

***Mycobacterium avium* subsp. *avium* in domestic pigeons (*Columba livia* f. *domestica*) diagnosed by direct conventional multiplex PCR: a case report**

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ABSTRACT: We report three pigeons euthanized in a small household breeding facility, where there was suspicion of an avian tuberculosis outbreak. For rapid identification of *Mycobacterium avium* subsp. *avium* direct conventional multiplex PCR was used. Nodular lesions were found on the livers of all three birds, the intestine of one bird and the kidney and ovaries of another. The liver samples and a further 18 tissue samples were examined. Acid-fast rods were detected in all the tissue samples after Ziehl-Neelsen staining. Isolation and diagnosis of *M. a. avium* (serotype 1 containing IS901) from 17 tissue samples was confirmed using conventional multiplex PCR.

Keywords: avian tuberculosis; mycobacteriosis; zoonosis; outbreak; rapid detection

Avian tuberculosis is a severe infectious disease in birds caused by *Mycobacterium avium* subsp. *avium* (*M. a. avium*). Avian tuberculosis in domestic or free living pigeons is probably rare and little is known about its pathogenicity and epizootiology in pigeons. In a study conducted in Turkey, none of the 28 domestic pigeons (*Columba livia* f. *domestica*) kept in the same aviary as four avian tuberculosis positive pheasants and peafowl presented any clinical signs of the disease. Nevertheless, none of them were tested for infection (Kul et al., 2005). *M. a. avium* can also infect different species of mammals including humans (Pavlik et al., 2000; Thorel et al., 2001; Ocepek et al., 2003; Cho et al., 2006; Shitaye et al., 2006; Moravkova et al., 2007; Pavlik et al., 2008).

M. a. avium is characterized by the presence of a specific insertion sequence IS901 within the subspecies genome (Pavlik et al., 2000; Mijs et al., 2002; Dvorska et al., 2003; Bartos et al., 2006; Schrenzel et al., 2008). *M. a. avium* can be diagnosed not only by conventional laboratory methods like gross pathology, histology, Ziehl-Neelsen microscopy and

culture, but also by PCR techniques. Members of *M. avium* species (*M. a. paratuberculosis*, *M. a. hominissuis*, *M. a. silvaticum*, and *M. a. avium*) can be differentiated by conventional multiplex PCR (cmPCR); this technique was also used for the successful direct (culture independent) detection of *M. a. paratuberculosis* in heavily infected tissues of infected sheep (Moravkova et al., 2008).

In this report we describe three pigeons from one household breeding facility where an outbreak of avian tuberculosis was suspected. *M. a. avium* was detected in pigeon tissues using direct conventional multiplex PCR (dcmPCR; Moravkova et al., 2008), which facilitated a rapid decision regarding the outbreak.

Case report

Clinical signs of diarrhoea, emaciation, reproductive disorders and sporadic death were observed in some pigeons in one household breeding facility in the southern part of the Moravia region, Czech

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Republic. The flock consisted of 437 birds of varying breeds. Once clinical signs were observed, a veterinary surgeon sent two dead pigeons to the State Veterinary Diagnostic Institute for examination. During their necropsy, nodular granulomatous lesions on craws of both birds, and the liver of one bird, were found. Many acid-fast rods (AFRs) were observed in microscopic smears of granulomas after Ziehl-Neelsen (Z-N) staining. Our laboratory was requested to assist with precise laboratory diagnostics. Due to their deteriorating condition (i.e. emaciation and/or inability to fly), three pigeons were selected, euthanized and sent for examination to our laboratory. The birds were necropsied and a gross examination of internal organs was performed. A total of 21 tissue samples (seven from each bird) were collected and examined by Z-N staining microscopy. DNA was isolated from liver tissue samples of all three pigeons and dcmPCR was carried out according to a previously described method (Moravkova et al., 2008). All 21 tissue samples, including three samples of liver, were cultured for the presence of mycobacteria as described previously (Fischer et al., 2000). Three isolates (one from each bird) were serotyped according to a previously described method (Wolinsky and Schaefer, 1973) modified by Sussland and Hrdinova (1976).

All three birds were emaciated and gross examination revealed nodular lesions (Table 1). Multiple yellowish granulomas 5–10 mm in size were observed in the liver and small and large intestines of pigeon No. 2. Severe hepatomegaly with multi-

ple nodular lesions (5 mm and 1 cm) was present in pigeon No. 3 (Figure 1). Small nodular lesions on the kidney and ovary were also observed after removal of the liver from the body cavity. Pigeon No. 1 only exhibited mild pathological lesions in the liver (Table 1). AFR's were microscopically seen in all of the 21 examined tissues. In all three Z-N positive liver tissue samples, *M. a. avium* was detected by dcmPCR through the detection of IS901 within 24 h of sample isolation. After three to four weeks of incubation, *M. a. avium* isolates of serotype 1 were obtained from all examined tissues (Table 1).

DISCUSSION

Reports of avian tuberculosis in domestic pigeons are generally rare and in the last two decades only two papers have been published dealing with this issue (Morita et al., 1994; Bougiouklis et al., 2005). A study done by Hejlíček and Tremel (1994) found that pigeons were considerably resistant to the causal agent of avian tuberculosis infection in infectious trials.

In our case it was not possible to determine the exact period in which the pigeons were infected. During epizootiological investigation, the breeder stated that he formerly raised also one flock of hens (for years) and that the last three hens from this flock had died due to white "poppy seed" lesions in the liver two years prior to this incident. It is thus probable that the flock became infected over the

Table 1. Results for the three tested pigeons

Tissue	No.1 (male)			No.2 (female)			No.3 (female)		
	PA	Z-N	culture	PA	Z-N	culture	PA	Z-N	culture
Liver	+	¹	+	++	¹	–	++	¹	+
Intestine	–	+	+	++	+	+	–	+	–
Kidney	–	+	+	–	+	+	+	+	+
Heart	–	+	+	–	+	+	–	+	+
Glandular stomach	–	+	+	–	+	+	–	+	–
Genitals	–	+	+	–	+	+	+	+	+
Bone marrow	–	+	+	–	+	+	–	+	–

PA = pathological anatomy (– tuberculous lesions not present; + moderate: few granulomas present; ++ severe: multiple granulomas present)

Z-N = direct tissue staining according to Ziehl-Neelsen for the detection of acid-fast rods (– not detected; + detected)

Culture examination for *M. avium* subsp. *avium* confirmed by the presence of IS901 by cmPCR in isolates (Moravkova et al., 2008; – negative, + positive)

¹*M. avium* subsp. *avium* confirmed directly in the tissue by dcmPCR (Moravkova et al., 2008)



Figure 1. Hepatomegaly with multiple tuberculous granulomas caused by *Mycobacterium avium* subsp. *avium* in pigeon No. 3

period of time when the pigeons were in contact with infected hens which are highly susceptible to *M. a. avium* infection (Shitaye et al., 2008a,b). It cannot be excluded, however, that the *M. a. avium* was introduced to the pigeon flock by infected free living birds (Hejlíček and Tremel, 1997). The question remains as to what was the reason for the massive infection of the examined birds. We speculate that the birds must have been somehow immunosuppressed, but we are not able to give a clear explanation.

M. a. avium infection in all three birds was confirmed within 24 h of DNA isolation using dcm-PCR, which has shown its usefulness for the rapid detection of this agent in cases such as the one described in this paper. Avian tuberculosis is a severe epizootic disease, the occurrence of which should be communicated to the State Veterinary Administration in the Czech Republic (Anonymous, 1999). Rapid diagnostic tests such as dcmPCR are attractive tools for the rapid detection of infection and the subsequent decision process compared with slow culture methods or microscopy which is lacking in sensitivity.

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