

## CASE REPORT

# Infectious coryza in a grey crowned crane (*Balearica regulorum*) recovered from captivity

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We report *Avibacterium paragallinarum* and *Klebsiella pneumoniae* coinfection in a grey crowned crane (*Balearica regulorum*). The crane was recovered from illegal captivity and released at a grey crowned crane (GCC) rehabilitation facility located at Akagera National Park in Rwanda. One year after being transferred, the bird presented with clinical signs suggesting a respiratory disease. Those signs included severe dyspnoea with mouth breathing, sneezing and nasal discharge. The crane was put on a 3-day treatment with antibiotics (ceftiofur 200 mg/ml at 50 mg/kg intramuscularly) and anti-inflammatory drug (meloxicam, intramuscular injection at a dose of 2 mg/kg), after which the crane seemed to have recovered. A month later, the same crane presented similar clinical signs and was treated with enrofloxacin at 10 mg/kg intramuscularly. Despite the treatment, the crane died 19 h later. At necropsy, adhesive air sacculitis and hydroperitoneum were observed, and a reddish fluid in air sacs and in the abdominal cavity was found. Also, a marked hepatomegaly and splenomegaly were observed. Samples were collected for laboratory examination. Molecular tests done on the tracheal and cloacal swabs revealed *A. paragallinarum* and *K. pneumoniae*, respectively. This is the first case of *A. paragallinarum* and *K. pneumoniae* coinfection reported in a grey crowned crane. Our study contributes to knowledge on the ecological distribution of both these pathogens in wild birds. It provides an opportunity to investigate further the clinical significance of infectious coryza in Rwanda's wild and domestic birds.

## KEYWORDS

*Avibacterium paragallinarum*, grey crowned crane, *Klebsiella pneumoniae*, Respiratory distress

## 1 | INTRODUCTION

*Gruidae* is a highly threatened bird family in the world. In particular, the grey crowned crane (GCC, *Balearica regulorum*) is a recognised endangered species by the International Union for Conservation of Nature (IUCN). There has been a sharp population decline of up to 80% of the species over the last 3 decades (BirdLife, 2016; Smith et al., 2016). The rapid decline is largely caused by illegal trade, captivity and habitat

destruction. These former two activities increase the risks of pathogen transmission between humans, livestock and wild birds.

*Avibacterium paragallinarum*, initially known as *Haemophilus paragallinarum*, is a gram-negative, non-motile bacterium known to cause infectious coryza. The bacterium is considered a significant challenge to the commercial poultry industry (Blackall et al., 2005). Infectious coryza is usually an upper respiratory disease affecting primarily domestic chicken (Wahyuni et al., 2018); however, it has also been reported

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in other bird species (Reece et al., 1981; Thenmozhi & Malmarugan, 2013).

*Klebsiella pneumoniae* is an important pathogen that causes infection in humans and animals. It is often associated with pneumonia, septicaemia and liver abscesses in infected individuals (Newire et al., 2013; Siu et al., 2011). *K. pneumoniae* is not uncommon in wild birds; thus, this poses a public health threat as a potential reservoir of multidrug-resistant *K. pneumoniae* (Navon-Venezia et al., 2017).

This case report describes a coinfection of *A. paragallinarum* and *K. pneumoniae* in a GCC recovered from illegal captivity in Rwanda and provides molecular insights into the isolated pathogens.

## 2 | CASE PRESENTATION

### 2.1 | Case history

In December 2015, a crane was recovered from illegal captivity and completed 60 days of quarantine before being released at a GCC rehabilitation facility located at Akagera National Park, Rwanda. The crane was able to fly and was free to fly in and out of the rehabilitation facility and liked to venture out of the National Park to nearby agricultural farms. In late November 2016, a guard at the GCC rehabilitation facility located in Akagera National Park reported that the crane was exhibiting abnormal behaviour. The crane was anorectic, listless, with a tendency to isolate itself. The crane was identified as ID 102. The veterinary clinician identified the crane as an adult weighing about 3 kg with a generally good body condition (body condition score of 4 on a scale of 1–5). The crane had severe dyspnoea with open mouth breathing, sneezing and nasal discharge. Also, it was lethargic and had a swollen abdomen. At auscultation, abnormal respiratory sounds were heard. Based on clinical signs, the examining veterinarian presumptively diagnosed infectious pneumonia. The crane was treated with an antibiotic (ceftiofur 200 mg/ml, intramuscular injection at 50 mg/kg, three injections at 3 days intervals) and an anti-inflammatory drug (meloxicam, single intramuscular injection at a dose of 2 mg/kg). After that course of treatment, the GCC's health status improved, and the crane was returned to the rehabilitation facility.

On the morning of the 19 December 2016, the same crane's health status had deteriorated. It was again isolated and treated with intramuscular injection of enrofloxacin at 10 mg/kg. Despite the care provided, it died the following day.

### 2.2 | Necropsy findings

The crane was submitted for necropsy, which was conducted on the same day by a veterinary pathologist. Macroscopically, there was severe congestion of the subcutaneous tissue, pectoral and abdominal muscles (Figure 1). Also, the trachea was severely congested. The pericardium and air sacs were filled with about 100 ml of blood-tinged fluid. There was also severe pleuropneumonia with air sacs adhered to the parietal pleura. In the abdominal cavity, splenomegaly and hep-

*Avibacterium paragallinarum* and *Klebsiella pneumoniae* are the main causes of respiratory infections in domestic bird species. These pathogens are rarely reported in coinfection and never reported in wild birds. While *Avibacterium paragallinarum* only causes disease in avian species, *Klebsiella pneumoniae* is zoonotic and causes human infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Illegal captivity and keeping wildlife as pets could potentially be a source of pathogen spillover events between wildlife, humans, and other livestock species.

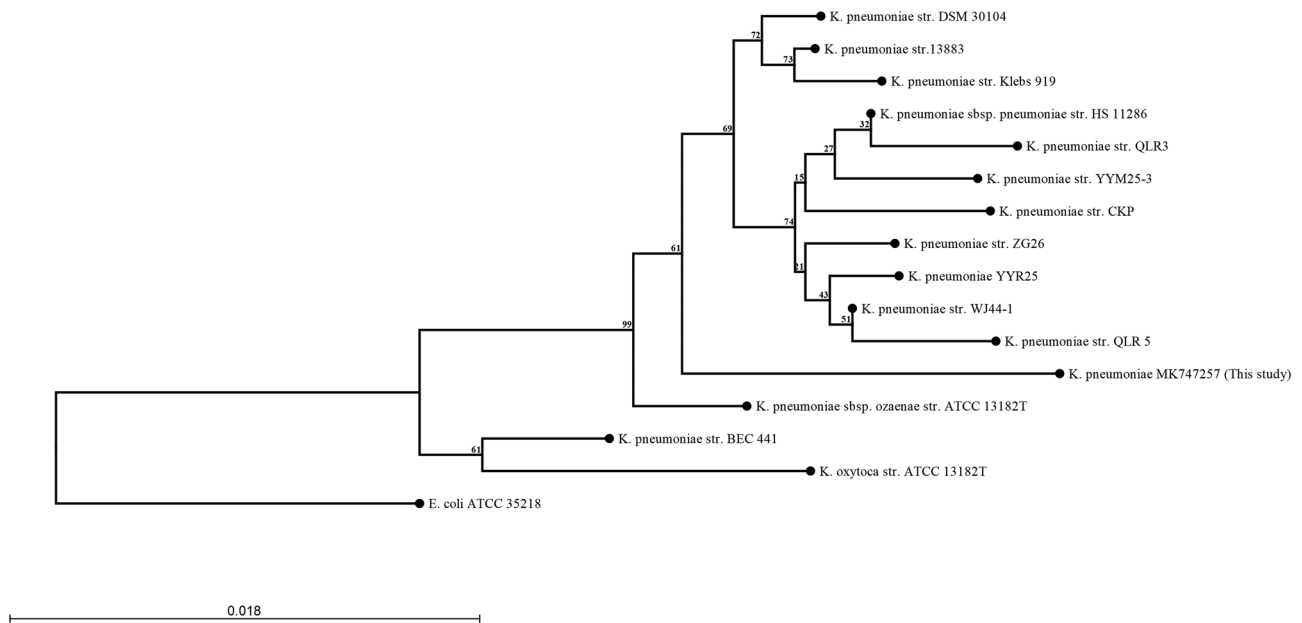
atomegaly were observed. Moreover, there were green strikes on the liver surface. The intestine had some remains of ingested food, and six intestinal roundworms were recovered from the large intestine (data not shown). Other organs had no significant macroscopic lesion.

### 2.3 | Laboratory findings

Prior to commencing treatment, cloacal and tracheal swabs were collected on FTA cards and sent to Molecular Diagnostic Services (Pty) Ltd, (a private laboratory based in Durban, South Africa). The DNA and total RNA were extracted using an in-house automated magnetic beads protocol. Both cloacal and tracheal swabs tested negative for Newcastle disease virus (NDV) and avian influenza virus (AIV) by PCR. 16S rRNA PCR and sequencing were also performed. The sequences were then submitted to BLAST/n at <http://www.ncbi.nlm.nih.gov/BLAST> to confirm the sequence identities (Altschul et al., 1990). Tracheal and cloacal swabs were PCR positive for *A. paragallinarum* and *K. pneumoniae*, respectively. Each sequence was aligned with its homologous sequences from *A. paragallinarum* and *K. pneumoniae*, using a strain of *Escherichia coli* as an outgroup. The alignment and phylogenetic tree for the nucleotide sequences were generated using the Neighbour-



**FIGURE 1** Severely congested trachea with a shiny mucosal surface



**FIGURE 2** Neighbour-joining method and the Kimura 2-parameter model were used to construct a phylogenetic tree showing the relationships between *Klebsiella pneumoniae* 16S rRNA gene sequences retrieved from GenBank and one isolate from this study (*K. pneumoniae* MK747257). The numbers displayed are bootstrap values (multiplied by 100) based on 10000 replicates (Dopazo, 1994). *Escherichia coli* strain ATCC 35218 was used as an outgroup. The scale bar represents the number of substitutions per nucleotide

joining distance algorithm, and the Kimura 2-parameter (K2P) model (Kimura, 1980) with 10,000 bootstrap replicates using CLC sequence viewer software (version 8; CLC Bio Inc., Massachusetts, USA) (Wen et al., 2018). The nucleotide sequences determined in this study were submitted to GenBank under accession numbers MK747257 and MK733284 for *K. pneumoniae* and *A. paragallinarum*, respectively.

The chicken *A. paragallinarum* isolates retrieved from the database formed distinct clusters phylogenetically distant from the isolate of this study (Figure 2), suggesting that the latter may be genetically different from genotypes causing infectious coryza in poultry. However, the probability of transmitting these pathogens between chickens and wild birds cannot be ruled out because these isolates are from different geographical locations. Moreover, only one isolate from the GCC was used.

On the other hand, the *K. pneumoniae* isolate from the GCC were indistinguishable from *K. pneumoniae* isolates from human clinical cases, implying more genetic similarity between them and the possibility of cross-infection between various hosts (Figure 3).

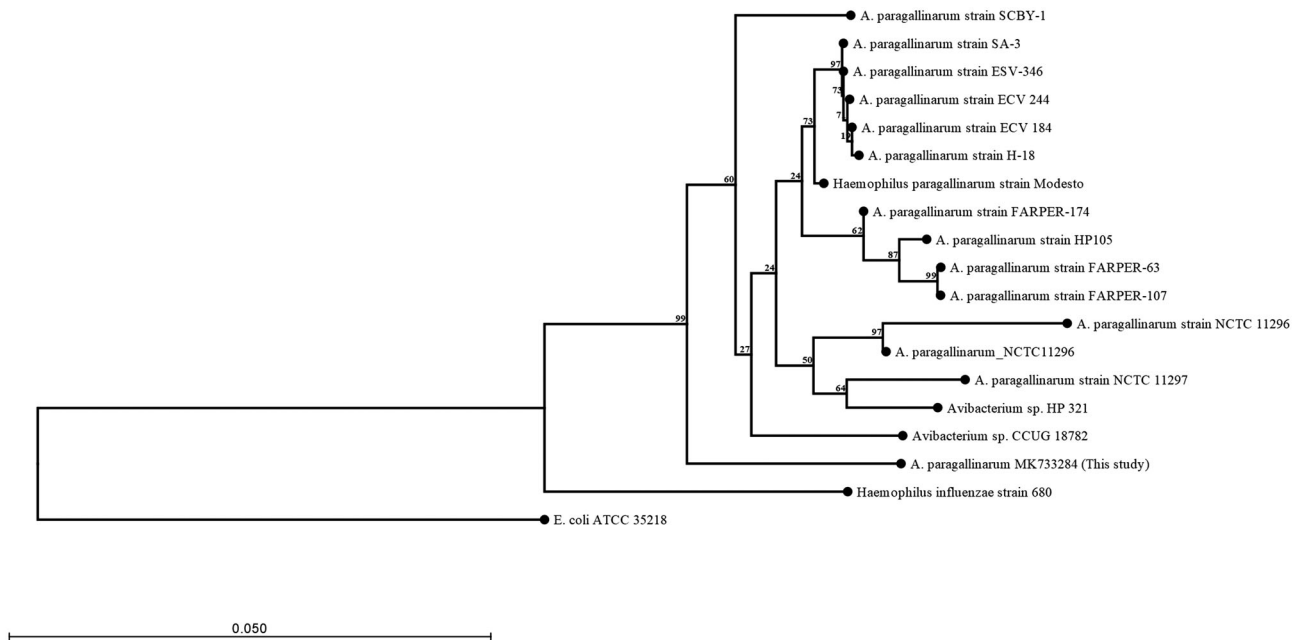
### 3 | DISCUSSION

We have presented data confirming infectious coryza caused by *A. paragallinarum* infection in grey crowned crane (*B. regulorum*). This report highlights the coinfection of a grey crowned crane by *A. paragallinarum* and *K. pneumoniae* supporting the severity of infectious coryza. Coinfection of *A. paragallinarum* with other opportunistic pathogens has been reported in the past. Naturally and experimentally infected birds display a severe clinical picture characterised by a respiratory distress syndrome (Kishida et al., 2004; Morales-Erasto et al., 2016).

The clinical signs described are in line with previous upper respiratory distress reports in other avian species (Blackall, 1999). The severe, extensive inflammatory lesions potentially resulted from complications due to *Avibacterium* spp. coinfection with other bacteria as previously reported (Morales-Erasto et al., 2016; Paudel et al., 2017). *K. pneumoniae* is generally reported as opportunistic bacteria that can occasionally cause pneumonia and a wide range of extra intestinal lesions such as liver abscesses in humans and animals (Brisse & Duijkeren, 2005; Davis & Price, 2016; Du et al., 2014). This opportunistic pathogen is reported in Rwanda as increasingly resistant to commonly used antibiotics in humans (Carroll et al., 2016; Ntirenganya et al., 2015). Animals are known possible reservoirs of resistant *K. pneumoniae* (Navon-Venezia et al., 2017) that can cross-infect humans in various ways, including close contact.

Infectious coryza is a well-known upper-respiratory tract disease of domestic chicken. The pathological condition has a significant economic impact on the poultry farming industry. It can lead to a substantial drop in egg production that can range between 14% and 41% and can cause mortality in chickens of 0.7%–10% (Thitisak et al., 1988), thus impeding farmers' economic development. Infectious coryza has not been reported in Rwanda, but it is highly prevalent in neighbouring countries (Byarugaba et al., 2007; Wambura, 2010).

To the best of our knowledge, this is the first report of *A. paragallinarum* and *K. pneumoniae* coinfection in a bird in Rwanda. These findings call for the need for further investigation of occurrence of these pathogens in various hosts in Rwanda. Evidence abounds elsewhere of wild birds transmitting pathogens to humans and livestock and has been well described (Bonnedahl & Järhult, 2014; Boseret et al., 2013; Shyaka et al., 2015). Moreover, *K. pneumoniae* is of clinical significance



**FIGURE 3** Neighbour-joining method and the Kimura 2-parameter model were used to construct a phylogenetic tree showing the relationships between *Avibacterium paragallinarum* 16S rRNA gene sequences retrieved from GenBank and one isolate from this study (*A. paragallinarum* MK733284). The numbers displayed are bootstrap values (multiplied by 100) based on 10,000 replicates (Dopazo, 1994). The scale bar represents the number of substitutions per nucleotide, and *Escherichia coli* strain ATCC 35218 was used as an outgroup

because it is a zoonotic pathogen and has been reported as increasingly prevalent and resistant to most antimicrobials in clinical settings in Rwanda (Ntirenganya et al., 2015; Umuhoza & Barton, 2014). These two identified pathogens were isolated from a GCC recovered from illegal captivity; thus, there is a possibility that these pathogens were acquired from, or spread to, other animal species such as poultry (*A. paragallinarum*) and/or humans (*K. pneumoniae*). Also, we cannot rule out the possibility that one or both pathogens infected the crane after recovery from captivity. The two hypotheses emphasise that keeping wild birds as pets could potentially be a source of pathogen spillover events between wild birds, humans and other livestock species.

Therefore, there is a need to study the broad epidemiology of public health and “One Health” pathogens, most importantly those threatening the livestock industry such as *K. pneumoniae* and *A. paragallinarum*. In this regard, it is imperative to adopt a holistic approach that would focus on generating data such as genome sequences of isolates from various hosts, including humans, livestock, and the environment. Such data could be used to better understand the epidemiology of various pathogens in humans, animals and the environment in Rwanda. Moreover, this could shed some light on the possible overlap between genotypes, implying a circulation of the identical genotypes between various hosts. This would be the first important step towards understanding the role of wild birds in the transmission cycle of various pathogens to humans and other animal species.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ETHICS STATEMENT

This study did not require ethical approval as it is linked to a clinical case. Animal care and all procedures employed adhered to World Organisation for Animal Health (OIE) Terrestrial Animal Health Code 2012 (use of animals in research and education). We obtained written consent from Rwanda Wildlife Conservation Association (RWCA) to publish this study.

#### AUTHOR CONTRIBUTIONS

Conceptualisation, funding acquisition, investigation, methodology, project administration and writing—review and editing: Olivier Nsengimana. Investigation, methodology, resources, writing – original draft and writing—review and editing: Gervais Habarugira. Investigation, methodology and writing—review and editing: Lonzy Ojok. Investigation and writing—review and editing: Deo Ruhagazi. Investigation and writing—review and editing: Albert Kayitare. Conceptualisation, data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing—original draft and writing—review and editing: Anselme Shyaka.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article. For any queries, please get in touch with the corresponding author.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.766>.

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## REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
- BirdLife, I. (2016). *Balearica regulorum*. The IUCN Red List of Threatened Species, 2016. e.T22692046A93334893.
- Blackall, P. J. (1999). Infectious coryza: Overview of the disease and new diagnostic options. *Clinical Microbiology Reviews*, 12, 627–632.
- Blackall, P. J., Christensen, H., Beckenham, T., Blackall, L. L., & Bisgaard, M. (2005). Reclassification of *Pasteurella gallinarum*, [*Haemophilus*] *paragallinarum*, *Pasteurella avium* and *Pasteurella volantium* as *Avibacterium gallinarum* gen. nov., comb. nov., *Avibacterium paragallinarum* comb. nov., *Avibacterium avium* comb. nov. and *Avibacterium volantium* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55, 353–362.
- Bonnedahl, J., & Järhult, J. D. (2014). Antibiotic resistance in wild birds. *Upsala Journal of Medical Sciences*, 119, 113–116.
- Boseret, G., Losson, B., Mainil, J. G., Thiry, E., & Saegerman, C. (2013). Zoonoses in pet birds: Review and perspectives. *Veterinary Research*, 44, 36.
- Brisse, S., & Duijkeren, E. (2005). Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. *Veterinary Microbiology*, 105, 307–312.
- Byarugaba, D. K., Minga, U. M., Gwakisa, P. S., Katunguka-Rwakishaya, E., Bisgaard, M., & Olsen, J. E. (2007). Virulence characterization of *Avibacterium paragallinarum* isolates from Uganda. *Avian Pathology*, 36, 35–42.
- Carroll, M., Rangaiahagari, A., Musabeyezu, E., Singer, D., & Ogbuagu, O. (2016). Five-year antimicrobial susceptibility trends among bacterial isolates from a tertiary health-care facility in Kigali, Rwanda. *American Journal of Tropical Medicine and Hygiene*, 95, 1277–1283.
- Davis, G. S., & Price, L. B. (2016). Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extraintestinal infections. *Current Environmental Health Reports*, 3, 128–135.
- Dopazo, J. (1994). Estimating errors and confidence intervals for branch lengths in phylogenetic trees by a bootstrap approach. *Journal of Molecular Evolution*, 38, 300–304.
- Du, Y., Luo, J., Wang, C., Wen, Q., Duan, M., Zhang, H., & He, H. (2014). Detection of drug-resistant *Klebsiella pneumoniae* in Chinese hares (*Lepus sinensis*). *Journal of Wildlife Diseases*, 50, 109–112.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- Kishida, N., Sakoda, Y., Eto, M., Sunaga, Y., & Kida, H. (2004). Co-infection of *Staphylococcus aureus* or *Haemophilus paragallinarum* exacerbates H9N2 influenza A virus infection in chickens. *Archives of Virology*, 149, 2095–2104.
- Morales-Erasto, V., Falconi-Agapito, F., Luna-Galaz, G. A., Saravia, L. E., Montalvan-Avalos, A., Soriano-Vargas, E. E., & Fernández-Díaz, M. (2016). Coinfection of *Avibacterium paragallinarum* and *Ornithobacterium rhinotracheale* in Chickens from Peru. *Avian Diseases*, 60, 75–78.
- Navon-Venezia, S., Kondratyeva, K., & Carattoli, A. (2017). *Klebsiella pneumoniae*: A major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Review*, 41, 252–275.
- Newire, E. A., Ahmed, S. F., House, B., Valiente, E., & Pimentel, G. (2013). Detection of new SHV-12, SHV-5 and SHV-2a variants of extended spectrum beta-lactamase in *Klebsiella pneumoniae* in Egypt. *Annals of Clinical Microbiology and Antimicrobials*, 12, 16.
- Ntirenganya, C., Manzi, O., Muvunyi, C. M., & Ogbuagu, O. (2015). High prevalence of antimicrobial resistance among common bacterial isolates in a tertiary healthcare facility in Rwanda. *The American Journal of Tropical Medicine and Hygiene*, 92, 865–870.
- Paudel, S., Hess, M., & Hess, C. (2017). Coinfection of *Avibacterium paragallinarum* and *Gallibacterium anatis* in specific-pathogen-free chickens complicates clinical signs of infectious coryza, which can be prevented by vaccination. *Avian Diseases*, 61, 55–63.
- Reece, R. L., Barr, D. A., & Owen, A. C. (1981). The isolation of *Haemophilus paragallinarum* from Japanese quail. *Australian Veterinary Journal*, 57, 350–351.
- Shyaka, A., Kusumoto, A., Chaisowwong, W., Okouchi, Y., Fukumoto, S., Yoshimura, A., & Kawamoto, K. (2015). Virulence characterization of *Campylobacter jejuni* isolated from resident wild birds in Tokachi area, Japan. *Journal of Veterinary Medical Science*, 77, 967–972.
- Siu, L. K., Fung, C. P., Chang, F. Y., Lee, N., Yeh, K. M., Koh, T. H., & Ip, M. (2011). Molecular typing and virulence analysis of serotype K1 *Klebsiella pneumoniae* strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. *Journal of Clinical Microbiology*, 49, 3761–3765.
- Smith, T., Page-Nicholson, S., Morrison, K., Gibbons, B., Jones, M. G., van Niekerk, M., Botha, B., Oliver, K., McCann, K., & Roxburgh, L. (2016). The African crane database (1978–2014): Records of three threatened crane species (Family: Gruidae) from southern and eastern Africa. *Biodiversity Data Journal*, 4, e9794.
- Thenmozhi, V., & Malmarugan, S. (2013). Isolation, identification and antibiogram pattern of *Avibacterium paragallinarum* from Japanese quails. *Tamil Nadu Veterinary and Animal Sciences*, 9, 253–258.
- Thitisak, W., Janviriyasopak, O., Morris, R. S., Srihakim, S., & van Kruedener, R. (1988). Causes of death found in an epidemiological study of native chickens in Thai villages. *Acta Veterinaria Scandinavica Supplement*, 84, 200–202.
- Umuhoga, C., & Barton, T. (2014). 983Outbreak of multi-drug resistant *Klebsiella pneumoniae*: A 4 month epidemiologic follow-up in a tertiary teaching hospital in Rwanda. *Open Forum Infectious Disease*, 1, S286–S286.
- Wahyuni, A., Tabbu, C. R., Artanto, S., Setiawan, D. C. B., & Rajaguguk, S. I. (2018). Isolation, identification, and serotyping of *Avibacterium paragallinarum* from quails in Indonesia with typical infectious coryza disease symptoms. *Veterinary World*, 11, 519–524.
- Wambura, P. N. (2010). Preparation and use of autogenous vaccine from *Avibacterium paragallinarum* (strain Tan 1–05) in layer chickens. *Tropical Animal Health and Production*, 42, 483–486.
- Wen, X., Su, H., Wang, Y., Pu, Z., Gao, J., Ji, Z., Yuan, X., Li, X., Zhang, W., Zhang, L., Long, Y., Yan, Y., & Shao, Z. (2018). Prevalence and natural course of occult hepatitis B virus infection in residents of 2 communities of Wuwei City, Gansu Province, China. *Journal of Viral Hepatitis*, 25, 281–288.

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