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Short Communication

Investigation of *Campylobacter jejuni* in microbiota of *Galerida cristata*, trapped in Southeast of Iran, Sistan

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Abstract

As a wild ground-foraging bird, crested lark (*Galerida cristata*) is widely distributed in Sistan area, located in the North of Sistan and Baluchistan Province, Iran. *Campylobacter jejuni* (*C. jejuni*) is known as a cause of acute diarrheal and Guillain-Barré syndrome in humans and can use the same wild birds as a carrier. The present study was aimed to determine if the target wild bird species can be regarded as a medium to carry *C. jejuni* or not. A total of 100 individual birds were trapped alive, and cloacal swab samples were collected from each animal. By amplifying *Hyp* and *hip* genes, we screened the presence of *C. jejuni* in the sampled specimens. The results indicated that generating an amplicon of 500 bp and 750 bp from control strain for *Hyp* and *hip* genes, but in all examined samples, no *C. jejuni* was detected. As a first microbiological investigation on a crested lark, our finding indicates that it likely plays no role in the epidemiology of infections caused by *C. jejuni*. Vast studies, with a variety of wild birds, and different geographical areas are proposed.

Keywords: Iran, microorganism, PCR, wild animals

Introduction

Crested lark (*Galerida cristata*), is a wild ground-dwelling bird. It is classified in the *Passeriformes* order, the *Alaudidae* family, and has a wide distribution in Sistan, in the North of Sistan and Baluchistan Province, Iran. Among the wild breeding birds, crested lark is the frequent breeder in Sistan. Moreover, it has excellent breeding ability (Mohammadi *et al.* 2017). Different investigations showed that some wild birds could host *Campylobacter* spp. as reservoirs of and the potential vectors for the transmission of Campylobacter to poultry, cattle, and humans (Abulreesh *et al.* 2007, Waldenstrom *et al.* 2002, Prince Milton *et al.* 2017).

Campylobacter jejuni, the causative agent of acute bacterial gastroenteritis or diarrhea in humans, is well colonized in the gastrointestinal tract of the birds; however, carrier birds show no recognizable clinical symptoms (Blaser 1997). Evidence has been indicated that possibly some birds can transmit diseases like Campylobacter infection to human beings (Prince Milton *et al.* 2017).

The prevalence of Campylobacter infection in humans and poultries has extensively been investigated (Ansari-Lari *et al*. 2011, Hamidian *et al*. 2011, Khoshbakht *et al*. 2013, Divsalar *et al*. 2019), while a few cases of wild birds has been considered in such studies such as wildfowl (Luechtefeld *et al.* 1980, Pacha *et al.* 1988), shorebirds (Fricker and Metcalfe 1984), gulls (Whelan *et al.* 1988, Lévesque *et al*. 2000), captive wild birds (Prince Milton *et al.* 2017), and corvids (Southern *et al.* 1990). However we found some similar investigations on birds inhabiting different rural and urban areas like Brown-eared bulbul, azure-winged magpie, crow, pigeon, pintail, and sparrow

(Kapperud and Rosef 1983, Fukuyama *et al.* 1986, Ito *et al.* 1988).

For the first time, we tried to investigate on the prevalence of the *C. jejuni* in crested larks as a nominated wild bird from *Alaudidae* family. We selected these birds because it is abundant species and can be found in rural areas of Sistan close to the humans. Meanwhile, Sistan, as one of the country's border areas, can provide a route for the entrance of the crested lark to Iran, and, thus, regional studies in wildlife could contribute to increasing the health of livestock population as well as the human population of the country.

Materials and Methods

This study was approved by the ethical committee of Faculty of Veterinary Medicine, University of Zabol, Iran with reference number of IRUOZECRA.2016.002. The sample size was calculated based on the assumption that the standard deviation (SD) of the prevalence of *campylobacter* spp. is 50%, due to the fact that the frequency of studied bacteria was unknown in examined birds. The margin of error was considered 10% at the confidence interval of 95%. The minimum sample size for the present study was estimated to be 100. From April to September 2017, crested lark was captured alive in a noninvasive way using mist nets and hand-made traps from different parts of Sistan (31°0'N -61°32'E). Using a sterile swab, inoculated in Cary and Blair Transport Medium Medium (Ibresco, Iran), we took samples from the cloaca of each animal, without any sacrificing. Subsequently, the fecal swabs were collected and transported to the laboratory for further analysis.

Reference strain (*C. jejuni* CCUG 11284), were used as controls (National Reference Laboratories, Applied Studies, and Diagnosis, Iran Veterinary Organization, Tehran, Iran). High Pure PCR Template Preparation Kit (Roche, Switzerland) was applied to extract DNA from the samples. Also, PCR (C-F:5'-GGCGTTCATTTGGCGAATTTGAA-3' and C-R: 5'-CCGCTGTATTGCTCATAGGGA-3') were used to target *Hyp* gene for the species-specific identification of *C. jejuni* (Raja *et al.* 2017). A primer pair including HIP400F (5'-GAAGAGGGTTTGGGTGGTG-3') and HIP1134R (5'-AGCTAGCTTCGCATAATA ACTTG-3') were, also, used for amplification of the *Hip* gene, which is absent from campylobacters other than *C. jejuni* (Linton *et al*. 1997). A 25-µL reaction mixture was set up in a 0.2-mL PCR tube. Briefly, the PCR master mix (Qiagen, Germany) consisted of 2.5 µL of 10x buffer, 1 µL of 10 mM dNTP, 1 µL of 25 mM MgCl2, 0.3 µL of 5 U/µl Hot start Taq DNA polymerase, 1 µL of each 10 µM primer (MWG, Germany), and 2.5 µL of DNA. PCR amplification was carried out by a thermocycler (Eppendorf, Germany) with program system consisting of an initial denaturation at 95°C for 6 min, followed by 30 cycles of denaturation at 95°C for 40 sec, annealing at 54°C for 25 sec, extension at 72°C for 40 sec, and a final extension step of 72°C for 1 min (Linton *et al*. 1997; Raja *et al*. 2017). Electrophoresis was performed on 2% agarose gel with a UV illuminator and visualized using a gel documentation system (BioRad, USA).

Results and Discussion

The primer pair was employed in the PCR with an annealing temperature of 54°C, generating an amplicon of 500 bp and 750 bp from control strain for *Hyp* and *hip* genes, respectively (Fig. 1). Molecular investigation showed that all the samples were negative for *C. jejuni* spp. because *Hyp* and *hip* genes representing the bacterium was not observed in the tested specimen (Fig. 1).

Two sets of primers were included in PCR reactions to differentiate *C. jejuni* from *C. coli*. *Hyp* gene is reported to be an exclusive marker for *C. jujeni* (Raja *et al.* 2017). Since *hip* gene may be harbored by both *C. jejuni* and *C. coli*, it was considered to use a primer pair for amplification of the *hip* gene, which is absent from campylobacters other than *C. jejuni* (Linton *et al.* 1997). Overall, our findings

revealed the absence of *C. jejuni* in examined birds in the study area.

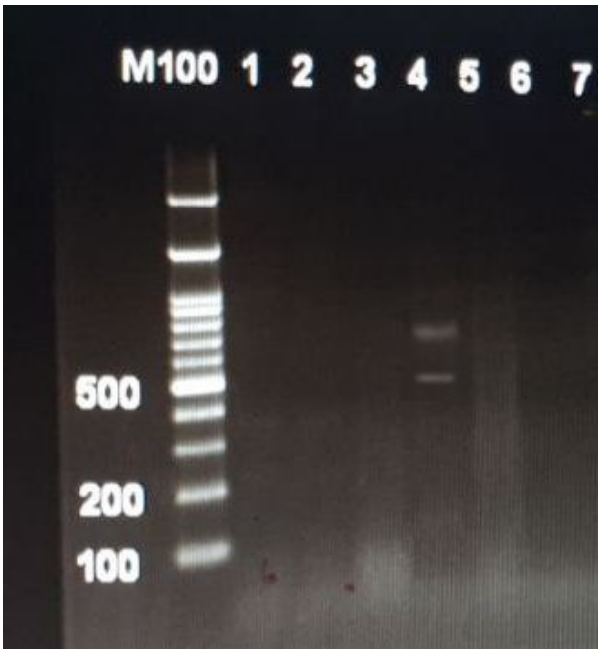

Figure 1. The electrophoresis result of the agarose gel of PCR products of *C. jejuni*. M100: 100 bp DNA ladder; Lanes 1, 2, 3, 6, 7: samples; Lane 4: Positive control containing *Hyp* (500 bp) and *hip* (750 bp) genes; Lane 5: Negative control (water instead of template DNA in PCR reaction)

Based on our literature review, none of the previous studies included crested lark.

In a survey in migratory birds, Waldenstrom *et al*. (2002) captured and sampled a large number of species of wild birds like *passeridae*, *paridae*, *fringillidae* families, etc. Their results suggested that the existence of *Campylobacter* spp. in different groups of wild birds is dependent on ecological parameters. Besides, the prevalence of Campylobacter infection was varied based on the taxa; the mean prevalence was 21.6% for all examined birds. Via PCR-based technique, *C. jejuni*, *C. lari*, and *C. coli* were reported to be 5.0%, 5.6%, and 0.9% of the birds, respectively (Waldenstrom *et al*. 2002). However, there was no data on Crested lark in Waldenstrom *et al*. (2002) investigation except a single species from *Alaudidae* family, *Alauda arvensis*. Moreover, of two birds tested, no isolate was identified as *Campylobacter* spp. and *C. jejuni*, which is consistent with our findings.

The prevalence of *Campylobacter* spp. has been included in Abulreesh *et al*. (2007) study, which has been done on various wild birds in different geographical areas. Megraud (1987) has found that the frequency of Campylobacter isolation from domestic swallow was 33% in Chile, while in France, it was observed 53% in domestic pigeons. Prince Milton *et al*. (2017) reported the prevalence rate of Campylobacter to be 2.94% in captive wild birds. The geographical distribution of Campylobacter, particularly in crested lark, needs further investigation and could be an explanation for findings observed in the present study.

Very few studies, relevant to our study design, have been carried out in Iran. Ehsan Nejad *et al*. (2015) have exhibited that 3% of fecal specimens collected from healthy ornamental and zoo birds in Tehran, Iran, have been polluted with *Campylobacter* spp. Besides, their study showed that 80% of contamination was related to *C. jejuni*.

Different birds have diverse feeding habits, habitat preferences, and geographic distributions (Waldenstrom *et al*. 2002), which affect their potential to be the host for common diseases like Campylobacter infection. Furthermore, Waldenstrom *et al*. (2002) have also demonstrated that the prevalence of *Campylobacter* spp. can depend on the animals foraging behavior. Therefore, more groups of generalist and specialist species concerning feeding behavior should be analyzed to find such relationships.

The target species in this study has a lower geographic range compare to other migrant water birds and probably doesn't make it to be the host for the infection. Those traveled shorter distances are more likely to be colonized than birds that travel longer distances (Waldenstrom *et al*. 2002). The bacterial population vary in the intestine resulting from their colonization ability. *C. jejuni* is an enteric pathogen. Our results indicated that the particular bacteria is unable to colonize in

Crested lark. This may be due to evolutionary changes in the birds resulted from lineage, which could obtain resistance to *Campylobacter* infection. However, this issue demands for more exploration.

Conclusion

The present investigation is the first study on the Crested lark regarding their potential to be host species for *C. jejuni*. From our data and examined samples, we didn't observe any possibility for this species to be the host for such a bacterium. Reviewing literature review on the similar designed studies emphasis different aspects of the species ecology and behavior in such a role. We have not any supporting proof at hand to confirm or reject such a hypothesis. We suggest that more populations from different areas of the country should be done on the whole family and compare the possible findings, which will help us to justify the outcomes more easily.

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References

Abulreesh H.H., Goulder R., Scott G.W. 2007. Wild birds and human pathogens in the context of ringing and migration. Ringing and Migration 23(4):193–200. DOI: 10.1080/03078698.2007.9674363

Ansari-Lari M., Hosseinzadeh S., Shekarforoush S.S., Abdollahi M., Berizi E. 2011. Prevalence and risk factors associated with Campylobacter infections in broiler flocks in Shiraz, southern Iran. International Journal of Food Microbiology144(3):475-9. DOI: 10.1016/j.ijfoodmicro.2010.11.003

Blaser M.J. 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. Journal of Infectious Diseases 176(Supl. 2): S103–S105.

Divsalar G., Kaboosi H., Khoshbakht R., Shirzad-Aski H., Ghadikolaii F.P. 2019. Antimicrobial resistance, and molecular typing of Campylobacter jejuni isolates, separated from food-producing animals and diarrhea patients in Iran. Comparative Immunology, Microbiology and Infectious Diseases 65:194-200. DOI: 10.1016/j.cimid.2019.06.001

Ehsannejad F., Sheikholmolooki A., Hassanzadeh M., Shojaei Kavan R.,Soltani M. 2015. Detection of cytolethal distending toxin (cdt) genes of *Campylobacter jejuni* and *coli* in fecal samples of pet birds in Iran. Iranian Journal of Veterinary Medicine 9(1):49–56. DOI: 10.22059/ijvm.2015.53233

Fricker C.R., Metcalfe N. 1984. *Campylobacters* in wading birds (Charadrii): incidence, biotypes and isolation techniques. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Serie B, Umwelthygiene, Krankenhaushygiene, Arbeitshygiene, präventive Medizin. 179(5):469–475.

Fukuyama M., Kamimura T., Itoh T., Saito K., Takahashi M., Sakai S., Murata M., Kohzaki K.I., Hara M., Shimizu T., Tabuchi K. 1986. Distribution of *Campylobacter jejuni* in wild birds and serogroup of isolates by slide agglutination technique. Nihon juigaku zasshi. The Japanese journal of veterinary science

48(3):487–493. DOI: 10.1292/jvms1939.48.487

Hamidian M., Sanaei M., Bolfion M., Dabiri H., Zali M.R., Walther-Rasmussen J. 2011. Prevalence of putative virulence markers in *Campylobacter jejuni* and *Campylobacter coli* isolated from hospitalized children, raw chicken, and raw beef in Tehran, Iran. Canadian Journal of Microbiology 57(2):143-8. DOI: 10.1139/w10-089

Ito K., Kubokura Y., Kaneko K., Totake Y., Ogawa M. 1988. Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. Journal of Wildlife Diseases 24(3):467–470. DOI: 10.7589/0090-3558-24.3.467

Kapperud G., Rosef O. 1983. Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. Applied and Environmental Microbiology 45(2):375–380.

Khoshbakht R., Tabatabaei M., Hosseinzadeh S., Shekarforoush S.S., Aski H.S. 2013. Distribution of nine virulence-associated genes in *Campylobacter jejuni* and *C. coli* isolated from broiler feces in Shiraz, Southern Iran. Foodborne Pathogens and Disease 10(9):764-70. DOI: 10.1089/fpd.2013.1489

Lévesque B., Brousseau P., Bernier F., Dewailly É., Joly J. 2000. Study of the bacterial content of ring-billed gull droppings in relation to recreational water quality. Water Research 34(4):1089–1096. DOI: 10.1016/s0043-1354(99)00266-3

Linton D., Lawson A.J., Owen R.J., Stanley J. 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. Journal of Clinical Microbiology 35(10):2568–2572.

Luechtefeld N.A., Blaser M.J., Reller L.B., Wang W.L. 1980. Isolation of *Campylobacter fetus* subsp. *jejuni* from migratory waterfowl. Journal of Clinical Microbiology 12(3):406–408.

Megraud F. 1987. Isolation of *Campylobacter* spp. from pigeon feces by a combined enrichment-filtration technique. Applied and Environmental Microbiology 53(6):1394–1395.

Mohammadi S., Molashahi F., Sabaghzadeh A. 2017. Breeding study of *Galerida cristata*, Linnaeus, 1758 in Zahak, southeastern of Iran. Animal Environment 8(4):77–82. [Persian]

Pacha R.E., Clark G.W., Williams E.A., Carter A.M. 1988. Migratory birds of central Washington as reservoirs of *Campylobacter jejuni*. Canadian Journal of Microbiology 34(1):80–82.

Raja S.S., Rao V.A., Babu R.N., Raja A., Abraham R.J.J., Thamizhannal M. 2017. Detection of *Campylobacter jejuni* in chicken meat by polymerase chain reaction. Indian Veterinary Journal 94(2):25–28.

Prince Milton A.A., Agarwal R.K., Priya G.B., Saminathan M., Aravind M., Reddy A., Athira C.K., Anjay Ramees T.P., Dhama K., Sharma A.K., Kumar A. 2017. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species of India. Iranian Journal of Veterinary Research 18(3):177–182.

Southern J.P., Smith R.M., Palmer S.R. 1990. Bird attack on milk bottles: possible mode of transmission of *Campylobacter jejuni* to man. Lancet 336(8728):1425–1427.

Waldenstrom J., Broman T., Carlsson I., Hasselquist D., Achterberg R.P., Wagenaar J.A., Olsen B. 2002. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Applied and Environmental Microbiology 68(12):5911–5917.

Whelan C.D., Monaghan P., Girdwood R.W., Fricker C.R. 1988. The significance of wild birds (*Larus* sp.) in the epidemiology of *Campylobacter* infections in humans. Epidemiology and Infection 101(2):259–267.