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High Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in Wild Ducks in the Middle Area of South Korea

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Abstract : *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes a significant economic burden in the animal production industry. The purpose of this study was to investigate the prevalence of MAP in the feces of wild duck populations residing along a riverside close to farms in the center of Korea. From wild Spot-billed (*Anas poecilorhyncha*) and Mallard (*Anas platyrhynchos*) ducks, 128 fecal samples were collected and analyzed using multiplex real-time PCR, sequencing, and nested PCR to confirm the presence of the organism. The molecular analyses showed that 44 samples (34.4%) were positive for MAP, suggesting a high prevalence of MAP in the wild duck population. Considering the nature and habitat of wild ducks, this result suggests that the organism was introduced from contaminated water from waste of nearby farms, and that the wild ducks may act as a transmitter of the organism to other wild birds or livestock.

Key words **:** wild duck, MAP, prevalence.

Introduction

Since the first description of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in 1895, it has been known as a pathogen that causes paratuberculosis or Johne's disease, an intestinal granulomatous infection in domestic (cattle, sheep, goats, and camelid) and wild ruminants (Cervidae and Bovidae) as well as wild Canidae, Mustelidae, and Herpestidae (1-6). Recent studies have also indicated the potential etiological role of MAP in the pathogenesis of Crohn's disease in humans, suggesting the organism may have zoonotic potential (7-9). In animals, the paratuberculosis infection occurs by ingesting the organism from the feces of infected animals or contaminated food or surface water (3). After infection, the infected animal can become a reservoir for disease transmission to other animals because of the long latent period between infection and the appearance of the first signs of disease. Therefore, the early detection and management strategy for the infected animals is important to reduce the spread of the disease in domestic and wild animal populations.

The purpose of this study was to investigate the prevalence of MAP in the feces of wild duck population which resides along a riverside close to farms. Since the wild ducks range the fields and riversides, and make contact with farm animals directly or indirectly (via sewage), the wild duck population can be an indirect marker of contamination of waste from farms.

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Materials and Methods

Fecal samples were collected from 49 wild Spot-billed (*Anas poecilorhyncha*) and 79 Mallard (*Anas platyrhynchos*) ducks in the Chungbuk province of South Korea. The fecal samples were collected into sterile conical tubes and bacterial DNA was isolated using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. To demonstrate the presence of the MAP genome in the samples, quantitative real-time PCR assays specific for the *IS900*, *F57*, and *ISMAP02* genes of MAP, respectively, were performed as described previously (Table 1) (10,11). Samples with positive amplification signals in the real-time PCR assays were re-tested in three ways to confirm the result. First, PCR amplicons were obtained from each sample using only the primer set corresponding to a specific target gene and examined by agarose gel electrophoretic analysis to verify that the PCR amplified the target gene with the expected molecular size. Second, PCR products with confirmed molecular sizes were sequenced for the intended target genes using the Big Dye Terminator v3.1 chemistry on an ABI 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and the homology between the deduced sequence and the known MAP strain type-specific sequence was analyzed with the BLAST search program (National Center for Biotechnology Information, USA). Third, a nested PCR target-

Table 1. Sequences of primers and probes used in this study

Target gene	Name	Sequence (5' to 3')	Product size (bp)	Reference
IS900	IS900qPCRF	GATGGCCGAAGGAGATTG	145	11
	IS900qPCRR	CACAACCACCTCCGTAACC		
	IS900qPCRTM	FAM-ATTGGATCGCTGTGTAAGGACACGT-BHQ		
F57	F57qPCRF	GCCCATTTCATCGATACCC	147	11
	F57qPCRR	GTACCGAATGTTGTTGTCAC		
	F57qPCRTM	FAM-CAATTCTCAGCTGCAACTCGAACACAC-BHQ		
ISMAP02	ISMAP02-for	CGC CAG GAA CGC AAA CAT	96	10
	ISMAP02-rev	GTG CAG GGT CGC TCT GAT G		
	ISMAP02-probe	FAM-ACTCCGCATCCAACAACTCACGCTG-BHQ		

ing a different region of the *ISMAP02* gene was performed as described previously (12) and the amplicon size was examined by electrophoretic analysis. A strain type of MAP (ATCC 19698) purchased from the American Type Culture Collection (Manassas, VA, USA) was used as the positive control for these procedures.

Results

Real-time PCR examination of the 128 fecal samples revealed that 44 of the samples (34.4%) were positive for the *ISMAP02* gene of MAP. On agarose gel electrophoresis, all 44 samples represented the amplicons with the expected molecular size of the *ISMAP02* gene in the MAP genome. Sequencing showed 99 to 100% identity to the *ISMAP02* gene sequence of MAP, suggesting that the PCR amplified its target sequence exactly. Finally, the nested PCR confirmed that all 44 samples also contained DNA matching a different region of the *ISMAP02* gene in the MAP genome (Fig 1).

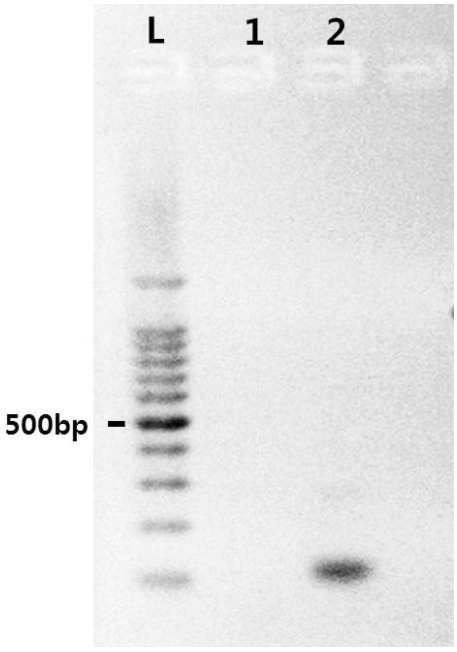

Fig 1. Detection of the 117 bp target product after amplification of the *ISMAP02* gene in a conventional nested PCR format, indicating the presence of *Mycobacterium avium* subsp. *paratuberculosis* in the feces of wild ducks. Lane L, 100-bp molecular marker; lane 1, blank; lane 2, 117 bp target product.

Discussion

Despite of the impact of MAP infection in the animal production industry, the existence and importance of wildlife reservoirs of MAP in the transmission cycle are still undetermined. While some investigations have recovered MAP from captive and free-ranging nondomestic animal species including all pseudoruminants and ruminants (except giraffids), and some nonruminant species such as primates, wild rabbits, foxes, and stoats (3,13-17), few wild bird species such as sparrows, snipes, starlings, and paradise shelducks have been reported to carry the pathogen (18,19).

In this study, a much higher MAP prevalence was detected than in previous studies of the wild bird population (13,18). Usually, carriage of MAP does not always indicate a true infection. In addition, only feces from the wild ducks were collected and analyzed in this study, leaving the possibility of a "pass-through" effect of the organism in the wild ducks. A previous study also indicated that no histopathologic evidence of the infection was identified in wild birds even if the fecal culture was positive for MAP (13). This result means that capture, necropsy, and histopathological examination for suspected MAP sufferers are necessary to quantify the pathogenic capacity of the organism for wild birds. However, it is also possible for MAP to be transmitted from contaminated environment to wild birds, from wild birds to wild birds, from wild birds to livestock, and/or from livestock to wild birds even if the wild birds are carriers for the pathogen and not true hosts (18). In particular, wild ducks live in groups, meaning that once one individual becomes infected, the organism effectively can be introduced quickly to most of the other individuals in the group.

In conclusion, the high prevalence of MAP detected in feces of the wild duck population in this study suggests 1) that the wild bird population can be affected by environmental contamination from farm waste and 2) that the infection or carriage of the organism in wild bird populations can be a source of readmission of the organism to farm animals regardless of the pathogenicity of the organism in the wild birds. Further study is necessary to identify that MAP can infect the wild duck population and to compare the MAP genotype isolated from wild ducks with MAP detected in farm animals to confirm the origin of the organism.

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