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Short Communication / *Kısa Bilimsel Çalışma*

Investigation of *Yersinia* spp. and *Aeromonas hydrophila* prevalences in Northern Bald Ibis (*Geronticus eremita)*

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Summary: Northern Bald Ibis (*Geronticus eremita)* is in the Red List of Threatened Species published by International Union for Conservation of Nature and Natural Resources (IUCN). The purpose of this study was to determine the frequency of *Aeromonas hydrophila* and *Yersinia* species in feces of Northern Bald Ibis. In the study, 29 (35.3%) *A. hydrophila* and 22 (26.8%) *Yersinia* species were isolated from stool samples. In isolated *Yersinia* species, 8 (9.7%) *Y. enterocolitica*, 5 (6%) *Yersinia spp*, 4 (4.8%) *Y. frederiksenii,* 3 (3.6%) *Y. intermedia* and 2 (2.4%) Y. *kristensenii* were identified. In conclusion, *Yersinia spp*. and *A. hydrophila* have been found at high rates in Northern Bald Ibis stool.

Key words: *Aeromonas hydrophila,* Northern Bald Ibis,*Yersinia* spp.

Kelaynaklarda *Yersinia* spp. ve *Aeromonas hydrophila* sıklığının araştırılması

Özet: Kelaynaklar (*Geronticus eremita*), Uluslararası Doğa ve Doğal Kaynakları Koruma Birliği tarafından yayınlanan kırmızı listede, türleri tehdit altında olan kuşlar olarak bildirilmiştir. Bu çalışmada, kelaynak dışkılarından *Aeromonas hydrophila* ve *Yersinia* türlerinin sıklığının araştırılması amaçlanmıştır. Çalışmada, 29 (%35.3) *A. hydrophila* ve 22 (%26.8) *Yersinia* türü izole edilmiştir. *Yersinia* türlerinin 8 (%9.7)'i *Y. enterocolitica*, 5 (%6)'i *Yersinia* spp., 4 (%4.8)'ü *Y. frederiksenii,* 3 (%3.6)'ü *Y. intermedia* ve 2 (%2.4)'si *Y. kristensenii* olarak identifiye edilmiştir. Sonuç olarak, kelaynak dışkılarından yüksek oranda *Yersinia* spp. ve *A. hydrophila* olduğu saptanmıştır.

Anahtar sözcükler: *Aeromonas hydrophila,* Kelaynak, *Yersinia* spp.

Northern Bald Ibis (*Geronticus eremita*) is in the Red List of Threatened Species published by IUCN. These birds are almost being extinct as very few of them left in the world, in Turkey for example it can be seen only in the Birecik district of Sanliurfa. Besides few left in Turkey, they're also visible in Morocco and lately in Syria. Their total population is estimated to be around 530 in the wild and about 1500 in captivity, of which about 100 in Sanliurfa area (2).

Aeromonas and *Yersinia* species are pathogenic or opportunistic pathogens for humans and animals (1). *Y. enterocolitica* and *Y. enterocolitica*-like organisms are widely distributed in nature. They can be isolated from humans, animals, food and environmental samples (14). *Y. pseudotuberculosis* and *Y. enterocolitica* are two species that are pathogenic for both humans and animals (16). Wild birds are thought to be a significant reservoir for *Y. pseudotuberculosis* (5).

Many *Aeromonas* species are free-living saprophytes commonly found in fresh water, soil and sewage (1). Animals and birds (particularly aquatic birds) can be fecal carriers of motile *Aeromonas* species (18). Reports of disease caused by *Aeromonas* species in livestock and companion animals, except for humans are rare. *A. hydrophila* has been associated with diarrhoea in piglets, abortion and reproductive problems in mares, septicaemia in dogs, acute deaths in aviary birds and conjunctivitis in a pet parrot (3).

The purpose of this study was to determine the frequency of *A. hydrophila* and *Yersinia* species in feces of Northern Bald Ibis.

In this study, stool samples were taken from Northern Bald Ibis cages in Birecik, Sanliurfa, Turkey. A total of 82 stool samples were collected and placed into sterile containers and examined within two hours of sampling. Samples were cultivated at the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, University of Harran.

Yersinia spp. were isolated, identified, and characterized on the basis of morphological, cultural, and biochemical characteristics as previously described (9, 17). Before direct plating, 5 ml of phosphate-buffered

saline (PBS, pH=7.4) enrichment broth was added to each tube containing fecal samples. PBS has been used to dilute stool samples. For cold enrichment, the tubes were incubated at +4°C and studied after 7, 14 and 21 days. For alkali treatment, 0.5 ml of the sample was mixed with 4.5 ml of 0.25% KOH solution for 20 s before being streaked onto CIN agar (Yersinia-selective agar base, CM0653; Yersinia selective supplement, SR0109, Oxoid, UK). This method was used after 7, 14 and 21 days of cold enrichment. CIN agar plates were used to isolate *Yersinia* and these plates were incubated at 30°C for 18 to 20 h.

For the isolation of *A. hydrophila*, an enrichment method was used. This procedure, 1 g of fecal sample was inoculated into 10 ml of alkaline peptone water (APW, pH= 8.4) (Alkaline peptone water, 101800, Merck, Germany) and incubated at 28 °C for 24 h. APW was further diluted (1:10) with PBS and samples were plated on Aeromonas Agar **(**Aeromonas medium base, CM0833; Ampicillin selective supplement, SR0136, Oxoid,UK) with an inoculating loop. All plates were incubated at 28 °C for 24 h. *A. hydrophila* was identified on the basis of morphological, cultural, and biochemical characteristics (9, 17).

In this study, 29 (35.3%) *A. hydrophila* and 22 (26.8%) *Yersinia* species were isolated from stool samples. In isolated *Yersinia* species, 8 (9.7%) *Y. enterocolitica*, 5 (6%) *Yersinia* spp., 4 (4.8%) *Y. frederiksenii,* 3 (3.6%) *Y. intermedia* and 2 (2.4%) *Y. kristensenii* were identified.

Several reports on the isolation of the *Yersinia* spp. from wild-living birds have been published (4, 12, 13). In Japan, Kato et al. (13) examined 500 birds representing nine species, recovered 34 isolates of *Yersinia* spp. They are identified the isolates as *Y . enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii*. In a total of 1370 samples belonging to wild passerine birds (fecal samples and cloacal swap), 41 *Y. enterocolitica*, 26 *Y. frederiksenii*, 20 *Y. kristensenii*, 9 Y. *intermedia* and 2 *Y. pseudotuberculosis* were isolated for a period exceeding one year (4). Of 540 apparently healthy, wild-living birds examined by Kapperud and Rosef (12) in Norway, 5 birds were infected with *Yersinia* species, 2 *Y. kristensenii*, 2 *Y. intermedia* and 1 *Yersinia* X2 but none were infected with *Y. enterocolitica*. Kapperud and Olsvik (11) recovered 6 isolates of *Yersinia spp* from 76 birds. Hamasaki et al. (8) tested 528 cloacal swaps from wild-living birds, and recovered 5 *Y. enterocolitica*, 2 *Y. frederiksenii*, 1 *Y. intermedia* and 3 *Y. pseudotuberculosis.* In this study, a total of 82 Northern Bald Ibis fresh faeces for the isolation of *Yersinia* species were analyzed. Eight (9.7%) *Y. enterocolitica*, 4 (4.8%) *Y. frederiksenii,* 3 (3.6%) *Y. intermedia*, 2 (2.4%) *Y. kristensenii*, and 5 (6%) *Yersinia* spp. were isolated and identified. In this study, similar *Yersinia* species were identified as described by other researchers (4, 8, 12, 13). Higher isolation rates in this study are thought to be related to dietary habits, animal species differences, sampling methods and enviromental conditions. Kaneuchi et al. (10) examined 822 stool samples of birds and found 138 (16.8%) *Y. enterocolitica*, 3 (0.4%) *Y. pseudotuberculosis*, 75 (9.1%) *Y. intermedia*, 74 (9%) *Y. frederiksenii*, 5 (0.6%) *Y. kristensenii* and 8 (1%) *Yersinia* spp. Compared with Northern Bald Ibis, bird species (gulls and ducks) cause higher prevalences rates (10).

Members of the genus *Aeromonas* are found in aquatic environments and animals. In addition, *A. hydrophila* is reported to be present among the intestinal flora in birds. *A. hydrophila* has been isolated from 10 (22.2%) out of 45 fecal samples derived from captive (15). Shane et al. (18) reported 20 (2%) isolations from about 1000 birds. Glunder (7) isolated *A. hydrophila* from nearly 3500 wild and pet birds. The researcher found higher isolations rates in carnivorous and insectivorous birds as 12.4% than granivorous and herbivorous species as 1.9% and omnivorous as 7.1% (7). Isolation rate of *A. hydrophila* in this study is higher (35%) than those reported before. The reason might be that Northern Bald Ibis is a carnivor bird and cross-contamination with housing conditions could affect bacterial colonisation in this species of birds. On the other hand, the intestinal carriage rate of *A. hydrophila* in grey herons (*Ardea cinerea*) was as high as 48% (6). This can be originated that the investigator worked with waterfowl (grey heron).

In conclusion, *Yersinia* species and *A. hydrophila* have been found at high rates in Northern Bald Ibis stool. Northern Bald Ibis can be under risk with regard to *Yersinia* and *Aeromonas* species. Further studies investigating other pathogens in stool of Northern Bald Ibis are necessary.

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