23.75), Nitrofurantoin (F300), Ofloxacin (OFX)(5), Enrofloxacin (ENR)(10) were placed on the surface of the Muller Hinton agar plates and incubated at 30°C for 24 h. The diameters of inhibition zones formed surrounding each isolate were measured inclusive diameter of the discs. Results were expressed as susceptible (≥21 mm); intermediate (16 to 20 mm) or resistant (≤15 mm) followed a standard range (Liasi *et al.*, 2009). All isolates were tested duplicate for each type of anti-biotoxic accordance with the criteria of the Clinical Laboratory Standards Institute, formerly guidelines (NCCLS, 2009). Antibiotic discs used and its concentrations were shown in Table 2.

Parasitological Examination

The birds were examined for external parasite and then necropsied for examining of intestine for helminthes parasites. Adult helminthes detected by naked eye were individually recovered, fixed and stored for later identification.

Influenza Virus Isolation and Identification

Oral and cloaca swabs were detected by direct by RT-PCR and by inoculation in 10-day-old embryonated specific- pathogen-free SPF eggs through allantoic sac route. The inoculated eggs were held at 37°C and candled daily for 5 days. The allantoic fluid was collected and tested by RT-PCR. Swabs were tested for the presence of influenza A viruses by detection of the Matrix (M) gene, using a viral RNA extraction kit (QIAamp, QIAGEN, Germany) and a one-step real-time RT-PCR kit (Quantitect, QIAGEN, Germany). The test was conducted in a Stratagene MX3005P real-time PCR machine (La Jolla, CA) using protocol that was previously described by Aly *et al.* (2008).

Results

Clinical and Post-Mortem Examination

All quails were apparently healthy. Post-mortem examination did not indicate any abnormalities except adult helminthes in the intestine and the ceca.

Occurrence of most Pathogenic Bacteria in Oral and Cloaca Swabs

As illustrated in Table 1, results of bacteriological examination revealed high percentage for lactose fermenter isolates (48.9%); including *E. coli* spp. (37.2%), *Salmonella* spp. (24.5%). *S. typhimurium* was identified in 3 out of 12 (25%) of *Salmonella* spp. isolates while none of *Salmonella* spp. isolates was identified as *S. Enteritidis* by PCR technique.

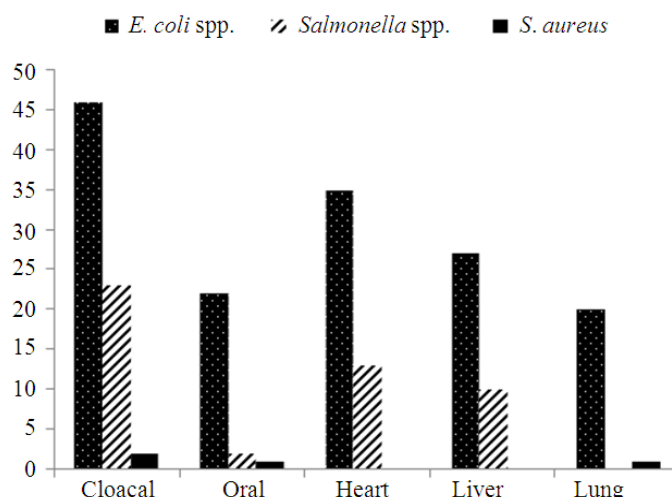


Fig. 2. Rate of isolation of *E. coli* spp. *Salmonella* spp. and *S. aureus* from different body sites

Table 1. Incidence of most zoonotic pathogens isolated from examined quails

Collection times	<i>E. coli</i> +ve %	<i>Salmonella</i> spp. +ve %	<i>S. aureus</i> +ve %	<i>Proteus</i> spp. +ve %	<i>Listeria</i> spp. +ve %	<i>Klepsi</i> ala spp. +ve %
1st (n=34)	11 11.7%	10 10.6%	7 7.4%	2 2.1%	1 1.06%	1 1.06%
2 nd (n=40)	19 20.2%	8 8.5%	10 10.6%	4 4.2%	2 2.1%	2 2.1%
3rd n=20	5 5.3%	5 5.3%	1 1.06%	1 1.06%	1 1.06%	0 0%
Total n=94%	35 37.2%	23 24.4%	18 1%	7 7.4%	4 4.2%	3 3.1%

Table 2. Comparative efficacy of antibiotic sensitivity of 16 antimicrobials against isolates collected from heart

Antimicrobial agent code	<i>E. coli</i> spp.			<i>Salmonella</i> spp.		
	S	M	R	S	M	R
	No %	No %	No %	No %	No %	No %
(T)	9 28.1%	5 15.6%	18 56.2%	2 16.6%	3 25%	7 58.3%
(CT)	2 6.25%	8 25%	22 68.5%	0 0%	2 16.6%	10 83.3%
(E)	21 65.6%	5 15.6%	6 18.7%	6 50%	3 25%	3 25%
(CN)	5 15.6%	7 21.8%	20 62.5%	2 16.6%	4 33.3%	6 50%
(AMC)	3 9.3%	5 15.6%	24 75%	1 8.3%	5 41.60%	6 50%
(SXT)	27 84.3%	3 9.3%	2 6.2%	8 66.6%	1 8.3%	3 25%
(CIP)	26 81.2%	4 12.5%	2 6.2%	5 41.6%	5 41.6%	2 16.6%
(SAM)	0 0%	7 21.8%	25 78.1%	1 8.3%	1 8.3%	10 83.3%
(CTX)	29 90.6%	3 9.3%	0 0%	8 66.6%	4 33.3%	0 0%
(DA)	5 15.6%	7 21.8%	20 62.5%	2 16.6%	4 33.3%	6 50%
ENR)	27 84.3%	5 15.6%	0 0%	8 66.6%	4 33.3%	0 0%
(OFX)	26 81.2%	5 15.6%	1 3.1%	6 50%	6 50%	0 0%
(F300)	28 87.5%	4 12.5%				

S = Sensitive, M = Moderate sensitive and R = Resistant

Results showed that the prevalence *S. aureus* isolates was 19.1%. 6.4% of the *S. aureus* isolates was coagulase positive strains. The lowest prevalence was *Proteus* spp. (7.4%) *L. monocytogenes* (4.3%) and *Klebsiella* (3.2%).

Bacterial Isolation from Examined Body Sites

Results tabulated in Fig. 2 revealed the percentage of bacterial species from examined body organs (liver, lung and heart). The highest rate of isolation from organs was from the heart followed by liver and lung. Examination of all isolates of *E. coli* spp. and *salmonella* spp. collected from heart by API 20 test and PCR revealed that 32/35 (91.4%) were *E. coli* spp. and 12/13 (92.3%) were confirmed as *Salmonella* spp.

Resistance Pattern of *E. coli* and *Salmonella* spp. Isolates

As illustrated in Table 2, *E. coli* and *Salmonella* spp. isolated from heart blood showed high resistance to multiple drugs. Over 50% of both *E. coli* and *Salmonella* spp. isolates were found resistant against sulbactin-ampicillin, amoxicillin and clavulanic, colistin, oxytetracycline, clindamycin and gentamycin. However, nitrofurantoin, enrofloxacin, trimethoprim and sulphamethoxazole, ciprofloxacin, ofloxacin were highly (effective) sensitive against *E. coli* and *Salmonella* spp. isolates.

Direct Parasitological Examination

Chewing lice were detected in between feather of wings and body in 35/94 (37.2%) of quails. Twenty seven (28.7%) of quails harbored one or two species of nematode helminthes. The first one was *Heterakis* spp. which was recovered from the caeca whereas the second was *Ascarida galli* spp. which was recovered from the small intestine.

Influenza A Virus Detection and Isolation Trials

The trials for detection and isolation of influenza viruses in oral and cloaca swabs by direct detection by RT-PCR and allantoic fluids from inoculated SPF embryonated chicken eggs were found negative.

Discussion

Importance of wild birds as potential vectors of some zoonotic disease has received recent renewed empirical interest. Studying the existence of pathogens in wild birds may serve as a useful tool for examining the spread of other diseases amongst birds and from birds to others. However, information regarding the normal gastrointestinal bacterial flora is limited for the majority of wild bird species, with the few well-studied examples concentrating on bacteria that are zoonotic and/or related to avian species of commercial interest.

Migratory bird's play an important role in transmission of the diseases because of their great

mobility from area to another which plays a role as potential vectors for disease may regard domestic animals and human health (Abulreesh *et al.*, 2007). Quails pass through seashore of Sinai during autumn migration from Central Europe. They arrive at the shore exhausted and easily caught in hunters' nets because they fly very low as they reach shore. Many of the residents of that areas hunt these birds easily and either eat or sell it out in the public markets. In the present study, results revealed that despite none of tested quails showed characteristic clinical symptoms or pathological lesions, a high occurrence of some zoonotic bacterial species were detected among them indicating asymptomatic or carrier infections. These results are inconsistent with previous studies (Leveque *et al.*, 2003; Benskin *et al.*, 2009; Hamad *et al.*, 2012). This finding could be explained for many reasons. First, the migratory quails can carry bacteria for several weeks without presenting any clinical signs. Second, it has been proven that the high ability of domestic quails to acquire infection more than in migratory quail (Medani *et al.*, 2002). Finally, the migratory quails that capable of travelling long distance start its flight from Europe, the diseased one might not be able to share in journey or die without reaching the transient area in Egypt.

The Sampling techniques for detecting incidence of some pathogenic bacteria are a major factor affecting detection rate of the pathogens. Isolation rate was differing according to the sampling sites. Our results revealed that higher isolation rate of bacteria species was in cloaca swabs in compare with oral swabs. In addition, among the internal organs, isolation rate from heart was much higher than that from lung and liver. These results were supported by previous study of Roy *et al.* (2012).

E. coli spp. and *Salmonella* spp. are the most potential pathogens causing food poisoning and posing a zoonotic hazard. In this study, the presence of *E. coli* was the predominant pathogen reached 37.2% of the examined quails. A nearly similar finding was previously recorded by Awadallah *et al.* (2013) who described a prevalence of (48%) in samples collected from wild birds in Egypt. A percentage of (38%) were reported by Rogers (2006) in California, USA. However, lower *E. coli* infection rates of (1-18.7%) was recorded in free living birds (Brittingham *et al.*, 1988). The variation in prevalence rates may be attributed to the localities and bird feeding habits. *Salmonella* spp. Isolation rate reached (21.6%) which was much higher than that was obtained by other studies (Vlahovic *et al.*, 2004; Hedawy and El-Shorbagy, 2007). Compared with other studies, higher isolation rate of *Salmonella* spp. in the current study may be attributed to the fact that wild birds may consume polluted food and water. This was previously supported by Fricker (1984) who stated that waste disposal sites increases the frequency of dispersion of enteric bacteria in migratory birds.

In the current study, *S. typhimurium* was confirmed in 3 out of 13 (23.07%) *S. typhimurium* is the most serotype commonly associated with wild birds (Benskin *et al.*, 2009). Wild quails could be considered as true reservoirs in transmission of zoonotic *E. coli* and *Salmonella* due to their indirect contact with human habitations (Ahmed *et al.*, 2011). Therefore, the potential role of migratory quails in transmission of infectious diseases should be considered.

In this study, the incidence *S. aureus* was 19.1%. A similar result was agreed with a previous study (Hamad *et al.*, 2012). Our results indicated that the migratory quails harbored some bacteria which might play a significant role in the epidemiology of some enteric bacterial pathogens. Similar results have been mentioned by Effat and Moursi (2005).

Detection of bacterial entero-pathogens in wild birds has applied through traditional microbiological techniques (Benskin *et al.*, 2009). Our isolation was done through traditional microbiological techniques. *E. coli* and *Salmonella* spp. Isolates from heart blood were further examined by API 20 test and PCR. Results indicated that API and PCR identifications were more rapid and accurate diagnostic techniques matched with traditional techniques.

In poultry, antimicrobial agents are often continuously supplied as antimicrobial growth promoters and this has resulted in increased antibiotic selection pressure for resistant bacteria, resulting in their fecal flora containing a relatively high proportion of resistant bacteria. The use of antibiotics is supposed to be the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. Our results regarding the antimicrobial resistance pattern of *E. coli* and *Salmonella* isolates were in agreement with Roy *et al.* (2006) and Salehi and Reza (2010) who found that the antimicrobial resistance pattern was 50% or more of that isolates were multi-drug resistant against sulbactin-ampicillin, amoxicillin and clavulanic, colistin, oxtetracycline, clindamycin and gentamycin. However, it disagreed with that obtained by Hedawy and Wassel (2005). High sensitivity was showed in 80% of *E. coli* isolates was to nitrofurantoin, enrofloxacin, trimethoprim-sulphamethoxazole, ciprofloxacin and ofloxacin. This finding was consistent with the findings of Blanco *et al.* (1997). In fact, contamination of foods with antibiotic resistant bacteria could be a major threat to public health Van *et al.* (2008). The diffusion of zoonotic bacteria resistant to antibiotics is an important concern for the treatment of human infections, because it can compromise the effectiveness of the therapy (Kilonzo-Nthenge *et al.*, 2008). The increase in awareness about zoonotic infections could provide a better plan for the prevention and treatment.

Our results revealed a high incidence of one ectoparasite (chewing lice) which was detected in between feather of both wings. This finding was similar to that detected by Nursel (2010) who detect chewing *lice* spp. in wild quails during autumn migration. In addition, two nematode parasite species were identified. The first was identified as *Heterakis* spp. which was similar the finding recorded by Alan *et al.* (1979). In addition, *Heterakis* spp. was well documented in relation to blackhead in quail and/or other gallinaceous birds. The second was *Ascaridia gali* which was recovered from the small intestine. This finding was agreed with the results of Williams *et al.* (2000) who found helminthes parasites in 28% of bobwhite quails.

Influenza viruses of several subtypes have been isolated from quails in North America, Europe, Asia and Africa through periodic surveillance and sporadic outbreaks. Quails could be intermediate host of IAVs Bahl *et al.* (2013). Wild quail may provide an optimal environment for the adaptation of wild bird AIV that could generate novel variants that can cross the species barrier to domestic poultry and human beings in spite of negative isolation trails against AIVs. Our data showed that AIVs were not detected in either oralorcloaca swabs of all quails. Similar results were clarified by Ferro *et al.* (2012) who mentioned that no virus was isolated from any of the suspicious samples tested. This finding might be attributed to short term of sample collection (autumn season). Therefore, the awareness to migratory birds as reservoir hosts for zoonotic pathogens should be raised.

Conclusion

Wild quail can be considered as potential vectors of zoonotic pathogens, including antimicrobial-resistant variants, which can be transferred to humans through direct contact or the food chain. Sensitivity of *E. coli* and *Salmonella* spp., strains to enrofloxacin, nitrofurantoin which considered the drug of choice in case of infections. Migratory quails could serve to disperse bacteria between widely separated locations during migration. The opportunities exist for new health problems in wildlife populations emerge as well as new reservoirs of zoonotic disease to form. This work is basis of continuing efforts to periodical screening for pathogen from wild quail to predict future disease risks for wildlife and humans. Stimulating awareness to hunters, handlers and consumer about zoonotic pathogens is an important measure.

Conflict of Interests

The authors declare no conflict of interests with respect to the research, authorship and/or publication of

this paper and has no direct financial relation with the commercial identities mentioned in this paper.

Acknowledgment

We thank Dr. Abdel SatarArafa for applying real time PCR and isolation trails for detection avian influenza virus.

Author's Contributions

The author and the co-author have equally contributed in the preparation development and publication of this manuscript.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

- Abulreesh, H. H., R. Goulder and G.W. Scott, 2007. Wild birds and human pathogens in the context ringing and migration. *Ring Migration*, 23: 193-200. DOI: 10.1080/03078698.2007.9674363
- Acha, P.N. and B. Szyfres, 1987. *Zoonosis and Communicable Diseases Man and Animals*. 3rd Edn., Pan American Health Organization, Scientific and Technical Publications, Washington, DC. USA.
- Ahmed, L.M., F.A. Al-Obaidi and S.M. Al-Shadeedi, 2011. Prevalence of some zoonotic bacteria in wild birds in Kirkuk city. *Al-Anbar J. Vet. Sci.*, 4: 11-17.
- Alan, K.A., H. Larry and J.H. Eve, 1979. Some parasitic and infectious diseases of bobwhite quail from Oklahoma. Oklahoma State University.
- Alvarez, J., M. Sota, A.B. Vivanco, I. Perales and R. Cisterna *et al.*, 2004. Development of a multiplex PCR technique for detection and epidemiological typing of salmonella in human clinical samples. *J. Clin. Microbial*, 42: 1734-1738. DOI: 10.1128/JCM.42.4.1734-1738.2004
- Aly, M.M., A. Arafa and M.K. Hassan, 2008. Epidemiological findings of outbreaks of disease caused by highly pathogenic H5N1 avian influenza virus in poultry in Egypt during 2006. *Avian Dis.*, 52: 269-277. DOI: 10.1637/8166-103007-Reg.1
- Awadallah, A.M., M.M. Abdallah and E.M. Rehab, 2013. Prevalence of zoonotic *Escherichia coli* and *Salmonella* in wild birds and humans in Egypt with emphasis on RAPD-PCR fingerprinting of *E. coli*. *Global Veterinaria*, 11: 781-788.
- Bahl, J., S. Krauss, D. Kühnert, M. Fourment and G. Raven, 2013. Influenza A virus migration and persistence in North American wild birds. *PLoS Pathogen*, 9: e1003570. DOI: 10.1371/journal.ppat.1003570
- Benskin, C.M., K. Wilson, K. Jones and I.R. Hartley, 2009. Bacterial pathogens in wild birds: A review of the frequency and effects of infection. *Biological Rev. Cambridge Philosophical Society*, 84: 349-373.
- Bertran, K., E. Perez-Ramirez, N. Busquets, R. Dolz and A. Ramis *et al.*, 2011. Pathogenesis and transmissibility of highly (H7N1) and low (H7N9) pathogenic avian influenza virus infection in red-legged partridge (*Alectoris rufa*). *Vet. Res.*, 42: 24. DOI: 10.1186/1297-9716-42-24
- Blanco, J.E., M. Blanco, A. Mora and J. Blanco, 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *Clin. Microbial*, 35: 2184-2185. PMID: 9230413
- Brittingham, M.C., S.A. Temple and R.M. Duncan, 1988. A survey of the prevalence of selected bacteria in wild birds. *J. Wildl. Dis.*, 24: 299-307. DOI: 10.7589/0090-3558-24.2.299
- Cole, D., D.J. Drum, D.E. Stalknecht, D.G. White and M.D. Lee *et al.*, 2005. Free-living Canada Geese and antimicrobial resistance. *Emerg. Infect. Dis.*, 11: 935-938. PMID: 15963291
- Edwards, P.R. and W.H. Ewing, 1972. *Identification of Enterobacter*. 3rd Edn., Minneapolis, Burgess Publishing Co. USA.
- Effat, A.E. and M.K. Moursi, 2005. Role of wild birds in transmission of protozoa and bacterial pathogens to domesticated birds in Ismailia province. *J. Egypt. Vet. Med. Assoc.*, 65: 297-325
- Ferro, P.J., O. Khan, C. Vuong, S.M. Reddy and L. LaCoste *et al.*, 2012. Avian influenza virus investigation in wild bobwhite quail from Texas. *Avian Dis.*, 56: 858-60. DOI: 10.1637/10197-041012-ResNote.1
- Fricke, C.R., 1984. A note on salmonella excretion in the black headed gull (*Larus ribibundus*) feeding at sewage treatment works. *J. Appl. Bacteriol.*, 56: 499-502. PMID: 6746468
- Hamad, M.A., A.M. Al-Aalim, S.Y.A. Al-Dabbagh and H.H. Ali, 2012. Detection of organ bacterial load in quails. *Iraqi. J. Vet. Sci.*, 26: 47-51.
- Hedawy, K.A.A. and F.A.A. Wassel, 2005. Studies on some bacterial agents causing mortalities in quail farms in Kena province. *Assuit Vet. Med. J.*, 53: 251-257.
- Hedawy, K.A.A. and M.M.A. El-Shorbagy, 2007. Role of some wild birds in transmitting some Bacterial agents among poultry farms in Sohag Governorate. *Assuit Vet. Med. J.*, 53: 251-257.

- Kahn, L.H., 2006. Confronting zoonoses, linking human and veterinary medicine. *Emerg. Infect. Dis.*, 12: 556-561. DOI: 10.3201/eid1204.050956
- Kilonzo-Nthenge, A., S.N. Nahashon, F. Chen and N. Adefope, 2008. Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and Guinea Fowl. *Poultry Sci.*, 87: 1841-1848. DOI: 10.3382/ps.2007-00156
- Kloos, W.E. and T.L. Bannerman, 1999. Staphylococcus and Micrococcus. In: *Manual of Clinical Microbiology*, Murray, P.R., E.J. Baron, M.A. Tenover, F.C. Tenover and R.H. Tenover (Eds.), Washington, D.C. American Society for Microbiology, pp: 264-282.
- Leveque, G., V. Forgetta, S. Morroll, A.M.L. Kenneth and M.S. Danielle *et al.*, 2003. Allelic Variation in *TLR4* Is Linked to Susceptibility to *Salmonella enterica* Serovar Typhimurium Infection in Chickens. *Infect. Immun.*, 71: 1116-1124. DOI: 10.1128/IAI.71.3.1116-1124.2003
- Liasi, S.A., T.I. Azmi, M.D. Hassan, M. Shuhaimi and M. Rosfariza *et al.*, 2009. Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product. *Budu. Malaysian J. Microbiol.*, 5: 33-37.
- McDaniels, A.E., E.W. Rice, A. Reyes, C.H. Johnson and R.A. Haugland *et al.*, 1996. Conformational Identification of *Escherichia coli*, a Comparison of Genotypic and Phenotypic Assays for Glutamate Decarboxylase and b-D-Glucuronidase. *Applied Environ. Microbiol.*, 62: 3350-3354.
- Medani, G.G., M.A. Mohamoud, M.M. Hussein and M.M. Sabry, 2002. Comparative study upon bacterial infection with special reference to *Enterobacteriaceae* in Japanese and migratory quails. *J. Egypt Vet. Med. Assoc.*, 62: 42-48.
- Middleton, J.H. and A. Ambrose, 2005. Enumeration and antibiotic resistance patterns of fecal indicator organisms isolated from migratory Canada geese (*Branta canadensis*). *J. Wildl. Dis.*, 41: 334-341. PMID: 16107668
- Mohamed, K.M., M.E. Hala and S. Soheir, 2001. Bacteriological and pathological studies on dead-in shell in quail farms in Ismailia. *Suez Canal Vet. Med. J.*, 1: 437-457.
- Monday, S.R. and G.A. Bohach, 1999. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J. Clin. Microbiol.*, 37: 3411-3414. PMID: 10488222
- NCCLS, 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-fourth edition; approved standard (NCCLS document M7-A4). Villanova, Pa: National Committee for Clinical Laboratory Standards.
- Nursel, A., 2010. The presence of chewing lice (Insecta: Phthiraptera) species on wild quails (*Coturnix coturnix*). *J. Anim. Vet. Adv.*, 9: 1377-1379. DOI: 10.3923/javaa.2010.1377.1379
- Rogers, K.H., 2006. Prevalence of pathogenic enteric bacteria in wild birds associated with agriculture in Humboldt County, California. MSc of Thesis, Humboldt State University, California, USA.
- Roy, P., V. Purushothaman, A. Koteeswaran and A.S. Dhillon, 2006. Isolation, characterization and antimicrobial drug resistance pattern of *Escherichia coli* Isolated from Japanese quail and their environment. *J. Appl. Poult. Res.*, 15: 442-446. DOI: 10.1093/japr/15.3.442
- Roy, S.R., M.B. Rahman, J. Hassan and K.N.H. Nazir, 2012. Isolation and identification of bacterial flora from internal organs of broiler and their antibiogram studies. *Microbes Health*, 1: 72-75. DOI: 10.3329/mh.v1i2.14094
- Salehi, M. and G. Reza, 2010. Phenotypic and genotypic properties of *Escherichia coli* isolated from colisepticemic cases of Japanese quail. *Trop. Anim. Health Prod.*, 42: 1497-1504. DOI: 10.1007/s11250-010-9583-5
- Smith, N., 1999. To field-dress a Deer, Pennsylvania game news, pennsylvania game commission, Harrisburg, PA. USDA-Food Safety Inspection Service, Department of Agriculture, USA.
- Stohr, K. and F.X. Melsin, 1997. The role of veterinary public health in the prevention of zoonosis. *Arch. Virol. J.*, 13: 207-218.
- Thontiravong, A., P. Kitikoon, S. Wannaratana, R. Tantilertcharoen and R. Tuanudom *et al.*, 2012. Quail as a potential mixing vessel for the generation of new reassorting influenza A viruses. *Vet. Microbiol.*, 160: 305-313. DOI: 10.1016/j.vetmic.2012.05.043
- Van, T.T.H., J. Chin, T. Chapmanb, L.T. Tranc and P.J. Coloe, 2008. Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *Int. J. Food Microbiol.*, 124: 217-223. DOI: 10.1016/j.ijfoodmicro.2008.03.029
- Vlahovic, K., B. Matica, I. Bata, M. Pavlak and Z. Pavicic *et al.*, 2004. *Campylobacter*, *Salmonella* and *Chlamydia* in free-living birds of Croatia. *Eur. J. Wildl. Res.*, 50: 127-132. DOI: 10.1007/s10344-004-0052-1
- Williams, C.K., W.R. Davidson, R.S. Lutz and R.D. Applegate, 2000. Health Status of Northern Bobwhite Quail (*Colinus virginianus*) in Eastern Kansas. *Avian Dis.*, 44: 953-956. DOI: 10.2307/1593071