

Potential risk factors for avian pathogenic *Escherichia coli* (APEC) transmission in Southern African chicken flocks: A review

Jerikias Marumure

Department of Physics, Geography and Environmental Science, Great Zimbabwe University,
P. O Box 1235, Masvingo, Zimbabwe

Corresponding author: jmarumure@gzu.ac.zw

Abstract

Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), appears to be of increasing concern in both poultry and human health in Southern Africa, but no conclusive studies on the potential risk factors associated with its spread in the region have been conducted. The aim of this narrative review was to assess the potential risk factors in light of APEC transmission. Information about these potential risk factors was gathered using well-known scholarly databases such as Web of Science, Scopus, ScienceDirect, PubMed, and Google Scholar. Anthropogenic factors, poultry housing conditions, access to contaminated water and feed, exposure of poultry to viral or other pathogens, and proximity of poultry farms to other poultry farms or other animals, have all been identified as potential risk factors. Researchers and poultry farmers will be better informed about the risk of transmission and ways to prevent the spread of APEC in the region as they gain a deeper insight of the underlying factors, resulting in increased savings and improved animal and human health.

Keywords: Avian colibacillosis; virulence genes; anthropogenic factors; zoonosis, ventilation

Introduction

Poultry meat is preferred over other meats by consumers due to its lower fat content, source of all essential amino acids, iron, and minerals. It is less expensive and subject to fewer

cultural or religious restrictions when compared to other meats when used as food (Adamski et al., 2017; Adeyanju and Ishola, 2014). Chickens, in particular, are selectively bred for faster and more uniform growth (Kemmett et al., 2013). *Escherichia coli* is a gram-negative, flagellated bacterium found in mammals' and birds' digestive and upper respiratory tracts (Pourabbas and Feizi, 2015). The presence of pathogenic *E. coli* in the environment or in poultry gastrointestinal or respiratory systems, on the other hand, is a potential risk factor for avian colibacillosis. Avian colibacillosis is caused by avian pathogenic *Escherichia coli* (APEC), an extraintestinal pathogenic *Escherichia coli* (ExPEC). The disease is characterized by airsacculitis, perihepatitis, pericarditis, salpingitis, omphalitis, coligranuloma, and cellulitis (Cunha et al., 2014). The bacterial strains have a diverse set of virulence factors, allowing them to escape from the gastrointestinal tract and spread to various internal organs, resulting in colibacillosis (Kazemnia et al., 2014). Depending on environmental variables and host stress, even less pathogenic strains with less virulence genes can infect organs and cause colibacillosis (Mbanga and Nyararai, 2015). In mammals, colibacillosis is largely an intestinal disease, but in poultry, it can produce localized or systemic disease, usually when the host's defense mechanisms are compromised (Kabir, 2010; Dhama et al., 2013). APEC infections are also regarded as a financial burden in the global poultry industry, owing to the high mortality rates (3–4% of birds on a farm) and spoilage of poultry carcasses during slaughter and storage (Kemmett et al., 2013; Dhama et al., 2013; Kazemnia et al., 2014; Solà-Ginés et al., 2015; Mohsenifard et al., 2016). According to a recent report by Bortolaia et al. (2016), poultry meat is one of the causes of food illnesses in humans. Pathogenic *E. coli* and other spoilage microorganisms in poultry continue to be a major concern for poultry farmers, suppliers, consumers, and public health officials worldwide. In most countries around the world, *Escherichia coli* is one of the microorganisms that has consistently been linked to foodborne illnesses (Kabir, 2010; Dhama et al., 2013).

Diarrhoea is a common symptom of *E. coli* infection, but it can be complicated by other syndromes such as fever, dysentery, shock, and general immunocompromisation, depending on the serotype (Mohammad and Reza, 2015). These characteristics of *E. coli* infections make them a major health concern for HIV/AIDS patients, as diarrhoea is one of the most common symptoms in such people. Treatment of such diseases may result in significant savings losses for countries, resulting in a decrease in the Gross National Product (GNP). According to the UNAIDS (2014) report, Southern Africa is regarded as the "epicentre" of

the global HIV epidemic, with HIV/AIDS prevalence rates of 15% and 27.4% in Zimbabwe and Swaziland, respectively.

Empirical evidence has indicated that multiple factors are at play in the establishment of APEC infections in chickens. Many reports have implicated poor poultry housing conditions, with high levels of contaminated faecal dust and ammonia (Kabir, 2010; Sadeyen et al., 2014), suboptimal or poor husbandry practices (Pan and Yu, 2014), contaminated water and feed, stress (Mbanga and Nyararai, 2015), underlying viral diseases (Sadeyen et al., 2014), contaminated eggs or vertical transmission (Pourabbas and Feizi, 2015), vectors and cannibalism (Zurek and Ghosha, 2014) and proximity to other animals and other poultry farms (Landman and Cornelissen, 2006; Pohjola et al., 2015), can increase chances of transmission of APEC in chickens. Despite the evidence, there are still concerns about the risk factors for APEC in Southern Africa. To the best of our knowledge, no studies have attempted to assess any links between the occurrence of APEC and the high incidence and prevalence of gastroenteritis in human populations, the impact of APEC on the health of HIV/AIDS patients, or the economies of Southern African countries. As a result, no colibacillosis control strategies have been investigated, nor has an understanding of the conditions that allow *E. coli* to survive in various environmental samples for lengthy periods of time been gained. The role of anthropogenic practices and environmental factors in APEC transmission between farmed birds, as well as between birds and their environment or humans, is still unknown.

A better awareness of APEC transmission would aid in the reduction and elimination of avian colibacillosis in poultry flocks, reducing economic losses in the poultry industry as well as the potential hazards to public health (Kabir, 2010). The current review provides a detailed account of the potential risk factors for the transmission of avian pathogenic *E. coli* in Southern Africa. To that end, closeness of poultry farms to other poultry farms or other animals has been examined, as well as anthropogenic factors, housing conditions for poultry, access to contaminated water and feed, exposure of chickens to viral or other diseases, and other variables.

Mechanisms of Transmission of APEC

Housing conditions, ventilation and stress

While direct contact has been shown to be a major factor in APEC transmission, the impact of poor housing conditions has also been reported (Christensen et al., 2021). Poor ventilation and/or excessive levels of dust particles or other chemical fumes in poultry houses, according to reports, harm the poultry respiratory system (Kabir, 2010; Sadeyen et al., 2014). The APEC may enter through scratches or wounds on the damaged respiratory tract resulting in airsacculitis, polyserositis, and possibly septicaemia (Christensen et al., 2021). High levels of ammonia (25 to 100 parts per million) can damage the epithelial lining or paralyze the cilia that line it, decreasing the ability of the bird to filter hazardous dust and bacteria from the respiratory system (Kabir, 2010). Ammonia levels are typically high in colder climates because ventilation is reduced to save money on heating (David et al., 2015). Lower ventilation rates cause the air inside poultry houses to become humid, which raises the moisture content in the litter and creates the ideal environment for bacteria to break down uric acid and release large amounts of ammonia, which can harm the poultry respiratory system and increase the risk of APEC transmission (David et al., 2015).

It is not advised to clean poultry houses while the birds are inside because doing so can result in the release of significant amounts of faeces-contaminated dust. APEC transmission in chicken flocks has been demonstrated to occur via inhalation of contaminated faecal dust (Dhama et al., 2013; Sadeyen et al., 2014). Dust has been demonstrated to harbour *E. coli* for a very long time (Davis and Morishita, 2005). To this end, *E. coli* was reportedly isolated from dust from livestock farms that had been kept at 40°C for up to 35 years (Schulz et al., 2016). Similarly, Rosa et al. (1997) isolated strains of *E. coli* with multi-resistance to antimicrobials such as trimethoprim and tetracycline from dust that had settled both indoors and outdoors of poultry houses in Mexico City. In Bindura (Zimbabwe), Mashoko et al. (2016) found high coliform counts (2.0 to 3.77 log CFU/g) in dust. Furthermore, loads of dust was seen on the windows and walls of most poultry houses in preliminary studies at chicken farms in and around Masvingo, which may raise the danger of APEC transmission in the birds kept there. Based on this and other reports, we believe that dust that circulates in and around poultry houses could be an important vehicle for APEC transmission. As a result, well-ventilated poultry houses are thought to limit the spread of APEC and other infections in chickens.

Furthermore, our preliminary research indicates that the type of bedding material used in poultry houses in Zimbabwe may be significant factors for the transmission of APEC in

chickens. There have been reports of using wire mesh or concrete flooring, dried grass, wood shavings, and crop residues as bedding for chickens (Gororo and Kashangura, 2016; Nunes et al., 2016). When walking regularly on hard, rough, or pointed surfaces, birds have been observed to develop tiny lesions on their feet (Heerkens et al., 2015). The lesions may act as ports of entrance for *E. coli* (Dhama et al., 2013). APEC can also be transmitted through wounds caused by rough handling by farm personnel and ectoparasite bites (e.g., lice, mites, ticks, fleas and flies), or through unhealed navels in chicks (Barnes and Gross, 1997). According to a number of studies carried out elsewhere, some farmers reused litter (bedding) for a number of growth cycles before completely cleaning the chicken houses (Pepper et al., 2021; Chinivasagam et al., 2016). However, farmers that use this method put their flocks at danger because mixing old poultry litter with old flocks' excrement may be a significant source of disease transmission (Pan and Yu, 2014). The reuse of litter may also be a risk factor in the spread of APEC.

According to reports, the temperature inside poultry houses affects APEC transmission in chickens (Omer et al., 2010). Pathogens are reportedly moderately resistant to environmental agents, but susceptible to disinfectants and high temperatures (Oosterik et al., 2014; Khoo et al., 2010; Dhama et al., 2013). Aside from diseases and nutritional challenges, extreme temperatures are known to cause stress in chickens, making them more susceptible to infections (Kapetanov et al., 2015). Following moderate stress caused by cold stress and corticosteroids, birds have been shown to acquire short-lived nonspecific resistance to colibacillosis (Dhama et al., 2013). Even with the least pathogenic strains, stress increases infection in poultry (Mbanga and Nyararai, 2015). Colibacillosis often appears when a bird has experienced a physical, dietary, toxic, or viral challenge or trauma (Nolan et al., 2013).

High densities, the onset of sexual maturity, and poor husbandry practices have been shown to cause stress in chickens (Sadeyen et al., 2014), which increases infection pressure. The caecal counts of bacteria like *Salmonella enteritidis* in chicks are also known to increase with higher housing densities (Asakura et al., 2001). The same might be presumed to apply to APEC transmission, but further study is needed because various bacteria may have different needs for growth.

Contaminated Water, Feed and Eggs

Water has the potential to be an important vehicle for the spread of APEC in poultry. Ozaki and Murase (2009) demonstrated that contaminated well water can introduce pathogenic *E. coli* serotypes into poultry flocks, leading to APEC transmission. The use of reclaimed wastewaters in urban chicken farming may aid in the transmission of APEC. Adefisoye and Okoh (2016) discovered multidrug resistance (MDR) in *E. coli* strains isolated from the discharged final effluents of two wastewater treatment facilities in South Africa's Eastern Cape Province. Final effluents of wastewater could be an equally important risk factor if the water is used in poultry farming. Contamination of feeds and feed ingredients has the potential to introduce new strains of the pathogens (Martins da Costa et al., 2007). There is also evidence that the type of diet fed to chickens can influence their intestinal microbiome. Certain dietary components have been shown to promote the growth of some intestinal bacteria while suppressing the growth of others (Conlon and Bird, 2015). Diets high in indigestible non-starch polysaccharides and animal protein have been shown to promote the growth of *Clostridium perfringens* in the chicken hindgut (Pan and Yu, 2014). While the factors that favour *C. perfringens* transmission may not be the same as those that favour APEC transmission, we believe that certain dietary components used in Southern African chicken feeds (e.g., groundnuts shells, fishmeal, bones) may increase the risk of APEC transmission. With the above reports in hand, it is necessary to establish a link between the prevalence or incidence of avian colibacillosis and the dietary components of chicken feed used in Southern Africa.

If eggs are contaminated with faeces, the yolk sac may become infected with *E. coli* during hatching, which is generally associated with high mortality rates (Dhama et al., 2013; Pourabbas and Feizi, 2015). Nwiyi et al. (2016) demonstrated that fowl typhoid and pullorum disease can be passed down from generation to generation via infected eggs. While the risk factors for APEC transmission may differ from those for fowl typhoid and pullorum disease, we believe that contaminated eggs may increase the risk of APEC transmission. Fumigation of eggs within two hours of laying, as well as removal of eggs with cracked shells or soiled with faecal material, has been shown to reduce avian colibacillosis cases (Shahein and Sedeek, 2014).

Underlying chicken diseases

Colibacillosis frequently co-occurs with other diseases, such as respiratory viral or Mycoplasma infections (Sadeyen et al., 2014), making diagnosis and management difficult

for farmers (Nolan, 2013). On the other hand, birds with functional immune systems are notably resistant to *E. coli* prevalent naturally in their surroundings (Hyline News, 2015). Immune impairment induced by acute infections, particularly infectious bursal disease, reovirus, Newcastle disease, infectious bronchitis, Marek's disease, and adenovirus, can make flocks more vulnerable to APEC infection (Pourabbas and Feizi, 2015; Cuperus et al., 2016). Furthermore, we are investigating how underlying infections and the existence of antibodies in the serum to conditions like infectious bronchitis virus and Newcastle disease virus may contribute to the spread of APEC.

Disease vectors and cannibalism

APEC transmission could occur through other animals in both housed and free-range chickens. In our survey, we discovered that birds of prey frequently gain access to poultry houses in Zimbabwe, potentially increasing the risk of pathogen transmission, including APEC. People, as well as fomites, rodents, chicken mites, and flies, can act as APEC vectors (Vandekerchove et al., 2004). It has been shown that flies and bugs can transfer bacteria that are identical to those found in animal dung and multidrug-resistant clonal lineages to new substrates (Zurek and Ghosha, 2014). Extended spectrum beta-lactamase (ESBL)-producing *E. coli* was reportedly isolated on flies collected in poultry houses (Blaak et al., 2015), raising the risk of APEC transmission even further. Rodent droppings could also be a significant source of APEC (Dhama et al., 2013). According to Armougom et al. (2016), rodent droppings can facilitate spread of antibiotic resistant genes among bacterial strains. Furthermore, flea infestations in poultry flocks have been shown to cause stress in the birds (Dhama et al., 2013). As a result, we hypothesize that fleas or other sucking parasites transmit APEC when they sucking blood from birds, and that flies may carry APEC from sick bird lesions to healthy birds and poultry feeds. In this study, we propose insect control as an important flock management practice for reducing APEC transmission. Furthermore, APEC was found to be transmitted between chickens via perking wounds and cannibalism (Kabir, 2010).

Proximity to other animals, poultry farms and poultry density

Distances between poultry farms have been shown to be a significant risk factor in the spread of avian diseases (Landman and Cornelissen, 2006; Wang et al., 2013). One of the most efficient approaches to decrease colibacillosis may be to reduce the number of chicken farms in an area, as well as the population of hens on individual farms (Vandekerchove et al.,

2004). Commercial poultry should be separated from village backyard flocks, which typically live in close proximity to wild birds if biosecurity measures are not strictly enforced (Wang et al., 2013; Pohjola et al., 2015). The exotic Newcastle disease outbreak in the United States between 2002 and 2003 reportedly began in backyard poultry flocks and spread to commercial flocks (Wang et al., 2013). Backyard flocks are typically exposed to transmissible infectious diseases such as avian influenza (AI) (Pohjola et al., 2015), and can contribute to disease transmission to commercial poultry and zoonosis. While APEC transmission differs from that of avian influenza and Newcastle viruses, we believe that interaction between farmed birds and other avian species, particularly village backyard flocks, increases the likelihood of APEC transmission in chickens.

Summary

Based on the information above, we postulated that the occurrence of APEC in Southern Africa is a product of interplay of factors as shown in Figure 1.

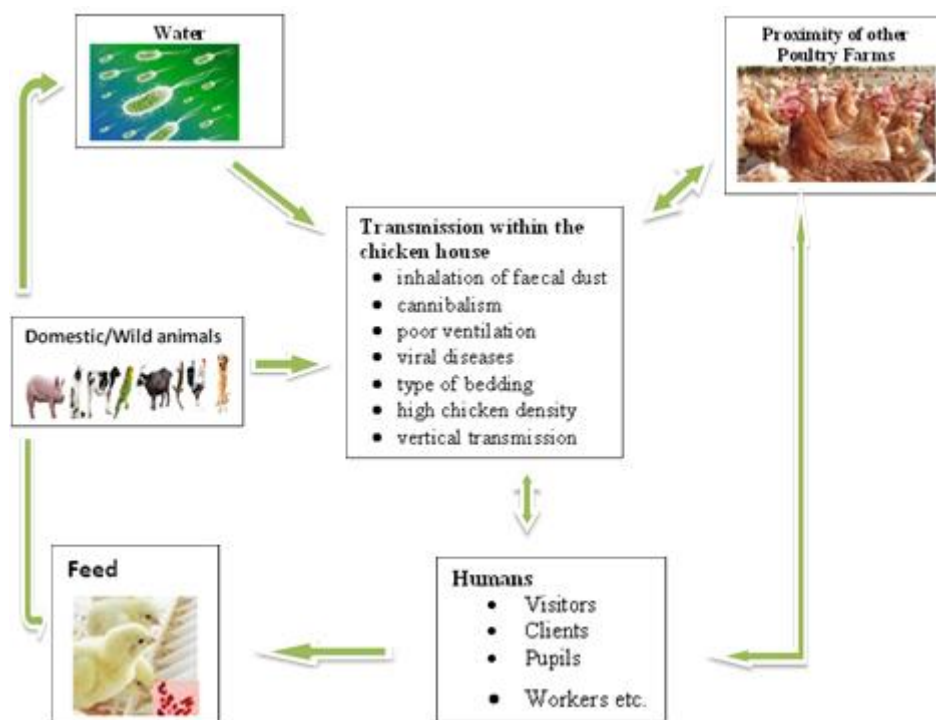


Figure 1. Potential risk factors for transmission of avian pathogenic *Escherichia coli* in chickens

Conclusion

Anthropogenic factors, poultry housing conditions, access to contaminated water and feed, exposure of poultry to viral or other pathogens, and proximity of poultry farms to other poultry farms or other animals, are some of the potential risk factors for the transmission of avian pathogenic *E. coli*. Among the risk factors, improving the housing conditions and hygiene is seen as the main preventive measure against the transmission of APEC during poultry production. Given that certain bacterial strains have been demonstrated to be transmitted to people through the food chain, it is therefore of significant concern that APEC is recognized as a hazard to food safety.

Recommendations

The occurrence of colibacillosis in chicken is a major cause for concern for food security and safety. The study recommends that control and prevention of APEC should be prioritized. Farmers should be made aware of potential risk factors that enhance APEC transmission in chickens. In order to gain a better knowledge of the potential risk factors, scientific data should be acquired through experiments or additional research in an African setting. Heritage-based solutions should be developed to minimize the spread of APEC in southern African chicken flocks.

References

- Adamski, M., Kuzniacka, J., & Milczewska, N. (2017). Preferences of consumers for choosing poultry meat. *Pol. J. Nat. Sci*, 32(2), 261-271.
- Adefisoye, M. A and Okoh, A. I. (2016). Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. *Microbiologyopen*. 5(1): 143–151, doi: 10.1002/mbo3.319
- Adeyanju, G. T. and Ishola, O. (2014). Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus*. 2014; 3: 139. doi: 10.1186/2193-1801-3-139
- Armougom, F., Bitam, I., Croce, O., Merhej, V., Barassi, L., Nguyen, T., Scola, B and Raoult. D. (2016). Genomic Insights into a New *Citrobacter koseri* Strain Revealed Gene Exchanges with the Virulence-Associated *Yersinia pestis* pPCP1 Plasmid. *Front Microbiol*. 2016; 7: 340. doi: 10.3389/fmicb.2016.00340

- Asakura, H., Tajima, O., Watarai, M., Shirahata, T., Kurazono, H. and Makino, S. (2001). Effects of rearing conditions on the colonization of *Salmonella enteritidis* in the cecum of chicks. *The Journal of Veterinary Medical Science*, 63, 1221 -1224.
- Awad, W. A., Hess, C., Khayal, B., Aschenbach, J. R. and Hess, M. (2014). *In Vitro* Exposure to *Escherichia coli* decreases ion conductance in the jejunal epithelium of broiler chickens. *PLoSOne*. 9(3): e92156. . doi: 10.1371/journal.pone.0092156
- Barnes, H.J. and Gross, W.B., (1997). *Colibacillosis, Diseases of Poultry*, 10th edn (pp. 131 - 141). Ames: Iowa State University Press.
- Blaak, H., van Hoek, A.H.A.M., Hamidjaja, R.A., van der Plaats, R.Q.J., Kerkhof-de Heer, L., de Roda Husman, A.M and Schets, F.M (2015). Distribution, Numbers, and Diversity of ESBL-Producing *E. coli* in the Poultry Farm Environment. *PLoS ONE* 10(8): e0135402. doi:10.1371/journal.pone.0135402
- Bortolaia, V., Espinosa-Gongora, C. and Guardabassi, L. (2016). Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. *Clin Microbiol Infect.* 22(2):130-40. doi: 10.1016/j.cmi.2015.12.003
- Chinivasagam, H. N., Estella, W., Rodrigues, H., Mayer, D. G., Weyand, C., Tran, T., ... & Diallo, I. (2016). On-farm *Campylobacter* and *Escherichia coli* in commercial broiler chickens: Re-used bedding does not influence *Campylobacter* emergence and levels across sequential farming cycles. *Poultry science*, 95(5), 1105-1115.
- Christensen, H., Bachmeier, J., and Bisgaard, M. (2021). New strategies to prevent and control avian pathogenic *Escherichia coli* (APEC). *Avian Pathology*, 50(5), 370-381.
- Cunha, M.P.V., de Oliveira, M.G.X., de Oliveira, M.C.V., da Silva. K.C., Gomes, C.R., Moreno, A.M. and Knobl, T. (2014). Virulence profiles, phylogenetic background, and antibiotic resistance of *Escherichia coli* isolated from turkeys with airsacculitis. *Scientific World Journal*. doi: 10.1155/2014/289024
- Cuperus, T., van Dijk, A., Matthijs, M.G.R., Veldhuizen, E. J.A and Haagsmana, H.P. (2016). Protective effect of *in ovo* treatment with the chicken cathelicidin analog D-CATH-2 against avian pathogenic *E. coli*. *Sci Rep.* 6: 26622. doi: 10.1038/srep26622
- Davis, M. and Morishita, T.Y., (2005). Relative ammonia concentrations, dust concentrations, and presence of *Salmonella* species and *Escherichia coli* inside and outside commercial layer facilities. *Avian Diseases*, 49: 30–35.

- Dhama, K.S., Chakraborty, R., Barathidasan, R., Tiwari, S., Rajagunalan, and Singh, S.D., (2013). *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance: A review. *Res. Opin. Anim. Vet. Sci.*, 3(6), 179-194. doi: 0.1637/10680100113RegR
- Heerkens, J.L., Delezie, E., Rodenburg, T.B., Kempen, I., Zoons, J., Ampe, B. and Tuytens, F.A., (2015). Risk factors associated with keel bone and foot pad disorders in laying hens housed in aviary systems. *Poult Sci.* 95(3):482-8. doi: 10.3382/ps/pev339.
- Hy-line International News, (2015). Colibacillosis in Layers: A review of the aetiology, routes of transmission, clinical signs, diagnosis and intervention strategies against colibacillosis in pullets and laying hens from Hy-Line International. <http://www.thepoultrysite.com/articles/3378/colibacillosis-in-layers-an-overview/>.
- Gororo, E., & Kashangura, M. T. (2016). Broiler production in an urban and peri-urban area of Zimbabwe. *Development Southern Africa*, 33(1), 99-112.
- Kabir, S.M.L., (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int J Environ Res Public Health*, 7: 89-114.
- Kapetanov, M., Pajić, M., Ljubojević, D. and Pelić, M. (2015). Heat stress in poultry industry, *Arhiv veterinarske medicine*, 8(2):87-101
- Kazemnia, A., Ahmadi, M. and Dilmaghani. M. (2014). Antibiotic Resistance Pattern of Different *Escherichia coli* Phylogenetic Groups Isolated from Human Urinary Tract Infection and Avian Colibacillosis. *IBJ*, 18(4): 219–224.
- Kemmett, K., Humphrey, T., Rushton, S., Close, A. and Wigley P., (2013). A longitudinal study simultaneously exploring the carriage of APEC virulence associated genes and the molecular epidemiology of faecal and systemic e. coli in commercial broiler chickens. Lakhota, R.L, Stephens, J.F. (1973). Drug resistance and R factors among enterobacteria isolated from eggs. *Poult. Sci.* 1955–1962.
- Khoo, L.L., Hasnah, Y., Rosnah, Y., Saiful, N., Maswati, M.A. and Ramlan, M. (2010). The prevalence of avian pathogenic *Escherichia coli* (APEC). in peninsular Malaysia. *MJVR*, 1(1): 27-31.
- Landman, W.J. and Cornelissen, R.A., (2006). *Escherichia coli* salpingitis and peritonitis in layer chickens: an overview. *Tijdschrift voor Diergeneeskunde*, 131(22): 814-822.

- Martins da Costa, P., Oliveira, M., Bica, A., Vaz-Pires, P. and Bernardo, F., (2007). Antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* isolated from poultry feed and feed ingredients. *Vet Microbiol*, 120: 122–131.
- Mashoko, E., Chingoto, T.D, and Chingwaru W (2016). Probing the Link between High Incidences of Diarrhoea, and the Microbial Quality of Dust, Freshwaters, and Ready-to-Eat Fruits and Vegetables in Bindura Town, Zimbabwe, Gillings Institute of Global Public Health, The Water Institute, University of North Carolina, USA
- Mbanga, J. and Nyararai, Y.O. (2015). Virulence gene profiles of avian pathogenic *Escherichia coli* isolated from chickens with colibacillosis in Bulawayo, Zimbabwe', *OJVR* 82(1), [http://dx. doi.org/10.4102/ojvr.v82i1.850](http://dx.doi.org/10.4102/ojvr.v82i1.850)
- Mohammad. J. and Reza E. D. (2015). Antimicrobial drug resistance pattern of *Escherichia coli* isolated from chicken farms with colibacillosis infection. *OJMM*, 05, 159-162. doi: 10.4236/ojmm.2015.54019
- Mohsenifard, E., Asasi, K., Sharifiyazdi, H. and Basaki, M. (2016). Phylotyping and ColV plasmid-associated virulence genotyping of *Escherichia coli* isolated from broiler chickens with colibacillosis in Iran. *Comp Clin Path.* 25: 1035–1042. doi:10.1007/s00580-016-2303-4
- Nolan, L. K. (2013). Overview of colibacillosis in poultry. <http://www.merckmanuals>.
- Nunes, Joao Carvalho, M. M. Carvalho, J. K. Sugui, F. A. Queiroz, A. E. Santana, M. E. Hata, A. L. O. Aiura, J. A. Oliveira, and S. A. Queiroz. "Effect of litter substrates on the performance, carcass traits, and environmental comfort of red-winged tinamou (*Rhynchotus rufescens*)."
Brazilian Journal of Poultry Science 18 (2016): 41-50.
- Nwiyi P.F., Char, K and Shoyinka, S.V.O. (2016). Molecular Detection of Salmonella Isolated from Poultry Farms In Abia State Southeast Nigeria. *Int. J. Curr. Microbiol. App. Sci* <http://dx.doi.org/10.20546/ijcmas.2016.507.108>
- Omer, M.M., Abusalab, S.M., Gumaa, M.M., Mulla, S.A., Omer, E.A., Jeddah, I.E., AL-Hassan, A.M., Hussein M.A. and Ahmed, A.M.,(2010). Outbreak of Colibacillosis among Broiler and Layer Flocks in Intensive and Semi intensive Poultry Farms in Kassala State, Eastern Sudan. *Asian J Poultry Sci*, 4: 173-181.
- Oosterik, L.H., Peeters, L., Mutuku, I., Goddeeris, B.M and Butaye, P. (2014). Susceptibility of avian pathogenic *Escherichia coli* from laying hens in Belgium to antibiotics and disinfectants and integron prevalence. *Avian Dis.* 58(2):2718.

- Ozaki, H. and Murase, T., (2009). Multiple routes of entry for *Escherichia coli* causing colibacillosis in commercial layer chickens. *J Vet Med Sci.* 71(12): 1685-1689.
- Pan, D. and Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes.* 5(1): 108–119.
- Pepper, C. M., Dunlop, M. W., and Walkden-Brown, S. (2021). An industry survey on litter management and re-use practices of Australian meat chicken growers. *Animal Production Science.*
- Pohjola, L., Rossow, L., Huovilainen, A., Soveri, T., Hanninen, M and Fredriksson-Ahomaa, M., (2015). Questionnaire study and postmortem findings in backyard chicken flocks in Finland. *Acta Veterinaria Scandinavica* 57:3 DOI 10.1186/s13028-015-0095-1
- Pourabbas, S. and Feizi, A., (2015). Clinical investigation, autopsy and some biochemical indices in broiler chickens following treatment with enrofloxacin against study by colibacillosis. *IJBPAS* 4(2): 719-727
- Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Johnson, T.J., Fakhr, M.K. and Lisa, K.N., (2006). Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology*, 151: 2097-2110.
- Sadeyen, J. R., Kaiser, P., Stevens, M. P. and Dziva, F. (2014). Analysis of immune responses induced by avian pathogenic *Escherichia coli* infection in turkeys and their association with resistance to homologous re-challenge. *Vet Res.* 45(1): 19. doi: 10.1186/129797164519
- Saidi B., Mafirakureva, P. and Mbanganga, J. 2012. Antimicrobial Resistance of *Escherichia coli* from chickens with colibacillosis in and around Zimbabwe. *Avian Dis.* 57: 152-154
- Schulz, J., Ruddat, I., Hartung, J., Hamscher, G., Kemper, N. and Ewers, C., (2016). Antimicrobial resistant *Escherichia coli* survived in dust samples for more than 20 years. *Front Microbiol.* 10; 7: 866.
- Shahein, E.H.A. and Sedeek, E. K. (2014). Role of spraying hatching eggs with natural disinfectants on hatching characteristics and eggshell bacterial counts. *Egypt. Poult. Sci.* 34(I):213-230.
- Singh, S.D., Tiwari, R. and Dhama, K. 2011. Avian colibacillosis, an economically important disease of young chicks. *Poultry World*, October issue. pp: 14-20.
- Sola-Gines, M., Cameron-Veas, K., Badiola, I., Dolz, R., Majo, Dahbi, G., Viso, S., Blanco, J., Piedra-Carrasco, N., Gonzalez-Lopez, J.J. and Migura-Garcia, L. (2015).

Diversity of MultiDrug Resistant Avian Pathogenic *Escherichia coli* (APEC) causing outbreaks of colibacillosis in broilers during 2012 in Spain. *PLoS One*. 10(11): e0143191.

Tivendale, K.A., Logue, C.M., Kariyawasam, S., Jordan, D., Hussein, A., Li, G., Wannemuehler, Y. and Nolan, L.K., (2010). Avian-pathogenic *Escherichia coli* strains are similar to neonatal meningitis *E. coli* strains and are able to cause meningitis in the rat model of human disease. *Infect. Immun.* 3412-3419.

UNAIDS (2014). THE GAP REPORT

Vandekerchove. D., De Herd. P., Laevens, H. and Pasmans. F., (2004). Risk factors associated with colibacillosis outbreaks in caged layer flocks, *Avian Pathology* 33(3): 337-342

Wang Y, Jiang Z, Jin Z, Tan H, Xu B (2013). Risk Factors for Infectious Diseases in Backyard Poultry Farms in the Poyang Lake Area, China. *PLoS ONE* 8(6): e67366. doi:10.1371/journal.pone.0067366

Westhuizen, W.A. and Bragg, R. R. (2012). Multiplex polymerase chain reaction for screening avian pathogenic *Escherichia coli* for virulence genes, *Avian Pathology*, 41:1, 33-40.

Wigley, P., Berchieri, A., Page, K.L., Smith, A.L. and Barrow, P.A., (2001). *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease free carriage in chickens. *Infect. Immun.* 7873 – 7879.