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Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species of India

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Summary

Campylobacteriosis is an important zoonotic disease and the prevalence of *Campylobacter* is largely unknown in the wildlife of India. A total of 370 samples, comprising of 314 fresh faecal samples from apparently healthy captive wild animals and birds, 30 stool swabs from animal care takers and 26 samples of the animals' food and water were collected from G. B. Pant High Altitude Zoo, Nainital, Kanpur Zoo, Wildlife Park, IVRI and the Post Graduate Research Institute in Animal Sciences (PGRIAS), Chennai, Tamilnadu from August 2014 to May 2015. Samples were processed for cultural isolation, direct PCR and multiplex PCR for species confirmation. To decipher the genetic diversity, the 16S rRNA gene was amplified, sequenced and analyzed. Based on isolation, the overall occurrence rate of *Campylobacter* spp. was 0.8% (3/370), being 2.94% (3/102) for captive wild birds. Three *Campylobacter jejuni* were isolated from silver pheasants, lady amherest pheasants and saras cranes. Direct PCR assay showed the overall occurrence rate of *Campylobacter* spp. to be 4.77% (15/315), being 1.58% (2/126) for captive wild ruminants, 5.81% (5/86) for non-ruminants and 7.84% (8/102) for birds. All the isolates were identified as *C. jejuni*.

Key words: *Campylobacter coli*, *Campylobacter jejuni*, Captive wildlife, India, Zoo

Introduction

Implementing the One Health Approach is crucial to the management and protection of humans, livestock, and wildlife health (Dhama *et al*., 2013). Research has shown the role wildlife plays in the emergence of livestock diseases in conjunction with human population growth and how wild animals act as reservoirs for several zoonotic diseases (Bengis *et al*., 2004; Dhama *et al*., 2013). Campylobacteriosis is an important zoonosis throughout the world. Thermophilic *Campylobacter* spp. have been isolated from the intestinal tracts of a wide variety of healthy and diseased warm-blooded animals, including poultry, swine, and captive and free-range wild animals (Colles *et al*., 2008, 2011; Koga *et al*., 2015; Mohan *et al*., 2015). *Campylobacter jejuni* and *Campylobacter coli* are recognized as the most frequent causes of acute diarrheal diseases in humans (Kapperud *et al*., 2003) and long-term sequels such as Guillain-Barré syndrome, reactive arthritis and irritable bowel syndrome (Haagsma *et al*., 2010). For this reason, greater attention has been given to *C. jejuni* and *C. coli* in this study as they are considered as the major contributing reservoirs of human campylobacteriosis.

Campylobacter jejuni is most frequently isolated from diarrheic and healthy captive and free-ranging nonhuman primates (Koga *et al*., 2015), black headed gulls (Broman *et al*., 2004), free living wild and migrating birds (Ito *et al*., 1988; Waldenstrom *et al*., 2002), elephants (Stoddard *et al*., 2005) and griffon vultures (Marin *et al*., 2014). *Campylobacter jejuni* has been reported to be associated with enteritis and hepatitis in captive ostrich chicks. The prevalence of *C. jejuni* in wild bird populations has been reported to vary from 2 to 60% (Waldenstrom *et al*., 2002; Waldenstrom *et al*., 2007; Colles *et al*., 2008; Marin *et al*., 2014; Hald *et al*., 2016). The existence of *Campylobacter* reservoirs in wildlife is a potential hazard to animal and human health; however, the prevalence of these species is largely unknown. To the best of our knowledge, no studies have evaluated the presence of *Campylobacter* in wild life in India. The present study aimed to investigate the prevalence of *Campylobacters* (*C. jejuni* and *C. coli*) in captive wild animals, birds and their foods as well as their human care takers across India and to find out the genetic diversity of the recovered isolates.

Materials and Methods

Study area and sample description

The study was performed from August 2014 to May 2015. The samples were collected from four zoological gardens and wildlife enclosures of India including the Pt. G. B. Pant High Altitude Zoo, Nainital, Uttarakhand, the

Kanpur Zoo, Kanpur, Uttar Pradesh, the Wildlife Park, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, and the Post Graduate Research Institute in Animal Sciences (PGRIAS), Kattupakkam, Chennai, Tamilnadu, India. A total of 370 samples, comprising of 314 fresh faecal samples from apparently healthy captive animals and birds, 30 stool swabs from the care takers and 26 water and food (meat, vegetables, animal and bird feed) samples provided to the animals and birds were collected. Of the 314 faecal samples collected, 126 were from 12 species of captive wild ruminants, 86 were from 17 species of captive wild non-ruminants and 102 were from 12 species of captive wild birds. Before sample collection, proper consent was obtained from the concerned authorities. Sample details are depicted in Table 1. Faecal samples were collected aseptically using readymade Cary-Blair medium transport swabs (Himedia, Mumbai, India) and water and feed samples were collected in suitable aseptic containers and transported immediately to the laboratory under chilled conditions.

Isolation and identification of *Campylobacter*

Bacteriological culture was performed in accordance with ISO 10272-1:2006 to detect *Campylobacter* spp. (ISO, 2006). Faecal and feed samples were pre-enriched in 1:10 vol/vol Bolton broth and pre-incubated at 37 ± 1°C for 5 ± 1 h. The pre-enriched broth was then incubated at 41.5 ± 1°C for 43 ± 1 h. Afterwards, 10 µL of the sample was cultured on modified charcoal cefoperazone deoxycholate selective agar plates (mCCDA) and incubated at 41.5 ± 1°C for 43 ± 1 h. *Campylobacter*-like colonies were purified on blood agar and identified to species levels by hippurate hydrolysis, oxidase, nitrate and indoxyl acetate hydrolysis, urease

Table 1: Sample description

S. No.	Captive wild ruminants		Captive wild non-ruminants		Captive wild birds	
	Animal species	No. of samples	Animal species	No. of samples	Bird species	No. of samples
1	Sambar deer *Rusa unicolor*	5	Leopard *Panthera pardus*	20	Golden pheasant *Chrysolophus pictus*	8
2	Himalayan goral *Naemorhedus goral*	8	Bengal tiger *Panthera tigris tigris*	9	Silver pheasant *Lophura nycthemera*	8
3	Barking deer *Muntiacus muntjak*	5	Striped hyena *Hyaena hyaena*	10	Cockatiel *Nymphicus hollandicus*	6
4	Thamin deer *Panolia eldii*	10	Tibetian wolf *Canis lupus chanco*	2	Lady amherest pheasant *Chrysolophus amhersitae*	12
5	Swamp deer *Cervus duvaucelii*	15	Jackal *Canis aureus*	5	Kalij pheasant *Lophura leucomelanos*	8
6	Nilgai *Boselaphus tragocamelus*	12	Himalayan black bear *Ursus thibetanus laniger*	7	Sun conure *Aratinga solstitialis*	6
7	Spotted deer *Axis axis*	32	Sloth bear *Melursus ursinus*	2	Red jungle fowl *Gallus gallus*	2
8	Blackbuck *Antilope cervicapra*	17	Hippopotamus *Hippopotamus amphibious*	6	Indian peafowl *Pavo cristatus*	4
9	Indian hog deer *Hyelaphus porcinus*	15	Indian rhinoceros *Rhinoceros unicornis*	3	White peafowl *Pavo cristatus mut. Alba*	3
10	Sika deer *Cervusnippon*	3	Gray langur *Semnopithecus entellus*	5	Saras crane *Grus antigone*	5
11	Chousingha deer *Tetracerus quadricornis*	2	Bonnet macaque *Macaca radiata*	5	Emu *Dromaius novaehollandiae*	*5*
12	Himalayan blue sheep *Pseudois nayaur*	2	Rhesus macaque *Macaca mulatta*	3	Ostrich *Struthio camelus*	35
13			Japanese macaque *Macaca fuscata*	2		
14			Palm civet *Paradoxurus hermaphrodites*	3		
15			Red panda *Ailurus fulgens*	2		
16			Leopard cat *Prionailurus bengalensis*	1		
17			Zebra *Equus quagga*	1		
Total		126		86		102

activity, catalase production tests and susceptibility to cephalotin and nalidixic acid.

Multiplex PCR for species identification

Multiplex PCR assay targeting the lipid gene, *lpxA* was used to confirme the species of *Campylobacter* (*C. jejuni* and *C. coli*) isolates as explained by Klena *et al*. (2004). The PCR control was obtained from the Division of Veterinary Public Health, ICAR-IVRI.

Direct PCR assay

Genomic DNA was isolated directly from 314 faecal samples from captive wildlife using QIAamp DNA Stool Mini Kit. The extracted DNA was subjected to PCR as described by Linton *et al*. (1997) by employing a primer that co-identifies *C. jejuni* and *C. coli* based on their 16S rRNA gene sequences.

Molecular diversity analysis by 16S rRNA gene sequencing

The 16S rRNA gene was amplified by PCR with self designed primers (F 5′TAC GGG AGG CAG CAG TRG GGA ATA3′, R5'WCA TCG TTT ASG GCG TGG ACT ACC3'). Sequencing of the amplified 16S rRNA gene of the *Campylobacter* isolates was carried out and analyzed using Gene Tool, DNA Star, Chromas Lite and MEGA software (version 5.0) for multiple sequence alignment, percent identity and phylogenetic tree construction. Percent identity and divergence were analyzed using MEGALIGN in DNA star.

Results

Out of the 370 samples obtained from different sources of wildlife, care takers, food and water screened for the presence of *Campylobacter* spp., three (0.8%) samples yielded characteristic bacterial colonies on mCCDA plates after 48 h of incubation, revealing characteristic Gram-negative, spiral or S shaped morphology and typical corkscrew motility, as observed by the hanging drop method. Biochemical testing showed all the isolates to be positive for catalase, oxidase, nitrate and indoxyl acetate hydrolysis. None of the isolates revealed positive reactions for urease activity. All three isolates were identified as *C. jejuni* based on multiplex PCR assay targeting the lipid gene *lpxA* (Fig. 1) and hippurate hydrolysis test, and all were found to be sensitive to nalidixic acid and resistant to cephalothin. All three *C. jejuni* isolates were obtained from captive wild birds, and no isolate was recovered from captive wild ruminants, non-ruminants, care takers and food and water samples.

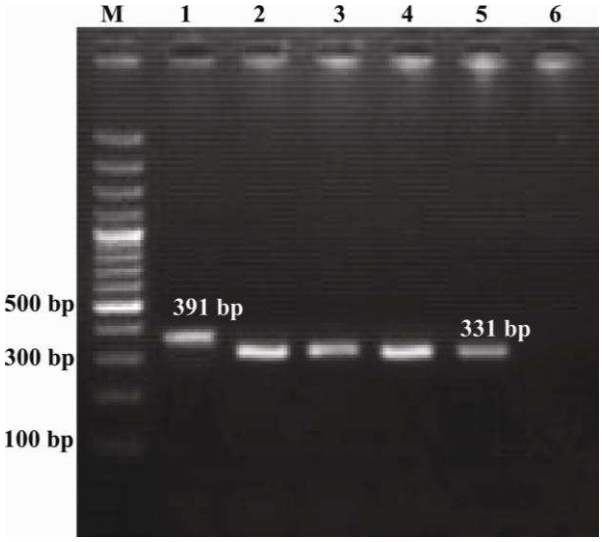

Fig. 1: Multiplex PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli*. Lane M: 100 bp to 1.5 kb DNA ladder. Lane 1: Positive control (*C. coli*-391 bp), Lane 2: Positive control (*C. jejuni*-331 bp), Lanes 3-5: *C. jejuni* from samples, and Lane 6: Negative control

The overall prevalence rate of *C. jejuni* and *C. coli* in captive wild birds was found to be 2.94% (3/102) and 0%, respectively. The three *C. jejuni* isolates were recovered from three different species of wild birds, namely, silver pheasant, lady amherest pheasant and saras crane. Based on cultural isolation, group-wise and zoo-wise (sampling area) distributions of *Campylobacter* are shown in Tables 2 and 3, respectively. Pearson's Chi-square and Fisher's exact tests showed that the isolation rate of *Campylobacter* among different groups and different zoos were not statistically significant (P>0.15 and P>0.79, respectively).

Based on direct PCR assay (Fig. 2), the occurrence of *Campylobacter* was found to be 4.77% (15/314). Eight positive samples were detected from wild birds (8/102, 7.84%), five from wild non-ruminants (5/86, 5.81%) and two from wild ruminants (2/126, 1.58%) including the samples that were already detected as positive from cultural isolation. The eight positive birds detected by direct PCR were a golden pheasant, two silver pheasants, a lady amherest pheasant, an Indian pea fowl, a saras crane and two ostriches. The five non-ruminants which were detected positive by direct PCR were a leopard, three striped hyenas and a jackal. The two ruminants

Table 2: Group-wise distribution of *Campylobacter*

S. No.	Group	No. of samples screened	No. positive (%)
1	Captive wild ruminants	126	0
2	Captive wild non-ruminants	86	0
3	Captive wild birds	102	3 (2.94%)
4	Care takers	30	0
5	Food and water	26	0
	Total	370	3 (0.81%)

Table 3: Zoo-wise distribution of *Campylobacter*

S. No.	Place of study	No. of samples screened	No. positive (%)
1	Nainital zoo	118	2 (1.69%)
2	Kanpur zoo	167	1 (0.59%)
3	IVRI deer par	41	0
4	PGRIAS ostrich enclosure	44	0
	Total	370	3 (0.81%)

which were detected positive by direct PCR were an Indian hog deer and a Himalayan blue sheep. Positive DNA samples were subjected to multiplex PCR for species identification and all the 15 samples were found to be *C. jejuni*.

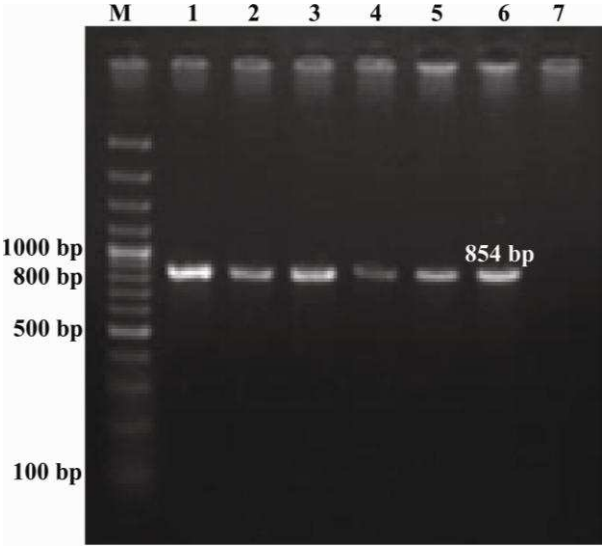

Fig. 2: Direct PCR assay to detect genus *Campylobacter* (*C. jejuni*/*C. coli*). Lane M: 100 bp to 1.5 kb DNA ladder. Lane 1: Positive control (*C. coli*), Lanes 2-6: Positive samples (*C. jejuni*/*C. coli*), and Lane 7: Negative control

All the three *C. jejuni* isolates (NSP7- Nainital zoo silver pheasant 7, NLAP2- Nainital zoo lady amherest pheasant 2, and KSCR3- Kanpur zoo saras crane 3) evolved from the same node, and the isolate NSP7 clustered with an already reported *Campylobacter* sequence of cattle faeces from India. Moreover, all three isolates clustered away from all the other reported sequences from various sources as indicated in the phylogenetic tree (Fig. 3). Analysis of sequences in the DNA Star of the three isolates indicated the percent identity among the isolates to be 96.7 to 98.3%, whereas the divergence was from 0.5 to 1.5 (Fig. 4). If isolates are numbered as 1, 2, 3 (1 being KSCR3 from Kanpur zoo saras crane 3, 2 being NSP7 from Nainital zoo silver pheasant 7 and 3 being NLAP2 from Nainital zoo lady amherest pheasant), the percent identity between 1 and 2, 1 and 3, and 2 and 3 were found to be 96.7, 97.2 and 98.3, respectively. The divergence between 1 and 2, 1 and 3 and 2 and 3 were 1.5, 0.5 and 0.7, respectively. Nucleotide sequences were deposited in the GenBank with the accession numbers KT000289, KT005218 and KT005219 using the National Centre for Biotechnology Information (NCBI, Bethsda, MD) Bankit submission tool (http:/www3.ncbi.nlm.nih.gov).

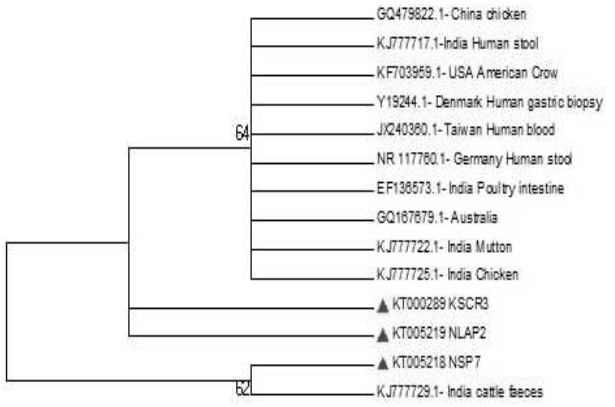

Fig. 3: Phylogenetic tree of *Campylobacter jejuni* isolates with the GenBank accession numbers

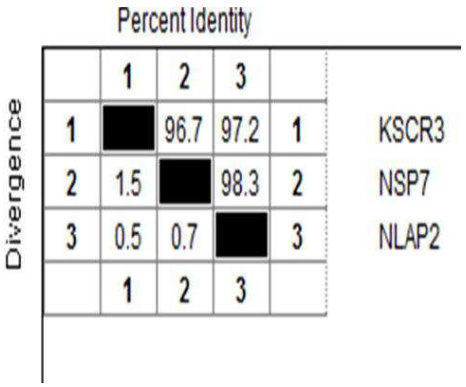

Fig. 4: Percent identity and divergence of *Campylobacter jejuni* isolates

Discussion

Animals kept at the zoo have been reported to be associated with bacterial infections and major health hazards, as their excretions can result in environmental contamination leading to morbidity and mortality of other animals as well as significant economic losses for the zoo. The aim of the present study was to determine the occurrence of *Campylobacter* amongst captive wildlife, their food and water and their caretakers.

In the present study, based on cultural isolation, the overall prevalence rate of *Campylobacter* was found to be 0.8% (3/370). For captive wild birds this rate was 2.94% (3/102). Three *C. jejuni* isolates were recovered from silver pheasants, lady amherest pheasants and saras cranes, which appears to be reported for the first time, based on the available literature and to the best of our knowledge. Our findings were in agreement with the observations of earlier researchers regarding the

incidence and prevalence of *Campylobacter* in wild birds (Adesiyun *et al*., 1998a, b; Waldenstrom *et al*., 2002; Hollamby *et al*., 2003; Hoar *et al*., 2007). Adesiyun *et al*. (1998b) reported a 2.5% prevalence of *C. jejuni* from birds in the Emperor Valley Zoo, Trinidad. In a study conducted on the wildlife of livestock farms, the overall prevalence was found to be 4.79% (Sippy *et al*., 2012), with isolates found only in wild birds, a finding which is in harmony with our study. In other studies, much higher prevalence rates (9 to 60%) have been reported (Kapperud *et al*., 1983; Ito *et al*., 1988; Yogasundram *et al*., 1989; Colles *et al*., 2008; Keller *et al*., 2011; Hald *et al*., 2016). Such variation may be due to the differences between the habitats and habits of free living, migrating and captive wild life. Since our study population was of captive nature, the prevalence of *Campylobacter* was found to be less as compared to the above mentioned studies. Birds are usually considered to be the reservoir of *Campylobacter* since the growth temperature range of these bacteria fits the body temperature of birds rather than that of mammals (Dhama *et al*., 2011). To correctly assess the impact of wild birds on *Campylobacter* epidemiology, it is essential to take into account the ecology of each bird species, i.e., its feeding habits, habitat preferences, migration patterns, life span, etc. Adesiyun *et al*. (1998a) reported less than 1% prevalence of *Campylobacter* spp., among 291 free-ranging mammals. Hollamby *et al*. (2003) recorded 0% prevalence in peafowls.

In the present study, no isolate was recovered from captive wild ruminants, non-ruminants, care takers, and food and water samples. Rosef *et al*. (1983) reported that *C. jejuni* was isolated from 1 out of 23 hares (4.34%), and no isolation (0%) was obtained from 3 species of cervids and rodents. Adesiyun *et al*. (1998b) conducted a study to record the prevalence of thermophilic *Campylobacter* species in animals kept at the Emperor Valley Zoo, Trinidad, and no isolate of *C. jejuni* was recovered from the animals. This difference may, at least in part, reflect the environments in which the animals live. Domestic mammals are subject to crowded conditions and the consequent frequent exposure to faecal excrements, thereby providing an ample opportunity for intra- and inter-specific cross contamination. On the other hand, it is highly probable that mammalian species differ in their susceptibility to intestinal colonization by *C. jejuni* and *C. coli*, regardless of the degree of exposure to these bacteria. In a recent work, *C. jejuni* from wild birds was found to be a constant source of human disease in the UK, indicating the existence of some unclear and uncertain epidemiological pathways between the wild bird reservoir and humans (Cody *et al*., 2015).

In the present study, the percent positivity by direct PCR was found to be higher compared to the isolation rate of culture methods. Although *C. jejuni* has already been reported in ostrich (Ling *et al*., 2012). Surprisingly, *C. jejuni* was detected in striped hyenas, jackals, leopards, Indian hog deer, blue sheep, Indian peafowl, lady amherest pheasants, saras cranes, and golden and silver pheasants for the first time in the world. The sensitivity of the culture method may be lower than that of DNA-based detection protocols due to sublethally injured or viable non-culturable cells in clinical materials. The low isolation rate may also be due to organisms that were not shed at the time of sampling. Being fastidious organisms that require microaerophilic conditions and capnophilic environments to survive, *Campylobacter* spp. may resort to viable but non-culturable forms if they were in adverse or stress conditions. Similar to our study, other researchers have also demonstrated the superior efficacy of PCR in the detection of *Campylobacter* from faecal samples which were previously declared negative by selective cultural and biochemical tests (Singh *et al*., 2011).

In conclusion, the present study reports the prevalence rate of *Campylobacter* to be 2.94% in captive wild birds (based on isolation) while no isolate was recovered from captive wild ruminants, non-ruminants, care takers, and food and water samples. However, based on direct PCR assay, the occurrence rate of the *Campylobacter* spp. was found to be 4.77%. The isolation of *C. jejuni* from silver pheasants, lady amherest pheasant sand saras cranes were reported for the first time in the world. We suggest that in order to discover the prevalence, magnitude and importance of *Campylobacter* infections in wildlife, extensive epidemiological studies be carried out with larger samples and a variety of wildlife enclosures, free-range wildlife and different geographical areas.

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Conflict of interest

The authors declare no conflict of interest.

References

Adesiyun, AA; Caeser, K and Inder, L (1998b). Prevalence of *Salmonella* and *Campylobacter* infections in animals at Emperor Valley Zoo, Trinidad, American Association of Zoo Veterinarians. J. Zoo Wildl. Med., 29: 237-239.

Adesiyun, AA; Seepersadsingh, N; Inder, L and Caesar, K (1998a). Some bacterial enteropathogens in wildlife and racing pigeons from Trinidad. J. Wildl. Dis., 34: 73-80.

Bengis, RG; Leighton, FA; Fischer, JR; Artois, M; Morner, T and Tate, CM (2004). The role of wildlife in emerging and re-emerging zoonoses. Rev. Sci. Tech., 23: 497-511.

Broman, T; Waldenstrom, J; Dahlgren, D; Carlsson, I; Eliasson, I and Olsen, B (2004). Diversities and similarities in PFGE profiles of *Campylobacter jejuni*

isolated from migrating birds and humans. J. Appl. Microbiol., 96: 834-843.

Cody, AJ; McCarthy, ND; Bray, JE; Wimalarathna, HM; Colles, FM; Jansen van Rensburg, MJ; Dingle, KE; Waldenstrom, J and Maiden, MC (2015). Wild bird associated *Campylobacter jejuni* isolates are a consistent source of human disease, in Oxfordshire, United Kingdom. Environ. Microbiol. Rep., 7: 782-788.

Colles, FM; Ali, JS; Sheppard, SK; McCarthy, ND and Maiden, MCJ (2011). *Campylobacter* populations in wild and domesticated Mallard ducks (*Anas platyrhynchos*). Environ. Microbiol. Rep., 3: 574-580.

Colles, FM; Dingle, KE; Cody, AJ and Maiden, MC (2008). Comparison of *Campylobacter* populations in wild geese with those in starlings and free range poultry on the same farm. Appl. Environ. Microbiol., 74: 3583-3590.

Dhama, K; Karthik, K; Chakraborty, S; Tiwari, R and Kapoor, S (2013). Wildlife: a hidden warehouse of zoonosis - a review. Int. J. Curr. Res., 5: 1866-1879.

Haagsma, JA; Siersema, PD; De Wit, NJ and Havelaar, AH (2010). Disease burden of post-infectious irritable bowel syndrome in the Netherlands. Epidemiol. Infect., 138: 1650-1656.

Hald, B; Skov, MN; Nielsen, EM; Rahbek, C; Madsen, JJ; Waino, M; Chriél, M; Nordentoft, S; Baggesen, DL and Madsen, M (2016). *Camylobacter jejuni* and *Campylobacter coli* in wild birds on Danish livestock farms. Acta Vet. Scand., 58: 11.

Hoar, BM; Whiteside, DP; Ward, L; Inglis, DG and Morck, DW (2007). Evaluation of the enteric microflora of captive whooping cranes (*Grus americana*) and sandhill cranes (*Grus canadensis*). Zoo Biol., 26: 141-153.

Hollamby, S; Sikarskie, JG and Stuht, J (2003). Survey of peafowl (*Pavo cristatus*) for potential pathogens at three Michigan zoos. J. Zoo Wildl. Med., 34: 375-379.

ISO 1072-1:2006. Microbiology of food and animal feeding stuffs- Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method.

Ito, K; Kubokura, Y; Kaneko, K; Totake, Y and Ogawa, M (1988). Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. J. Wildl. Dis., 24: 467-470.

Kapperud, G; Espeland, G; Wahl, E; Walde, A; Herikstad, H; Gustavsen, S; Tveit, I; Natas, O; Bevanger, L and Digranes, A (2003). Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am. J. Epidemiol., 158: 234-242.

Kapperud, G and Rosef, O (1983). Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* sp., and *Salmonella* sp. in Norway. Appl. Environ. Microbiol., 45: 375-380.

Keller, JI; Shriver, WG; Waldenström, J; Griekspoor, P and Olsen, B (2011). Prevalence of *Campylobacter* in wild birds of the Mid-Atlantic region, USA. J. Wildl. Dis., 47: 750-754.

Klena, JD; Parker, CT; Knibb, K; Ibbitt, JC; Devane, PM; Horn, ST and Konkel, ME (2004). Differentiation of *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpx*A. J. Clin. Microbiol., 42: 5549-5557.

Koga, T; Aoki, W; Mizuno, T; Wakazono, K; Ohno, J; Nakai, T; Nomiya, T; Fujii, M; Fusegawa, K; Kinoshita, K; Hamada, T and Ikeda, Y (2015). Antimicrobial resistance in *Campylobacter coli* and *Campylobacter jejuni* in cynomolgus monkeys (*Macaca fascicularis*) and eradication regimens. J. Microbiol. Immunol. Infect., 50: 75-82.

Ling, YE; Saleha, AA and Jalila, A (2012). Detection of *Campylobacter* and *Salmonella* in ostrich. J. Vet. Malaysia. 24: 6-8.

Linton, D; Lawson, AJ; Owen, RJ and Stanley, J (1997). PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J. Clin. Microbiol., 35: 2568-2572.

Marin, C; Palomeque, M; Marco-Jiménez, F and Vega, S (2014). Wild griffon vultures (*Gyps fulvus*) as a source of *Salmonella* and *Campylobacter* in eastern Spain. PLoS One., 9: e94191.

Mohan, V (2015). Faeco-prevalence of *Campylobacter jejuni* in urban wild birds and pets in New Zealand. BMC Res. Notes. 8: 1.

Rosef, O; Gondrosen, B; Kapperud, G and Underdal, B (1983). Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild animals in Norway. Appl. Environ. Microbiol., 46: 855-859.

Singh, H; Rathore, RS; Singh, S and Cheema, PS (2011). Comparative analysis of cultural isolation and PCR based assay for detection of *Campylobacter jejuni* in food and faecal samples. Braz. J. Microbiol., 42: 181-186.

Sippy, R; Sandoval-Green, CM; Sahin, O; Plummer, P; Fairbanks, WS; Zhang, Q and Blanchong, JA (2012). Occurrence and molecular analysis of *Campylobacter* in wildlife on livestock farms. Vet. Microbiol., 157: 369-375.

Stoddard, RA; Gulland, FMD; Atwill, ER; Lawrence, J; Jang, S and Conrad, PA (2005). *Salmonella* and *Campylobacter* spp. in northern elephant seals, California. Emerg. Infect. Dis., 11: 1967-1969.

Waldenstrom, J; Broman, T; Carlsson, I; Hasselquist, D; Achterberg, RP; Wagenaar, JA and Olsen, B (2002). Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Appl. Environ. Microbiol., 68: 5911-5917.

Waldenstrom, J; On, SL; Ottvall, R; Hasselquist, D and Olsen, B (2007). Species diversity of *Campylobacteria* in a wild bird community in Sweden. J. Appl. Microbiol., 102: 424-432.

Yogasundram, K; Shane, SM and Harrington, KS (1989). Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Louisiana. Avian Dis., 33: 664-667.