Article

Avian tuberculosis of zoonotic importance at a zoo on the Bogotá Andean plateau (Sabana), Colombia

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Abstract — Given that exposure to captive wild animals at circuses or zoos can be a source of zoonotic infection, a case and control study was carried out with a collection of exotic fowl at a zoo in Bogotá, Colombia. The presence of *Mycobacterium avium-II* was directly related to the death of birds kept in the original enclosure, and of 50% of a group of sentinel birds. Failure to detect the organism in a control group of birds outside the enclosure indicated that the infection was limited to the original enclosed area. We demonstrated that *M. gordonae-IV* was disseminated in all organs from 1 bird with macroscopic granulomatous lesion, a finding which has not been reported previously. We emphasize the importance of establishing handling norms to reduce the risk of zoonotic transmission.

Résumé — Tuberculose aviaire d'importance zoonotique dans un zoo sur le plateau andéen de Bogotá (Sabana), en Colombie. Vu que l'exposition à des animaux sauvages en captivité dans les cirques ou les zoos peut devenir une source d'infection zoonotique, une étude de cas-témoin a été réalisée avec une collection d'oiseaux aquatiques exotiques à un zoo de Bogotá, en Colombie. La présence de *Mycobacterium avium-II* a été directement reliée à la mort d'oiseaux gardés dans un enclos original et de 50 % d'un groupe d'oiseaux sentinelles. La non-détection de l'organisme dans un groupe d'oiseaux témoins à l'extérieur de l'enclos a indiqué que l'infection s'est limitée à l'enclos original. Nous avons démontré que *M. gordonae-IV* a été disséminé dans tous les organes en provenance d'un oiseau avec une lésion granulomateuse macroscopique, un résultat qui n'a pas été signalé antérieurement. Nous insistons sur l'importance d'établir des normes de manipulation afin de réduire le risque de transmission zoonotique.

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Introduction

Zoonotic diseases are a worldwide challenge associated with globalization, international mobility, increase in cattle ranching, and climate change. The challenge must be met while using sustainable agro-industrial practices and maintaining the environment and free-roaming wildlife. Exposure to captive wild animals at circuses or zoos is a source of zoonotic infection, that is not often reported (1).

Avian mycobacteriosis is a bacterial disease affecting several bird species (2); it is highly contagious and chronic. The disease is characterized by granulomatous lesions (3) and a variety of clinical presentations. It is widely distributed throughout the world (4), and recently, it has been reported as being prevalent in wild and domestic fowl (5). In most countries, its distribution and incidence are unknown due to the absence of bacteriological studies (4) that identify the species that are involved. This is a weakness of many epidemiological surveillance systems.

The etiologic agent most frequently involved in avian mycobacteriosis is *Mycobacteirum avium* (6), although there are reports on the isolation of *M. tuberculosis* (7,8), *M. bovis* (9), and *M. genavense* (10–12). *Mycobacteirum avium* is often assigned to the *M. avium-intracellulare* complex (MAC), given the difficulty in differentiating members of this group through culture and biochemical methods. It is frequently present in soil and water (13), and has been reported as the 2nd most common mycobacterial species infecting both animals and human beings (14). Ubiquitous environmental exposure makes prevention difficult (15).

In humans, lymphadenitis is one of the most frequent clinical presentations of mycobacteriosis. *Mycobacteirum avium* is isolated in 70% to 80% of cases involving the MAC (16) and is responsible for the most prevalent disseminated infection in AIDS patients (17). There is increased interest in its epidemiology as human-to-human transmission has not been proved. It

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Table 1. Number of birds and samples by group and methodology

Methodology	Number of birds	Number of samples	Number of cultures or PCR tests	Number of isolates or genotypic positives
GROUP 1				
Microbiological	5	21	38 cultures	10 isolates
Molecular	5	20	20 PCRs	10 genotypic identification
GROUP 2				
Microbiological	5	25	75 cultures	0 isolates
Molecular	5	25	25 PCRs	1 genotypic identification
GROUP 3				
Microbiological	10	48	98 cultures	8 isolates
Molecular	10	46	46 PCRs	14 genotypic identification
GROUP 4				
Microbiological	12	20	40 cultures	0 isolates
Molecular	12	20	20 PCRs	0 PCR products

PCR — polymerase chain reaction.

is assumed that human infection occurs through contact with birds. Nosocomial infections transmitted through water pipes have been described (18) and contaminated water is considered the principal reservoir for AIDS patients.

Nongranulomatous or atypical avian mycobacteriosis is difficult to detect at necropsy (19), as histopathologic examination of tissues stained with hematoxylin and eosin (H&E) does not detect the bacteria. This, along with the lack of effective vaccines or appropriate drug treatment programs, associated mortality, survival of the bacteria in the soil, and the absence of adequate procedures to clean and disinfect contaminated sites make it difficult to diagnose and control (20), especially in birds kept in zoos (4).

This study was conducted because of the finding of granulomatous lesions and acid-fast bacteria in H&E and Ziehl-Neelsen stained tissues, respectively, during necropsy of 3 birds that died while being kept in the same enclosure at a zoo: 2 *Porphirura martinica* (tinguas) and a *Burhinus bistriatus* (alcaraván). Conventional microbiological methods were used to isolate and identify the mycobacterial species. Morphological, biochemical, and antituberculosis drug sensitivity characteristics were determined. A polymerase chain reaction (PCR) for detection of gene *hsp*65 and enzymatic restriction analysis of the PCR product were used to identify the species involved.

Materials and methods

A case-control study was carried out between September, 2003 and December, 2004 at a zoo located on the Bogotá Plateau (Colombia). The birds were confined in an enclosure 3 m tall by 5 m wide and 15 m long. Its walls were constructed of cement up to 1 m from the ground, followed by glass windows, and ending with wire mesh. The roof had plastic roof tiles and the floor was earth; there was a waterer made out of cement. The various species of birds were separated by wire mesh. The research was approved by the ethics committees of both participating centers.

Population

The birds that were investigated belonged to a collection of exotic fowl at the zoo. In addition, 15 domestic fowl were introduced as part of the research. The birds were divided into 4 groups.

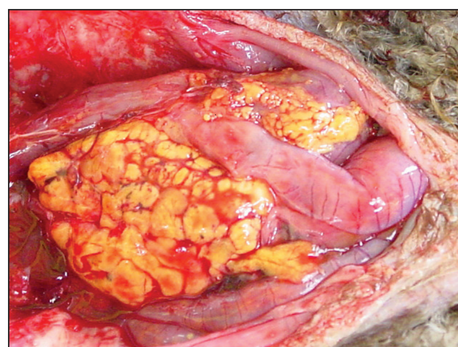

Figure 1. Granulomatous lesion in the peritoneum, caused by *M. gordonae-IV.*

Group 1 consisted of the 5 birds that were kept in the same enclosure in which acid-fast positive cases had been detected. The group included a *Burhinus bistriatus* (alcaraván), a *Speotito cunicularia* (mochuelo de hoyo), 2 *Ortalis motmot columbiana* (guacharaca variable), and a *Chamaepetes goudotii goudotii* (pava maraquera). All birds in this group died during the study. Necropsies were conducted and samples of lung, spleen, intestine, and liver were taken in sterile tubes for microbiology and molecular studies. In the case of the *B. bistriatus* (alcaraván), muscle and bone tissue were also examined. A blood sample with EDTA for culture and molecular studies was taken from the ulnar vein of the *C. g. goudotii* before it died.

Group 2 (experimental control) comprised five 5-week-old *Hy-line brown* pullets from a traditional breeding farm. The apparently healthy animals were delivered directly from the farm and then slaughtered. Samples of spleen, liver, intestine, lung, and blood were collected and tested for infection by *Mycobaterium.*

Group 3 (sentinel bird group) was made up of 10 birds with the same characteristics as those of Group 2, and were taken directly from the farm to the zoo enclosure. They were kept there for 49 wk, during which 2 birds died. Ulnar vein blood samples were taken from the remaining 8 birds, and then they were slaughtered. Necropsy was carried out and samples of liver,

Table 2. Mycobacteria species identified by samples, group, and species of bird

	Bird			
Group	Name	Sample	Molecular method	Microbiological method
1	*Chamaepetes g. goudotii* *(Pava maraquera)*	Spleen, liver Intestine, lung	*M. avium-II* *M. avium-II*	MAC *Mycobacterium* sp.
1	*Burhinus bistriatus* (alcaraván)	Liver, muscle Bone	*M. avium-II* *M. avium-II* + *M. fortuitum-III*	MAC *MAC* + *M. chelonae*
1	*Speotito cunicularia* (mochuelo de hoyo)	Lung, liver	*M. avium-II*	*Mycobacterium* sp.
2	*Gallus gallus* (gallina)	Intestine	*Mycobacterium* sp.	No isolate
3	*Gallus gallus* (gallina)	Spleen Liver, lung	*M. avium-II* *M. avium-II*	MAC No isolate
3	*Gallus gallus* (gallina)	Lung Spleen	*M. avium-I* *M. avium-I*	MAC No isolate
3	*Gallus gallus* (gallina)	Spleen	*Mycobacterium* sp.	No isolate
3	*Gallus gallus* (gallina)	Liver Spleen	*M. chelonae-I* *M. chelonae-I*	*M. chelonae* No isolate
3	*Gallus gallus* (gallina)	Intestine	*Mycobacterium* sp.	No isolate
3	*Gallus gallus* (gallina)	Spleen, liver, Intestine Lung	*M. gordonae-IV* *M. gordonae-IV* *M. gordonae-IV*	*M. gordonae* *M. gordonae* *Mycobacterium* sp.
3	*Gallus gallus* (gallina)	Lung Spleen	ND *Mycobacterium* sp.	*Mycobacterium* sp. No isolate

ND = not done.

Table 3. Distribution of samples according to methodology and positivity

Sample		Microbiological study			Molecular study			
			Positives			Positives		
Tissue	*n*	Total	*n*	% (95% CI)	Total	*n*	% (95% CI)	*P*
Lung	23	23	5	21.7 (3.9–63.0)	23	6	26.1 (5.5–66.5)	0.729
Spleen	19	19	3	15.8 (1.7–61.8)	19	7	36.8 (8.8–77.0)	0.140
Liver	23	23	5	21.7 (3.9–63.0)	23	6	26.1 (5.5–66.5)	0.729
Intestine	22	22	2	9.1 (0.6–25.8)	22	4	18.2 (2.6–60.9)	0.660
Blood	23	23	0	0	22	0	0	
Muscle	1	1	1	100	1	1	100	
Bone	1	1	1	100	1	1	100	
Total	112	112	17	15.2 (5.0–32.6)	111	25	22.5 (10.4–41.0)	0.161

95% CI — 95% confidence interval.

lung, spleen, and intestine were collected. This group was set up to evaluate the transmissibility of the causal agent.

Group 4 (external dissemination control group) was made up of 12 birds kept in enclosures different from the affected one. Three of these birds died, and lung, spleen, intestine, and liver samples were taken during necropsy. The other 9 birds survived the study and blood samples were taken as before.

All tissue samples were homogenized using a disposable pellet pestle in a tissue homogenizer and were decontaminated with sodium hydroxide.

Microbiology

The following culture media were prepared in the laboratory and used to isolate mycobacteria: biphasic medium of solid Ogawa-Kudoh medium and liquid Sauton tween modified albumin (MSTA), biphasic medium Lowenstein Jensen/MSTA, and Stonebrink modified by Giraldo (ST-G) (8). The Ziehl-Neelsen stain was done on cultures to determine the presence of acid fast bacilli (AFB) and the cultures were then identified phenotypically by the Atlanta CDC standard methodology (21).

Genotyping

DNA was extracted for PCR by thermal shock (22). Samples that showed amplification of a fragment of 439 pb with the PCR that targeted gene *hsp65*, indicating the presence of *Mycobacterium,* were identified by enzymatic restriction of the amplified fragment with *BstE-II* and *Hae-III* enzymes (23). The mycobacteria species were determinined based on the sizes of the digestion fragments. The molecular weights of the bands were compared with those registered in the PRASITE

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(http://app.chuv.ch/prasite) data base, thus determining the specific patterns for the different species and their variants (24).

Statistical analysis

Statistical analysis was carried out by using EpiInfo 2002 (CDC, Atlanta, Georgia, USA). The proportions of positivity, according to the diagnostic tests in terms of confidence intervals (95% CI) and *P*-values were compared using Fisher's exact test.

Results

The numbers of birds and samples in each group are shown in Table 1 according to the methodology that was used.

Microbiology

In group 1, there were 3 positive birds with 9 positive samples, 4 of which were identified as *MAC,* 4 as *Mycobacterium* sp. and 1 with *MAC* + *M. chelonae* (Table 2). In group 3, 5 positive birds were detected with 8 positive samples that phenotypically corresponded to: 2 *MAC,* 2 *Mycobacterium* sp., 3 nonpigmented *M. gordonae,* and 1 *M. chelonae.* In groups 2 and 4, there were no mycobacterial isolations and no positive birds.

Molecular studies

In group 1, 3 positive birds with 9 positive samples consisted of 8 *M. avium-II* and 1 co-infection by *M. avium-II* + *M. fortuitum-III.* In group 3, there were 7 positive birds with 14 positive samples that consisted of 3 *M. avium-II,* 2 *M. avium-I,* 4 *M. gordonae-IV,* 2 *M. chelonae-I,* and 3 *Mycobacterium* sp.

In group 2, 1 positive bird yielded a sample that was positive for *Mycobacterium* sp. and in group 4 there were no positive birds. *Mycobacterium avium* was the species with the widest distribution in the various samples examined by conventional microbiology as well as molecular methodology, with which 2 varieties could be differentiated. *Mycobacterium avium-II* was the species found in the highest proportion, above 50%, independently of the methodology, followed by *M. gordonae-IV.* One of the 4 birds from which *M. avium-II* was isolated was young; the other 3 were adults. Likewise, the bird from group 2 in which *Mycobacterium* sp. was reported, was also young.

The PCR showed an increase in sensitivity compared with the traditional microbiology for the spleen, intestine, and liver samples but the increase was not statistically significant (Table 3). Samples of lung and liver allowed a greater proportion of diagnoses by culture, while the spleen samples were the most efficient when evaluated through PCR.

Discussion

Mycobacteria were detected in 50% of the birds in the original enclosed area and in 30.4% of the sentinel birds (group 3). A shorter duration of exposure and different species of birds may have contributed to this difference. In the control sentinel group, 50% of contaminated birds were detected by culture and 70% by PCR ($P = 0.464$), reflecting the ability of PCR to detect the lower concentrations of the bacteria that are present in the early stages of infection (22).

The presence of *M. avium-II* was directly related to the pathology observed in the birds kept in the original enclosure. *Mycobacterium avium-II* was also related to the pathology observed in the 50% of the sentinel birds that died. This type of *M. avium,* which corresponds to *M. avium* subsp. *avium,* in both the old and modern classifications (25), has been accepted as the causative agent of avian tuberculosis. It has been associated with dissemination in AIDS patients, in children with lymphadenitis, and in adolescents and elders with chronic lung tuberculosis. It is a different subspecies from the one causing infections in pigs but the question of host specificity among members of the MAC is still unclear (26). *M. avium* subsp. *avium* may have wildlife as a major reservoir with wild birds being responsible for its excretion into water and soil where it can remain for long periods of time and infect various animal species and human beings (27).

Mycobacterium avium-I was present exclusively in one of the 8 sentinel birds that were slaughtered at the end of the study, which may suggest that this *Mycobacterium* might not be as virulent in birds as *M. avium-II.*

Macroscopic granulomatous lesions were observed in the lung and spleen of only 1 of the 3 birds from group 3 in which *Mycobacterium* sp. was detected. This illustrates the great sensitivity of the molecular methodologies used to detect early infection by mycobacteria. A very interesting finding was the microbiological and molecular demonstration of *M. gordonae-IV* disseminated in all the organs studied from 1 bird with macroscopic evidence of granulomatous lesions. This had not been reported in the literature prior to this study. In humans, this mycobacterium has caused pathology and has frequently been reported in treated and untreated water (15).

In another bird of the sentinel group, *M. chelonae-I* was detected and isolated in association with macroscopic granulomatous lesions in the liver and spleen. This bird may have been immunosuppressed, favoring the dissemination of this potentially pathogenic mycobacterium (28). Within group 4, the external dissemination control group, mycobacteria were not detected by microbiology or PCR, indicating that the infection was likely limited to the original enclosure in which the infected birds were identified originally.

Samples of intestine showed low positivity, contrary to what might be expected in an organ where initial colonization occurs (9). This might be due to the abundant microbial flora, which would make isolation of mycobacteria difficult.

The results lead to the conclusion that the birds in the sentinel group became infected within the original enclosure and that the agent was able to infect 70% of these birds. Through this study, a definite diagnosis of avian mycobacteriosis caused by *M. avium-II* was established in a zoo on the Bogota Andean Plateau (Sabana).

The findings in this study highlight the importance of establishing handling norms to reduce the risk of zoonotic transmission. Since some studies (29) have shown a high proportion of mycobacteriosis by *M. avium-II* in immunosupressed human patients, there is a need to establish routinely the immunologic state of those handling the birds. Such procedures should be performed exclusively by immunocompetent individuals. The soil of enclosures is the biggest source of infection due to the spread of bacilli contained in the feces from birds infected with

M. avium, which can remain viable in soil for more than 4 y. In the case of carcasses burned and buried 1-m deep, this time could be as long as 27 mo (4). It is therefore a priority to establish disinfection protocols of enclosures of exotic birds, which should be reviewed periodically and when the birds become infected with mycobacteria.

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