Examination of *Mycobacterium avium* subsp. *avium* distribution in naturally infected hens by culture and triplex quantitative real time PCR

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ABSTRACT: *Mycobacterium avium* subsp. *avium* (*MAA*) is the etiologic agent of avian tuberculosis, a chronic contagious disease described in a wide variety of domestic and wild bird species. The aims of this study were to assess the advantages of triplex quantitative real time PCR (qPCR) in comparison with culture testing for distribution of *MAA* in the organs of hens displaying varying degrees of clinical symptoms of the disease. From one small flock of ten hens and one cock with a history of weight loss, 98 tissue samples were examined in total. Pathological lesions were observed in six hens from which two were clinically ill. A total of 12 samples were positive by culture and 16 were positive by IS*901* and IS*1245* qPCR, confirming *MAA* infection. In conclusion, qPCR was a faster and more reliable alternative method in comparison with conventional culture analysis. Due to the detection of *MAA* in the muscle tissue of one hen, consumption of under cooked meat originating from infected fowl could pose a threat to immunosuppressed individuals.

Keywords: chicken; mycobacteriosis; non-tuberculous mycobacteria; food safety; meat; zoonosis

Mycobacterium avium subsp. *avium* (*MAA*) is the etiologic agent of avian tuberculosis, a chronic contagious disease described in a wide variety of domestic and wild bird species (Thorel et al., 1997; Tell et al., 2001; Kriz et al., 2010; Skoric et al., 2010) as well as other animals (Pavlik et al., 2008; Pate et al., 2009). Galliformes, in particular domestic fowl, are considered as the principal reservoir of the disease (Hejlicek and Treml, 1995). Birds mainly become infected through ingestion of the pathogen, although the respiratory route has not been excluded (Tell et al., 2001). Affected birds are in poor physical condition, emaciated, lethargic and weak. The manifestation of the disease can vary, apart from the classical tuberculosis symptoms, lesions can also present themselves only in the intestinal tract. Miliary tuberculosis has been characterised previously in several cases of heavily infected birds (Thorel et al., 1997; Prukner-Radovcic et al., 1998; Tell et al., 2001).

Due to the long incubation period needed for the development of avian tuberculosis, clinical signs are observed only in advanced stages of the disease (Biet et al., 2005), thus it might not be diagnosed in time to prevent the spread among fowl. Infected birds are capable of shedding *MAA* to the surrounding environment (Shitaye et al., 2008a; Kazda et al., 2009), which poses a danger of the spread of infection through fowl as well as other animals and humans. Immunocompromised individuals are especially susceptible to *MAA* infections (Falkinham, 1996; Griffith et al., 2007).

Timely diagnosis of avian tuberculosis is the most promising preventative measure. Diagnosis relies on isolation of the causative agent, which in some cases is problematic due to the long time needed for growth, or its inability to grow *in vitro* (e.g. *M. genavense*). Also, viable but non-cultivable mycobacteria could be present in the specimen (Beran

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et al., 2006). Surveys of prevalence and distribution of *MAA* in chickens have been carried out using culture as well as serological testing (Shitaye et al., 2008a,b). Different culture methods for the recovery of *MAA* have also been tested previously (Shitaye et al., 2009a).

Culture independent molecular tests have been used previously for the detection and identification of species belonging to the *M. avium* complex directly from tissue samples (Tell et al., 2003; Ikonomopoulos et al., 2009; Manarolla et al., 2009). The detection of avian mycobacteria in previous studies used more general approaches, such as amplification and sequencing of the *hsp*65 and 16*S rRNA* genes. This method was determined to not be specific enough for the identification of *MAA*, as all the subspecies of *M. avium* species have 100% homology in their 16*S rRNA* gene sequences and are therefore not distinguishable from one another. With the discovery of insertion elements in the mycobacterial genome, the determination of *M. avium* subspecies has since became more precise (Shamputa et al., 2004; Moravkova et al., 2008). *MAA* is characterised by the presence of 2 to 17 copies of the IS*901* insertion sequence (Dvorska et al., 2003; Inglis et al., 2003) and a single copy of IS*1245* (Johansen et al., 2007). To the authors' knowledge, no study for the specific identification of *MAA* in the tissue of naturally infected hens has previously been reported. We have used the presence of the specific insertion sequences IS*901* and IS*1245* for triplex real time quantitative PCR (qPCR) with an internal amplification control (IAC; Slana et al., 2010).

The aim of this study was to assess the advantages of qPCR in comparison with culture testing for the determination of *MAA* distribution in organs of hens with qualitative and quantitative differences in disease symptoms. Additionally, we aimed to detect possible risks posed by human consumption of infected chicken.

MATERIAL AND METHODS

Samples origin

One cock and a flock of ten hens were selected for this study because of their previous weight loss and poor physical condition. Hens were euthanised in the dissection room of the Veterinary Research Institute (Brno) and tissue samples were obtained from nine different organs (Table 1). Ninety eight samples were examined in total. The samples were stored at –70°C until further processing.

Clinical signs and pathological examination

The health status of the hens was evaluated for signs of emaciation and weakness. During the dissection, organs were observed and examined for the presence of tuberculous lesions.

Mycobacterial culture and identification of isolates

Tissue samples were cultured according to Fischer et al. (2001). Briefly, 1 g of tissue was homogenised and decontaminated using 4 ml of a 1M HCl solution. After neutralisation, 80 µl of the suspension were inoculated onto two egg-based solid media (Herrold's and Stonebrink) and one liquid serum media according to Sula (Kubin et al., 1986). Incubation was performed at 37°C for two months. The cultures were checked after one week to rule out fast growing mycobacterial species or possible contamination and then every two weeks for the following two months. DNA was extracted from single colonies of the isolates as described by Moravkova et al. (2008). Further identification was done by conventional PCR for the presence of specific mycobacterial DNA and *MAC* classification (Wilton and Cousins, 1992); and for presence of specific insertion sequences for subspecies identification (Moravkova et al., 2008).

DNA isolation

A commercially available kit (DNeasy Blood & tissue kit, Qiagen, Hilden, Germany) with a slightly modified protocol (Slana et al., 2010) was used for the isolation of DNA from 50 mg of tissue samples. Briefly, the tissue samples were incubated in the presence of ATL buffer (Qiagen) and proteinase K (Sigma, St. Louis, MO, USA) at 56°C with shaking at 1400 rpm until tissue lysis was complete. The mycobacterial cells were disrupted using 0.1 mm zirconia silica beads (BioSpec, Bartlesville, OK, USA) in the MagnALyser instrument (Roche Molecular Diagnostic, Mannheim, Germany) at 6400 rpm for 60 s. After the addition of 96% ethanol, the samples

were loaded in two consecutive steps onto a column and washed with each provided washing buffer twice. The DNA was eluted in 100 µl of pre-heated TE buffer (Amresco, Solon, OH, USA) after 3 min of on-column incubation.

Real Time qPCR

Real time quantitative PCR was performed as a triplex reaction for the specific detection of IS*901*, IS*1245* and IAC (Slana et al., 2010). All three targets were amplified in the same reaction on a semi-competitive principle with two sets of primers. Primers targeting IS*1245* also amplified the IAC. All of the samples were analysed in duplicate. In the case of inhibition (i.e. negative IAC), the DNA isolation was repeated. Every experiment included a negative DNA isolation control. Quantification was based on the principle that the amount of starting material is proportional to the number of cycles required to cross the threshold point of fluorescence. Standards prepared from plasmids containing the target diluted in the range of 10^5 to 10^0 copies made up the calibration curve. According to the number of copies of IS*901* (2 to 17, mean number 12) and IS*1245* (single copy) per cell in *MAA*, the quantity of the cells present in the sample was calculated.

RESULTS

Clinical signs and pathological lesions

Two hens (No. 3 and 5) were very weak and emaciated with deformation of the breast bone. Hen number five was also showing signs of apathy. Pathological lesions were observed in six hens. Most often these lesions were present in the liver (in five hens), less frequently in the spleen (in three cases) and sporadically in the small intestine (one hen; Table 1).

Culture examination

In total there were 12 isolates of *MAA* obtained by culture examination. Isolates were confirmed by conventional PCR for the presence of IS*901* and IS*1245*. Colony forming units (CFU) were counted in the positive samples.

Table 1. Distribution of *Mycobacterium avium* subsp. *avium* and tuberculous lesions found in the organs of 11 naturally infected hens

Hen No.	Liver			Spleen			Intestine			Lung			Kidney			Ovaries			Heart			Bone			Muscle		
	P	C	Q	P	C	Q	P	C	Q	P	C	Q	P	C	Q	P	C	Q	P	C	Q	P	C	Q	P	C	Q
1	–	–	–	–	+	10^3	+	+	10^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	+	–	–	–	+++	10^4	–	+++	10^3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+++	10^3	+	+	10^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	+	10^1	+	++	10^2	–	+++	10^8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10^2
6	+	–	–	–	–	–	–	+	10^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	10^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10^1	–	–	–	–	–	–	–	–	–	–	–	–
9	+	–	–	+	+++	10^7	–	+++	10^5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	–	–	–	–	–	–	–	–	10^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

P = pathological lesions (+ = yes, – = no)
C = culture examination (CFU = colony forming units: – = 0 CFU; + = 1–50 CFU; ++ = 50–100 CFU; +++ = ≥100 CFU)
Q = qPCR results (quantification per gram of tissue according to calibration curve)

Culture on solid media proved to be more successful, as only three samples were positive in liquid media. Using this method, infection was confirmed in six (54.5%) out of eleven hens (Table 1).

Real time quantitative PCR

In nine hens (81.8 %) *MAA* was detected by qPCR. From the 98 tissue samples (originating from 11 animals) examined by qPCR, 16 (16.3%) were positive for the presence of both IS*901* and IS*1245*, confirming infection with *MAA*. All samples tested gave a clear signal for IAC. Although quantification was calculated for both targets, the numbers of *MAA* presented here are based on IS*901* copy number. The *MAA* DNA was present in at least one of the tissues examined in nine hens. From all the tissues examined, the highest numbers of positive samples were obtained from the small intestine of seven hens, with quantification ranging from 10^2 to 10^8 mycobacterial cells per gram of tissue. In five hens, the spleen was positive with up to 10^7 cells per gram of tissue, and in the other organs positivity was sporadic. The examined heart, lungs, ovaries and bone marrow samples were negative. In two hens (hens No. 5 and 11) none of the tissues tested were positive. In three other hens, *MAA* was detected by triplex qPCR only in the small intestine. The remaining hens were positive in the spleen and liver, and in one case in kidney tissue. Hen No. 5 tested gave most positive results with mycobacterial DNA detection in five organs: liver, spleen, small intestine and breast muscle (Table 1).

DISCUSSION

The distribution of *MAA* infection determined in this study suggests that the hens became infected through the alimentary tract, which has been described in other studies as the most common route of infection (Tell et al., 2001). *MAA* was found by qPCR in the intestine in seven of the eleven hens examined. In concordance with the pathognomic lesions for avian tuberculosis in hens, the organs most affected apart from the intestine were the liver and spleen (Thorel et al., 1997; Shitaye et al., 2008a,b).

In previous studies concerned with the detection of mycobacteria in tissue samples without culturing, mycobacteria were identified by sequencing of the hyper variable region of the 16*S rRNA* gene (Manarolla et al., 2009), a fragment of the *hsp65* gene (Tell et al., 2003) or a PCR reaction for *Mycobacterium* sp. identification and species identification with insertion sequence-specific PCR reactions (Miller et al., 1999; Ikonomopoulos et al., 2009). Our detection method was based on a specific and sensitive triplex reaction which enables identification of *MAA* (Slana et al., 2010).

Besides *MAA*, *M. genavense* has been sporadically isolated from avian tissue, and found to be an etiological agent of avian tuberculosis, especially in pet birds (Hoop et al., 1996; Manarolla et al., 2009). Nevertheless, *MAA* remains the most prevalent causative agent of avian tuberculosis in domestic hens (Gonzalez et al., 2002, Shitaye et al., 2008a). *MAA* have also been isolated as non-tuberculous mycobacteria (NTM) species from opportunistic infections in humans (Pavlik et al., 2000; Koh et al., 2002; Glassroth, 2008). In previous studies pulmonary as well as disseminated infections were described (Horsburgh et al., 1994).

The routes of infection with *MAA* are not yet fully understood and evidence of human to human transmission is lacking. At present, the environment and infected animals remain the main reservoir for NTM including *MAA* (Kazda et al., 2009; Krizova et al., 2010)). Although various sources of infection in the environment exist (i.e. birds' faeces), it can be assumed that human infection can be acquired through the ingestion of contaminated food. *MAA* was detected in the muscles of one hen in this study (Table 1).

Different species of NTM including *M. avium* species have been isolated from raw meat and meat products in previous studies (Alonso-Hearn et al., 2009; Shitaye et al., 2009b; Mutharia et al., 2010; Slana et al., 2010). A direct method for mycobacterial detection in meat enables more sensitive determination of this specific pathogen associated with human disease. Although a small study sample was used, the single finding of *MAA* in meat destined for consumption has raised the question of current food safety standards.

From the comparison of methods used in this study for determining the presence of *MAA* in tissues, we can draw the conclusion that qPCR is a faster and also reliable alternative to conventional culture methods. Using qPCR, we were able to confirm *MAA* infection in all of the positive culture samples as well as additional samples infected by low numbers of *MAA* cells (under 10^2). Due to the detection of *MAA* in the muscles of one hen, food quality control and food safety must be taken into

consideration. Consumption of under cooked meat originating from infected fowl could pose a serious threat to immunosuppressed patients. Further studies are needed to assess the prevalence of potentially pathogenic mycobacteria in meat and the risk of human consumption of contaminated meat.

REFERENCES

Alonso-Hearn M, Molina E, Geijo M, Vazquez P, Sevilla I, Garrido JM, Juste RA (2009): Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from muscle tissue of naturally infected cattle. Foodborne Pathogens and Disease 6, 513–518.

Beran V, Havelkova M, Kaustova J, Dvorska L, Pavlik I (2006): Cell wall deficient forms of mycobacteria: a review. Veterinarni Medicina 51, 365–389. http://vri.cz/docs/vetmed/51-7-365.pdf

Biet F, Boschiroli ML, Thorel MF, Guilloteau LA (2005): Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium-intracellulare* complex (*MAC*). Veterinary Research 36, 411–436.

Dvorska L, Bull TJ, Bartos M, Matlova L, Svastova P, Weston RT, Kintr J, Parmova I, Van Soolingen D, Pavlik I (2003): A standardised restriction fragment length polymorphism (RFLP) method for typing *Mycobacterium avium* isolates links IS*901* with virulence for birds. Journal of Microbiological Methods 55, 11–27.

Falkinham JO (1996): Epidemiology of infection by nontuberculous mycobacteria. Clinical Microbiology Reviews 9, 177–215.

Fischer O, Matlova L, Dvorska L, Svastova P, Bartl J, Melicharek I, Weston RT, Pavlik I (2001): Diptera as vectors of mycobacterial infections in cattle and pigs. Medical and Veterinary Entomology 15, 208–211.

Glassroth J (2008): Pulmonary disease due to nontuberculous mycobacteria. Chest 133, 243–251.

Gonzalez M, Rodriguez-Bertos A, Gimeno I, Flores JM, Pizarro M (2002): Outbreak of avian tuberculosis in 48-week-old commercial layer hen flock. Avian Diseases 46, 1055–1061.

Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K (2007): An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. American Journal of Respiratory and Critical Care Medicine 175, 367–416.

Hejlicek K, Treml F (1995): Comparison of pathogenesis and epizootiology signification of avian mycobacteriosis in different sorts of domestic and free-living synanthropic fowl. Veterinarni Medicina 40, 187–194.

Hoop RK, Bottger EC, Pfyffer GE (1996): Etiological agents of mycobacterioses in pet birds between 1986 and 1995. Journal of Clinical Microbiology 34, 991–992.

Horsburgh CR, Metchock B, Gordon SM, Havlik JA, Mcgowan JE, Thompson SE (1994): Predictors of survival in patients with *AIDS* and disseminated *Mycobacterium avium* complex disease. Journal of Infectious Diseases 170, 573–577.

Ikonomopoulos J, Fragkiadaki E, Liandris E, Sotirakoglou K, Xylouri E, Gazouli M (2009): Estimation of the spread of pathogenic mycobacteria in organic broiler farms by the polymerase chain reaction. Veterinary Microbiology 133, 278–282.

Inglis NF, Stevenson K, Heaslip DG, Sharp JM (2003): Characterisation of IS*901* integration sites in the *Mycobacterium avium* genome. FEMS Microbiology Letters 221, 39–47.

Johansen TB, Olsen I, Jensen MR, Dahle UR, Holstad G, Djonne B (2007): New probes used for IS*1245* and IS*1311* restriction fragment length polymorphism of *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *hominissuis* isolates of human and animal origin in Norway. BMC Microbiology 7, 14.

Kazda J, Pavlik I, Falkinham III JO, Hruska K (eds.) (2009): The Ecology of Mycobacteria: Impact on Animal's and Human's Health. 1st ed. Springer; xviii+522pp. http://www.springer.com/public+health/book/978-1-4020-9412-5

Koh WJ, Kwon OJ, Lee KS (2002): Nontuberculous mycobacterial pulmonary diseases in immuniocompetent patients. Korean Journal of Radiology 3, 145–157.

Kriz P, Slana I, Mrlik V, Moravkova M, Kralova A, Krizova K, Pavlik I (2010): *Mycobacterium avium* subsp. *avium* in domestic pigeons (*Columba livia* f. *domestica*) diagnosed by direct conventional multiplex PCR: a case report. Veterinarni Medicina 55, 87–90. http://www.vri.cz/docs/vetmed/55-2-87.pdf

Krizova K, Matlova L, Horvathova A, Moravkova M, Beran V, Boisselet T, Babak V, Slana I, Pavlik I (2010): Mycobacteria in the environment of pig farms in the Czech Republic between 2003 and 2007. Veterinarni Medicina 55, 55–69. http://www.vri.cz/docs/vetmed/55-2-55.pdf

Kubin M, Wisingerova E, Pekarek J, Prochazka B (1986): The patterns of complex and partially purified mycobacterial antigens in macrophage-migration inhibition testing. Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene Series A – Medical Microbiology Infectious Diseases Virology Parasitology 261, 362–369.

Manarolla G, Liandris E, Pisoni G, Sassera D, Grilli G, Gallazzi D, Sironi G, Moroni P, Piccinini R, Rampin T

(2009): Avian mycobacteriosis in companion birds: 20-year survey. Veterinary Microbiology 133, 323–327.

Miller JM, Jenny AL, Ellingson JL (1999): Polymerase chain reaction identification of *Mycobacterium avium* in formalin-fixed, paraffin-embedded animal tissues. Journal of Veterinary Diagnostic Investigation 11, 436–440.

Moravkova M, Hlozek P, Beran V, Pavlik I, Preziuso S, Cuteri V, Bartos M (2008): Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and heavily infected tissues. Research in Veterinary Science 85, 257–264.

Mutharia LM, Klassen MD, Fairies J, Barbut S, Gill CO (2010): *Mycobacterium avium* subsp. *paratuberculosis* in muscle, lymphatic and organ tissues from cows with advanced Johne's disease. International Journal of Food Microbiology 136, 340–344.

Pate M, Moravkova M, Krt B, Pavlik I, Ocepek M (2009): Genotypimg of *Mycobacterium avium* subsp. *avium* isolates from domestic animals in Slovenia by IS*901* RFLP. Veterinarni Medicina 54, 270–279. http://www.vri.cz/docs/vetmed/54-6-270.pdf

Pavlik I, Svastova P, Bartl J, Dvorska L, Rychlik I (2000): Relationship between IS*901* in the *Mycobacterium avium* complex strains isolated from birds, animals, humans, and the environment and virulence for poultry. Clinical and Diagnostic Laboratory Immunology 7, 212–217.

Pavlik I, Jahn P, Moravkova M, Matlova L, Treml F, Cizek A, Nesnalova E, Dvorska-Bartosova L, Halouzka R (2008): Lung tuberculosis in a horse caused by *Mycobacterium avium* subsp. *avium* of serotype 2: a case report. Veterinarni Medicina 53, 111–116. http://www.vri.cz/docs/vetmed/53-2-111.pdf

Prukner-Radovcic E, Culjak K, Sostaric B, Mazija H, Sabocanec R (1998): Generalised tuberculosis in pheasants at a commercial breeding farm. Zeitschrift fur Jagdwissenschaft, 44, 33–39.

Shamputa IC, Rigouts L, Portaels F (2004): Molecular genetics methods for diagnosis and antibiotic resistance detection of mycobacteria from clinical specimens. APMIS, 112, 728–752.

Shitaye JE, Matlova L, Horvathova A, Moravkova M, Dvorska-Bartosova L, Treml F, Lamka J, Pavlik I (2008a): *Mycobacterium avium* subsp. *avium* distribution studied in a naturally infected hen flock and in the environment by culture, serotyping and IS*901* RFLP methods. Veterinary Microbiology 127, 155–164.

Shitaye JE, Matlova L, Horvathova A, Moravkova M, Dvorska-Bartosova L, Trcka I, Lamka J, Treml F, Vrbas V, Pavlik I (2008b): Diagnostic testing of different stages of avian tuberculosis in naturally infected hens (*Gallus domesticus*) by the tuberculin skin and rapid agglutination tests, faecal and egg examinations. Veterinarni Medicina 53, 101–110. http://www.vri.cz/docs/vetmed/53-2-101.pdf

Shitaye JE, Beran V, Svobodova J, Moravkova M, Babak V, Pavlik I (2009a): Comparison of the conventional culture, the manual fluorescent MGIT system and the automated fluorescent MGIT 960 culture system for the detection of *Mycobacterium avium* ssp. *avium* in tissues of naturally infected hens. Folia Microbiologica 54, 137–141.

Shitaye JE, Horvathova A, Bartosova L, Moravkova M, Kaevska M, Donnelly N, Pavlik I (2009b): Distribution of non-tuberculosis mycobacteria in environmental samples from a slaughterhouse and in raw and processed meats. Czech Journal of Food Sciences 27, 194–202. http://www.agriculturejournals.cz/publicFiles/08683.pdf

Skoric M, Fictum P, Frgelecova L, Kriz P, Slana I, Kaevska M, Pavlik I (2010): Avian tuberculosis in a captured Ruppell's griffon vulture (*Gyps ruppellii*) – a case report. Veterinarni Medicina 55, 348–352. http://www.vri.cz/docs/vetmed/55-7-348.pdf

Slana I, Kaevska M, Kralik P, Horvathova A, Pavlik I (2010): Distribution of *Mycobacterium avium* subsp. *avium* and *M. a. hominissuis* in artificially infected pigs studied by culture and IS*901* and IS*1245* quantitative real time PCR. Veterinary Microbiology 144, 437–448.

Tell LA, Woods L, Cromie RL (2001): Mycobacteriosis in birds. Revue Scientifique et Technique-Office International des Epizooties, 20, 180–203.

Tell LA, Leutenegger CM, Larsen RS, Agnew DW, Keener L, Needham ML, Rideout BA (2003): Real-time polymerase chain reaction testing for the detection of *Mycobacterium genavense* and *Mycobacterium avium* complex species in avian samples. Avian Diseases 47, 1406–1415.

Thorel MF, Huchzermeyer H, Weiss R, Fontaine JJ (1997): *Mycobacterium avium* infections in animals. Literature review. Veterinary Research 28, 439–447.

Wilton S, Cousins D (1992): Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. PCR Methods and Applications 1, 269–271.

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