



Presence of *Mycobacterium* sp in the feces of captive wild birds in three municipalities of the State of Jalisco

Presencia de *Mycobacterium* sp en las heces de aves silvestres cautivas en tres municipios del Estado de Jalisco

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Data of the Article

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Abstract

To determine the presence of *Mycobacterium* sp in captive wild birds in the state of Jalisco, Mexico, an experimental study was carried out in private hatcheries of three municipalities in the state of Jalisco, covering Guadalajara, Tesistan, and Tonalá to identify the presence of alcohol-acid resistant bacilli (ABB) in stool samples stained with the use of the Ziehl-Neelsen stain, stool samples were taken from 76 birds under captive conditions. Of the 33 birds analyzed in the municipality of Guadalajara, 4 tested positive for *Mycobacterium* sp., Giving 0.12 % positivism in this municipality, while, in Tesistan, of 21 birds analyzed, only one specimen tested positive for *Mycobacterium* sp., Giving 0.4 % positivism. In the municipality of Tonalá, a positive specimen was found out of 22 birds analyzed, giving 0.45 % of bird's positive for *Mycobacterium* sp. Of the 76 birds analyzed, 0.7 % were positive for *Mycobacterium* sp. Species such as *Eupsittula canicularis*, *Amazona autumnalis*, *Ara militaris*, *Alopochen aegyptica*, and *Pavo cristatus* tested positive for *Mycobacterium* sp. This work contributes to documenting the cases of *Mycobacterium* sp., In captive wild birds to take measures that guarantee the health of wild birds.

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Resumen

Con el objetivo de determinar la presencia de *Mycobacterium* sp en aves silvestres cautivas del estado de Jalisco, México se realizó un estudio de tipo experimental en criaderos particulares de tres municipios del estado de Jalisco, abarcando Guadalajara, Tesistán y Tonalá con el fin de identificar la presencia de bacilos alcohol-acido resistentes (BAAR) en muestras de heces teñidas con el uso de la tinción Ziehl-Neelsen, se tomaron muestras de excremento de 76 aves en condiciones de cautiverio. De las 33 aves analizadas en el municipio de Guadalajara, 4 dieron positivo a *Mycobacterium* sp., dando un 0.12 % de positivismo en este municipio, mientras que, en Tesistán, de 21 aves analizadas solo un ejemplar dio positivo a *Mycobacterium* sp., dando un 0.4 % de positivismo. En el municipio de Tonalá de 22 aves analizadas se encontró un ejemplar positivo, dando 0.45 % de aves positivas a *Mycobacterium* sp. De las 76 aves analizadas el 0.7 % dio positivo a *Mycobacterium* sp. Especies como *Eupsittula canicularis*, *Amazona autumnalis*, *Ara militaris*, *Alopochen aegyptica* y *Pavo cristatus* dieron positivo a *Mycobacterium* sp. Este trabajo contribuye a documentar los casos de *Mycobacterium* sp., en aves silvestres cautivas para tomar medidas que garanticen la salud de las aves silvestres.

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## Introduction

Many species of birds have been the cause of *Mycobacterium avium* disease<sup>1</sup>, according to Bernarde-Illi et al.<sup>2</sup> *M. avium* is common where there are a large number of birds, such as zoological institutions, since the presence of an infected bird in captivity, such as a zoo exhibitor, can increase the number of mycobacteria in that environment<sup>3</sup>, according to Chiodini et al.<sup>4</sup>, the incidence is high in intensively reared birds and can be commonly detected in animal feces. Infected<sup>5</sup>, the source of infection is unknown and variable, such as bird remains, which depends on the species, and whether the birds are in captivity or free<sup>6</sup>, in bird necropsies it ranges from 0.5 % to 14 %<sup>7-9</sup>, including parrots<sup>10</sup>, to confirm the presence of *M. avium*<sup>11</sup>, the Ziehl-Neelsen (ZN) stain allows the observation of alcohol-acid resistant bacilli (ABB), according to VanDerHeyden<sup>9</sup>, mycobacterial infections detected at autopsy of pet birds, *M. avium* is considered the most important pathogen a cause of tuberculosis in domestic birds<sup>12</sup>, the diagnosis of *M. avium* is based on the demonstration of ABB by microscopy<sup>13</sup>, as *M. avium* has a thick cell wall, rich in mycolic acids, mycosides, glycolipids, and sulpholipids. makes it hydrophobic and impervious to aqueous stains without heat, so heat must be applied in ZN staining<sup>14</sup>.

One of the potential sources of contamination of *M. avium* is fecal matter<sup>15</sup>. *M. avium* is a Gram-positive ABB<sup>16</sup>, as ZN staining is a quick, easy, and low-cost technique<sup>17</sup>, it allows us to differentiate bacteria into two groups: those that resist discoloration with alcohol-acid and those that are not<sup>18</sup>, to Selvakumar et al.<sup>19</sup>, the sensitivity of this stain to identify ABB is 74 % and a specificity of 98 %. In humans, *M. avium* is capable of first producing lymphadenitis, lung disease, and the spread of the infection, particularly in immunosuppressed individuals or under transplant

therapy<sup>20</sup>. According to Aranaz et al.<sup>21</sup>, the stress experienced by birds in captivity triggers the incidence of *M. avium*, it is also considered by Kriz et al.<sup>22</sup> as a potential zoonotic risk due to contact with infected animals.

With insufficient data on the pathogenic potential of *Mycobacterium* sp., in captive animals and given that the information regarding *Mycobacterium* sp., infection in birds is relatively limited and incomplete, due to a lack of information on the apparently long incubation, an inadequate identification test, and the difficulty of discovering infected birds<sup>23</sup> It is intended that this study be focused on providing information about the presence of *Mycobacterium* sp in wild birds under captive conditions in the state of Jalisco and the use of tools for its diagnosis as an efficient and economical method for the diagnosis of this zoonotic disease<sup>24</sup>.

## Materials and methods

*Study Design.* From June to December 2019, an experimental study<sup>25</sup> was carried out, based on the samples of feces from captive birds from hatcheries belonging to individuals, which are dedicated to the reproduction, maintenance, and commercialization of birds. with the permits of SEMARNAT (Secretary of the Environment and Natural Resources) for its operation.

*Study zone.* The study was carried out in the state of Jalisco, covering the municipalities of Guadalajara, Tesisitan, and Tonalá, located in the center of the country and located within the metropolitan area of Guadalajara.

*Collection and transfer of samples.* 33 birds from Guadalajara, 21 from Tesisitan and 22 from Tonalá,

their excreta were evaluated. The presence of mycobacteria in the faeces<sup>26</sup>, for this reason, the excrement samples were taken from the soil, which was previously cleaned and disinfected, it was preferred to make the collections in the morning after feeding, we waited for the birds to defecate and with a sterile swab was stored in a test tube, for transfer to the laboratory, following the Biosafety recommendations Jorge et al.<sup>27</sup>, later they were stored in thermal coolers with cooling gel inside and kept at a temperature of 4 °C until processed, as this technique is easy to perform, it was preferred to carry out each test in each hatchery, that is, the staining equipment and the microscope were taken to each place in order to reduce

the transfer times of the samples. After being collected, they were processed within 20 min.

*Identification of Alcohol Acid Resistant Bacilli (ABB)*. Smears were made of the feces that were previously collected and stained with it with the indicated staining, which is made up of a set of reagents and the ZN reagent<sup>28,29</sup>, it was observed under a microscope with 100X magnification in oil immersion, following the recommended technique<sup>30,31</sup>. The description of the bacilli was followed according to Bartos et al.<sup>32</sup>, following the WHO recommendations<sup>33</sup>.

## Results

**Table 1 Bird species with the presence of *Mycobacterium* sp in their fecal material, municipality of Tesisitan, in 2019**

Species	Sampled specimens N=21	Positive n=1
Pavo real ( <i>Pavo cristatus</i> )	9	0
Coquena ( <i>Numida meleagris</i> )	6	0
Ganso egipcio ( <i>Alopochen aegyptica</i> )	6	1
<b>Total</b>	21	0.4 %

Table 1 indicates that of the 21 birds analyzed, only one Egyptian goose (*A. aegyptica*) tested positive for *Mycobacterium* sp, which represents 0.4 % positivism.

**Table 2 Bird species with the presence of *Mycobacterium* sp in their fecal material, municipality of Guadalajara, in 2019**

Species	Sampled specimens N=33	Positive n=4
Tucán pecho amarillo ( <i>Ramphastos sulfuratus</i> )	7	0
Perico atolero ( <i>Eupsittula canicularis</i> )	1	1
Loro copete rojo ( <i>Amazona autumnalis</i> )	6	1
Loro montañés ( <i>Amazona finschi</i> )	3	0
Loro frenteazul ( <i>Amazona aestiva</i> )	1	0
Pavo real ( <i>Pavo cristatus</i> )	1	0
Guacamaya verde ( <i>Ara militaris</i> )	8	2
Cotorro tamaulipeco ( <i>Amazona viridigenalis</i> )	1	0
Tucaneta esmeralda ( <i>Aulacorhynchus prasinus</i> )	1	0
Cacatúa ninfa ( <i>Nymphicus hollandicus</i> )	1	0
Cacatúa Alba ( <i>Cacatua moluccensis</i> )	1	0
Guacamaya roja ( <i>Ara macao</i> )	2	0
<b>Total</b>	33	0.12 %

Table 2 0.12 % of the stool samples were positive for *Mycobacterium* sp.

Table 3 Bird species with the presence of *Mycobacterium* sp in their fecal material, municipality of Tonalá, in 2019

Species	Sampled specimens N=22	Positive n=1
Lechuza ( <i>Aegolius acadicus</i> )	1	0
Buho gigante ( <i>Bubo virginianus</i> )	1	0
Perico Nanday ( <i>Aratinga nenday</i> )	1	0
Loro cabeza amarilla ( <i>Amazona oratrix</i> )	1	0
Guacamaya verde ( <i>Ara militaris</i> )	1	0
Pavo real ( <i>Pavo cristatus</i> )	4	1
Avestruz ( <i>Struthio camelus</i> )	3	0
Caracara ( <i>Caracara cheriway</i> )	3	0
Urraca real cara negra ( <i>Calocitta colliei</i> )	2	0
Faisán de collar ( <i>Phasianus colchicus</i> )	1	0
Halcón Harris ( <i>Parabuteo unicinctus</i> )	2	0
Hocofaisan ( <i>Crax rubra</i> )	1	0
Chonche ( <i>Penelope purpurascens</i> )	1	0
<b>Total</b>	<b>22</b>	<b>1</b>

Table 3 shows 0.45 % of birds positive for *Mycobacterium* sp, specimens of *S. camelus* were negative.

## Discussion

Several types of *Mycobacterium* sp., the disease occurs in vertebrates, both in mammals, birds, reptiles, amphibians, and fish, it is considered that they contribute to its global distribution since there are few studies published so far<sup>34</sup>. *Mycobacterium* sp., the infection has been reported in parrots<sup>35</sup>, raptors<sup>36</sup>, pigeons, and ducks<sup>37</sup>.

To prevent wild animals in captivity from becoming sources of infection for humans and other species, it is necessary to carry out routine diagnostic tests, supplemented with management measures to detect and eliminate sick animals, thus avoiding the disease spread<sup>38</sup>.

In aquatic species, Cromie et al.<sup>39</sup> observed a 70 % reduction in mortality. In the municipality of Guadalajara, 0.12 % of the stool samples were positive for *Mycobacterium* sp., while in the municipality of

Guadalajara, out of 33 birds sampled, 4 were positive for *Mycobacterium* sp., and in the municipality of Tesisitan de in the 21 birds analyzed, only one specimen of *Al. aegyptiacus* tested positive for *Mycobacterium* sp., giving 0.4 % positivism. In the municipality of Tonalá, out of 22 birds analyzed, 0.45 % of birds were positive for *Mycobacterium* sp., one of them being *S. camelus* itself, which has been reported in this species by García et al.<sup>40</sup>.

Species such as *E. canicularis*, *A. autumnalis*, *A. militaris*, *A. aegyptica* and *P. cristatus* were positive for *Mycobacterium* sp., coinciding with what was reported by Fowler<sup>41</sup>.

A 0.7 % positivism is reported to *Mycobacterium* sp of the 76 birds analyzed.

Finally, mycobacteria have inhabited our environment and even more so in animals in captivity, apparently, with the data provided in this research work, it seems that the mere fact of a rapid diagnosis

would be the first initiative that allows us to give foot to collect data of its incidence.

But I must also emphasize that a ZN stain is not an accurate diagnosis of *M. avium* identification, but it would allow us to control the spread of the disease and why not say it, it could even be an initiative that avoids infection to human.

The data are alarming since the sample collection sites are animals that are exposed or commercialized and if there is an infection we could with this technique and in the measure that it is carried out with relative frequency we would help to avoid the spread of this pathogen.

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### Conflicts of interest

There were no conflicts of interest between the author and the institution to develop the work interventions.

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### Ethical considerations

During the work carried out, each captive wild bird was treated with dignity and respect, and in each enclosure where the samples were collected, it was avoided to alter their environment.

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