Retrospective Evaluation of Clinical Signs and Gross Pathologic Findings in Birds Infected With Mycobacterium genavense

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High prevalence of *Mycobacterium genavense* within flocks of pet birds

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Highlights

- *M. genavense* is the primary cause of mycobacteriosis in parrots and song birds
- Up to 91% of pet birds in the same flock were found infected with *M. genavense*
- Viral co-infections were common and may be important risk factors
- Improved antemortem diagnostics to evaluate zoonotic risks is urgently needed

Abstract

Mycobacterium genavense is regarded as the primary cause of mycobacteriosis in psittaciform and passeriform birds, which are commonly kept as pets. In humans, *Mycobacterium genavense* is especially pathogenic for young, old, pregnant and immunocompromised people (YOPIs). In birds, only few studies, mainly case reports, exist and there is still little information about occurrence and relevance of this zoonotic pathogen.

In this first pilot study concerning the prevalence of *Mycobacterium genavense* within flocks of naturally infected pet birds, real-time PCR examinations of 170 individual passeriform and psittaciform birds, including commonly kept budgerigars, lovebirds and zebra finches as well as gold finches and weaver finches, were conducted to determine the infection rate in six different aviaries. Antemortem examinations of faeces and cloacal swabs were compared with postmortem examinations of tissue samples to evaluate the reliability of antemortem diagnostics. Additional ophthalmologic examinations were performed to evaluate their diagnostic potential. Molecular examinations for viral co-infections, including circovirus, polyomavirus and adenovirus, were conducted to identify potential risk factors.

PCR results revealed a detection prevalence of *Mycobacterium genavense* in the flocks varying from 3% to 91% based on postmortem testing, while antemortem diagnostics of

faecal samples and swabs showed 64% discrepant (false negative) results. Ophthalmologic examinations were not useful in identifying infected birds within the flocks. Viral co-infections, especially with polyomavirus, were common.

It has to be assumed that *Mycobacterium genavense* infections are widespread and underdiagnosed in companion birds. Viral infections might be an important risk factor. There is urgent need to improve antemortem diagnostics.

Introduction

Mycobacterium genavense is a non-tuberculous slow-growing, fastidious mycobacterium, which cannot be grown on standard solid media (Böttger et al., 1993; Böttger et al., 1992). At the beginning of the 1990s it was identified for the first time in pet birds as an etiological agent of mycobacteriosis (Hoop et al., 1993), a common disease (Forster et al., 1988), for which for many years *Mycobacterium avium* ssp. *avium* had been regarded as the main and prevalent causal pathogen. Meanwhile *Mycobacterium genavense* has been described as the primary cause of mycobacteriosis in psittaciform and passeriform birds (Holsboer Buogo et al., 1997; Hoop et al., 1996; Manarolla et al., 2009; Palmieri et al., 2013; Schrenzel, 2012; Shivaprasad and Dhillon, 2005; Shivaprasad and Palmieri, 2012).

Birds are very popular pets. About 4.6 million pet birds were held in Germany in 2016, they lived in 4% of the German households (Heimtierbedarf, 2017). The psittaciform species budgerigar (*Melopsittacus undulatus)* as well as the passeriform species canary (*Serinus canaria*) and zebra finch (*Taeniopygia guttata*) belong to the most common birds kept in close contact to humans. Zoonotic diseases like mycobacteriosis due to non-tuberculous mycobacteria originating from such pet birds therefore are demanding high attention.

In humans, diseases due to *Mycobacterium genavense* have especially been found in people with immunosuppression, for example due to an infection with human immunodeficiency virus (HIV) (Böttger et al., 1992; Tortoli et al., 1998). But descriptions of infections in immunocompetent people also exist (Liberek et al., 1996). The source of infection and the reservoir in the environment of *Mycobacterium genavense* is unknown (Schrenzel, 2012; Tortoli et al., 1998). *Mycobacterium genavense* has been isolated from tap water (Hillebrand-Haverkort et al., 1999). Other non-tuberculous mycobacteria like *Mycobacterium avium* ssp. *avium* and *Mycobacterium intracellulare* were considered to be environmental (Schrenzel et al., 2008) and birds were hypothesized as potential reservoir (Tortoli, 2003).

Shedding via faeces has been regarded as the primary source of infection in birds with mycobacteria (Tell et al., 2001) and it is likely to be so with *Mycobacterium genavense* too (Lennox, 2007). Due to the occurrence of respiratory lesions, an airborne infection with *Mycobacterium genavense* has also been discussed in pet birds (Manarolla et al., 2007; Manarolla et al., 2009; Portaels et al., 1996). In captive psittaciform and passeriform birds, clinical signs have been described as unspecific (Bercovier and Vincent, 2001; Palmieri et al., 2013; Ramis et al., 1996; Shivaprasad and Dhillon, 2005). It was observed, that, until late disease stages, mycobacterial shedding is low in number and intermittent, therefore negative results cannot rule out mycobacteriosis (Tell et al., 2004; Tell et al., 2003; Tell et al., 2001).

Mycobacterium genavense was described not to be very contagious and to have a low pathogenicity, because pet birds in contact with infected birds did not show any signs of infections (Hoop et al., 1993; Hoop et al., 1994; Manarolla et al., 2007). Only one study up till now described, that several birds from the same owner were infected suggesting that an animal-to-animal spread of *Mycobacterium genavense* is probable (Manarolla et al., 2009). *Mycobacterium genavense* has been shown to be a relevant infecting agent of parrots in zoological gardens in Poland, where it was detected in faeces of 8.8% out of 261 clinically healthy psittaciform birds by a conventional PCR protocol (Ledwoń et al., 2008).

In this pilot study, in order to get first information on the risk for humans arising from bird populations kept in close contact to humans, we determined the infection prevalence of *Mycobacterium genavense* within pet bird flocks. We additionally compared, for the first time, the detection sensitivity of *Mycobacterium genavense* antemortem from faecal and swab samples and postmortem from tissue samples via real-time PCR. Additional ophthalmologic examinations were conducted in two aviaries to evaluate, if they can serve as a supportive antemortem diagnostic tool. In order to identify possible risk factors for mycobacterial infections in birds, additional examinations for viral co-infections were performed.

Materials and Methods

Birds

Six captive bird flocks with a total of 170 individual birds of the orders Passeriformes and Psittaciformes were included in this study. These bird flocks originated from different habitats i.e. private breeding facility, university, zoological garden and research facility.

In aviary A, 42 budgerigars (*Melopsittacus undulatus*) and in aviary B, ten Eurasian goldfinches (*Carduelis carduelis*) were held. The aviary C included 20 weavers (17 *Ploceus vitellinus*, one *Foudia madagascariensis* and two *Euplectes orix*) and eight Fischer's lovebirds (*Agapornis fischeri*), which were all kept together in a mixed flock. There were 31 zebra finches (*Taeniopygia guttata*) in aviary D and 23 zebra finches in aviary E. Thirty-six budgerigars were kept in aviary F. The examined bird species are listed in table 1.

In each of the captive bird populations presented here, single birds had died due to mycobacteriosis, as shown during pathological investigations, or acid-fast rods had been detected via Ziehl-Neelsen staining in pooled faeces of the whole flock. As requested by the bird owners, a total depopulation of the bird flocks had to be done due to standard operating procedures because of the zoonotic risk, animal welfare reasons and to stop spread of disease.

Examination of tissue samples for *Mycobacterium genavense*

Each individual bird from every aviary was examined for *Mycobacterium genavense*, to determine the prevalence of infection in the single flocks. Tissue samples from a pool of organs collected from every bird including heart, liver, lung, trachea, kidney, spleen, intestine, proventriculus, ventriculus, brain and integument were first shredded using a tissue homogenisator (Precellys®, VWR, Darmstadt, Germany). DNA was then extracted from the organ pool sample of each bird using the DNeasy blood and tissue mini kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Negative controls without clinical

materials were included in every extraction run. A real-time PCR examination was conducted according to a published protocol (Schmitz et al., 2018). The forward primer 5´-AAA CAG CGT CAG GAA ATC-3´ and the reverse primer 5´-GTG GGA CGA AGA TGT AGT-3´ as well as the Taqman probe 5´-FAM-AAC CGC TAT CTA CAT CCG CAG-TAMRA-3´ targeting the hypothetical 21 kDa protein gene (Chevrier et al., 1999) were used in combination with the QuantiFast Pathogen PCR +IC Kit, (Qiagen, Hilden, Germany). Reactions included 0.4 µmol/L, each, of the *Mycobacterium genavense* primers and 0.16 µmol/L of the *Mycobacterium genavense* probe. Real-time PCR assays were run using the Mx3000P real-time PCR device (Agilent Technologies, Santa Clara, California, USA) employing 50 cycles of denaturation at 94°C for 15s and annealing-elongation at 60°C for 30s and were analysed with the software MxPro (Agilent Technologies, Santa Clara, California, USA). Reactions were evaluated as being positive if their fluorescence curves were sigmoidal and the fluorescence values exceeded the threshold values calculated by the software. All PCR runs included negative extraction controls as described above and at least one sample known to contain *Mycobacterium genavense* DNA as a positive control.

In a blinded study the sensitivity of the *Mycobacterium genavense* real-time PCR had been determined before as 10^5 bacteria x g^{-1} feces by spiking feces of pigeons known to be free of *Mycobacterium genavense* with defined numbers of cultured mycobacteria. Five different *Mycobacterium genavense* reference strains (strain Oberschleissheim, Bavarian Health and Food Safety Authority, Germany as well as strains 177414/08, 1603/04, N86/04 and N87/04 obtained from the Institute for Medical Microbiology, University of Zurich, Switzerland) had been correctly identified using this assay. Fifteen reference strains including nine mycobacterial species and *Mycobacterium avium* ssp. different from *Mycobacterium genavense* as well as faecal samples without mycobacteria had all been identified correctly negative in this blinded evaluation study (Schmitz et al., 2018).

Examination of faeces or cloacal swabs for *Mycobacterium genavense*

Faeces pooled from three consecutive days were collected from individual living birds in aviary A (42 birds) and aviary B (seven available birds out of ten). DNA was extracted using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer and examined via real-time PCR following the protocol described above.

Twenty-one birds of aviary D and all 23 zebra finches of aviary E had cloacal swabs taken directly whilst euthanasia. DNA was extracted using the DNeasy blood and tissue mini kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer and then examined via real-time PCR as described above.

Ophthalmology

Ophthalmological examinations of 35 living birds from aviary A and of seven birds from aviary B were conducted using a headband ophthalmoscope (Heine, Herrsching, Germany) and a 90 dioptric double aspheric lens (Volk Optical Inc., Mentor, Ohio, USA) as well as a slit lamp (SL-15, Kowa, Tokyo, Japan). The intraocular eye pressures (IOD) were measured using a rebound tonometer (Icare® TonoVet, Kruuse, Langeskov, Denmark).

Screening for co-infections with circovirus, polyomavirus and adenovirus

DNA from tissue samples of all birds extracted as described above was used to screen for co-infections with circovirus, polyomavirus and adenovirus. Pools of DNA extracts of four individuals were examined via PCR for circovirus, polyomavirus and adenovirus according to published protocols (Halami et al., 2008; Johne et al., 2005; Rinder et al., 2018; Wellehan et al., 2004). If DNA of circovirus or polyomavirus was detected, PCR examination was repeated for the individual birds of the respective pool. To confirm the identity of the PCR products, direct sequencing of PCR products was performed (GATC, Köln, Germany) and Blast analysis (blast.ncbi.nlm.nih.gov) was conducted.

Results

Detection of *Mycobacterium genavense* DNA

Using real-time PCR, DNA of *Mycobacterium genavense* was detected in postmortem tissue samples of 10% of the budgerigars (4 out of 42) in aviary A, in 50% of the Eurasian goldfinches (5 out of 10) in aviary B, and in 85% of the weavers (17 out of 20) but none (0%) of Fischer's lovebirds (0 out of 8) of aviary C. For the zebra finches in aviaries D and E, prevalences in postmortem tissue samples of 74% (23 from 31 birds) and 91% (21 out of 23 birds) respectively, were detected. In aviary F only one bird of 36 (3%) was found to be infected with *Mycobacterium genavense* postmortem. The overall prevalence thus varied between 3% in budgerigars in aviary F and 91% in zebra finches in aviary E (Table 1).

Antemortem DNA of *Mycobacterium genavense* was not found in any of the faeces (3-day pools) of the individual birds in aviary A and B. Thus, DNA of *M. genavense* was not detected by antemortem examination of the faeces in five birds, which had positive tissue samples (Table 2).

The results of the antemortem examinations of the cloacal swabs in aviary D and E revealed *Mycobacterium genavense* DNA in 29% (6 out of 21) and 39% (9 out of 23) of the examined birds. Comparison of these results with those of the postmortem examination of the same birds showed an antemortem detection rate of only 38% (6 out of 16) and 43% (9 out of 21) respectively (Table 2).

All the birds with negative postmortem tissue results were also negative in antemortem faeces or swab investigations while, in total, 27 negative antemortem PCR results (from faeces or swab investigations) were obtained from 42 birds with positive postmortem PCR results (from tissue samples). Thus 64% false negative results were obtained by antemortem sampling.

Ophthalmology

Two out of 35 budgerigars of aviary A revealed eye lesions. In one bird, a chorioretinitis localized inferior to the pecten oculi and hyperpigmentation in a widespread central fundus area was detected in the right eye. The left eye showed several small foci of chorioretinitis disseminated over the fundus oculi. In this bird, DNA of *Mycobacterium genavense* was detected in the tissue sample. The second budgerigar had a cataracta immatura in the left eye. *Mycobacterium genavense* DNA was not found in this bird. In aviary B, only one *M. genavense* positive Eurasian goldfinch had a defect localized superfically in the corneal epithel. All the other birds did not show any lesions of the eyes.

The intraocular eye pressures and pupillary reflexes in all examined birds (aviary A and B) were in the normal range and no further abnormalities were noted.

Detection of circovirus, polyomavirus and adenovirus DNA

Circovirus DNA was detected in a single zebra finch from aviary D and was identified as *Zebra finch circovirus* by sequencing. There was no circovirus DNA found in the other birds included in the examination.

Polyomavirus DNA was not detected in birds of aviaries A, C and F, while four Eurasian goldfinches (40%) of aviary B were shown to be infected as indicated by a positive DNA detection. Positive polyomavirus PCR results were obtained in 100% of the zebra finches of aviary D and in 96% (n = 22) of the zebra finches of aviary E. Sequencing and molecular characterisation revealed the species *Serinus canaria polyomavirus 1* (Canary polyomavirus) in all birds (Table 1).

Adenovirus DNA was detected in five out of 17 vitteline masked weavers (*Ploceus vitellinus*) corresponding to a prevalence of 25% in all the weavers. It also was detected in three out of 42 budgerigars (7%) in aviary A and in three out of 36 budgerigars (8%) from aviary F.

Co-infections

With regard to polyomavirus, it was noted, that in aviary B all the polyomavirus-infected Eurasian goldfinches (40%) were also infected with *Mycobacterium genavense.* In this flock, only one Eurasian goldfinch infected with *Mycobacterium genavense* did not show an additional infection with polyomavirus. Almost all zebra finches in aviary D and E, as shown in table 1, were infected with polyomavirus (100% and 96% respectively) and most of these birds were also infected with *Mycobacterium genavense* (74% and 91% respectively). In aviary E, two birds were found to be infected with polyomavirus but not with *Mycobacterium genavense* and one bird was found to be infected with *Mycobacterium genavense* but not with polyomavirus. In aviary D, eight birds with polyomavirus infections did not show an additional *Mycobacterium genavense* infection. In aviary C, where 85% of the weavers were infected with *Mycobacterium genavense* and 25% with adenovirus, only one adenovirus-infected bird was not found to be concurrently infected with *Mycobacterium genavense*. While 10% of the budgerigars of aviary A were infected with *Mycobacterium genavense,* co-infections with polyomavirus or circovirus were not detected. In aviary F the bird infected with *Mycobacterium genavense* also was infected with adenovirus. Two other birds in the flock were found to be infected with adenovirus too but not with *Mycobacterium genavense* .

Discussion

In this pilot study, which is, to our knowledge, the first report on the prevalence of *Mycobacterium genavense* within flocks of psittaciform and passeriform birds commonly kept as companion birds, we could show that this infection can be widespread in aviaries and clinically healthy birds. Up to 91% of individuals in the captive bird populations of this investigation were infected with *Mycobacterium genavense*. This contradicts the view that *Mycobacterium genavense* is infecting only single birds of a flock as presumed by authors of other studies and case reports (Hoop et al., 1993; Hoop et al., 1994; Manarolla et al., 2007; Portaels et al., 1996). Our results are supported by a case report on diamond doves (*Geopelia*

cuneata, order Columbiformes) held in a zoological garden where acid-fast bacilli were detected in formalin-fixed paraffin-embedded tissue slides of all 23 birds of the flock (Haridy et al., 2014).

Reasons for the varying infection prevalence within the flocks are unknown but might include differences in host species or bird order susceptibility, virulence of mycobacterial strains involved, infection pressure due to amount of shedded microorganisms accumulating in the environment or exposure time and age of the birds. It is noteworthy that, in the mixed flock of weavers and Fischer's lovebirds included in our study, infections with *Mycobacterium genavense* were detected exclusively in the weaver finches but not in the lovebirds suggesting differences in bird species or bird order susceptibility. However, a *Mycobacterium genavense*-infection has previously been described in a lovebird (Michels, personal communication 2014) arguing at least against an absolute resistance of the bird genus *Agapornis*. There are hints in other studies that psittacine and passeriform bird might respond differently to *Mycobacterium genavense* (Schmitz et al., 2018).

In humans, immunosuppression due to HIV infection was shown to be an important risk factor leading to an increased individual susceptibility. We thus firstly focussed on viral co-infections in order to identify risk factors and included circovirus, polyomavirus and adenovirus which are known to occur in both zoological orders of birds and which are suspected to induce immunosuppression in birds.

Interestingly co-infections with polyomavirus were commonly detected in our flocks. Aviaries with high polyomavirus infection prevalence also contained relatively more birds with *Mycobacterium genavense* infections. A single zebra finch was additionally infected with zebra finch circovirus, a virus species which has only recently been identified (Rinder et al., 2015; Rinder et al., 2017). This pilot study thus may reveal the first evidence to suggest, that birds, which are immunocompromised due to viral infections, might be increasingly

prone to infections with *Mycobacterium genavense*. This view is supported by, to our knowledge, the only other study concerning co-infections in birds naturally infected with *Mycobacterium genavense*, which revealed infections with additional viral pathogens including polyomavirus and circovirus in 52% of the affected birds (Schmitz et al., 2018).

In the weavers, infections with polyomavirus could not be detected with the PCR protocols used here. There are no scientific reports on polyomavirus infections in Ploceidae so far, and it is thus unknown whether the PCR protocols might even be applied. A broad-range adenovirus assay (Wellehan et al., 2004) was successfully used to detect, for the first time, adenovirus infections in this bird family (Rinder, personal opinion). Information on pathogenicity of these viruses is still lacking. In the Fischer's lovebirds of the same aviary, infections with circovirus, polyomavirus and adenovirus were not detected supporting the view that viral infections might represent an important risk factor.

Mycobacterium genavense infections are commonly identified in budgerigars (Michels, personal communication 2014). Whether a young age of the birds contributed to the low detection prevalence of 3% and 10% of the budgerigars in aviary F and A is speculative. In our case, this relatively low detection prevalence might also reflect a good immune status of the birds as infections with circovirus and polyomavirus, which are common in budgerigars and are the etiological agents of Psittacine Beak and Feather Disease (PBFD) and Budgerigar Fledgling Disease (also known as French Molt), respectively, were not detected. Adenovirus infections have been described in psittaciform and non-psittaciform birds (Hulbert et al., 2015) but their role as pathogens is still unclear. Nevertheless, only six aviaries were included in our pilot study, and our hypothesis of viral infections representing important risk factors has to be confirmed in a larger number of aviaries or in larger case-control studies.

Avian mycobacteriosis is a diagnostic challenge for clinicians (Dahlhausen et al., 2012; Tell et al., 2003). In our study, detection of *M. genavense* in living birds was very unreliable using

3 day-pool faecal samples or cloacal swabs, and a rate of 64% discrepant (false negative) results compared to the postmortem analysis of pooled tissues was obtained. One possible reason might be a low diagnostic sensitivity of the molecular assay. The detection limit of the real-time PCR assay used in this study but in combination with a differing DNA extraction protocol has been determined to be 10^5 mycobacteria x g^{-1} faeces (Schmitz et al., 2018). Sensitivities were not described for the PCR assays used in other publications for the detection or differentiation of *Mycobacterium genavense* (Chevrier et al., 1999; Holsboer Buogo et al., 1997; Ledwoń et al., 2008; Manarolla et al., 2009; Portaels et al., 1996; Ramis et al., 1996). They are thus not available for comparison but our diagnostic findings match the results of other studies (Tell et al., 2003). The reported low and intermittent faecal shedding of mycobacteria until late disease stages (Tell et al., 2004; Tell et al., 2003; Tell et al., 2001) might represent an important factor in limiting the success of antemortem diagnosis. Alternatives to the detection of shedded mycobacteria, like serological and intra-dermal testing for immune reactions against *Mycobacterium genavense* are not available nor useful in pet birds, and *Mycobacterium genavense* is very difficult to culture (Dahlhausen et al., 2012; Tell et al., 2003). Improved early antemortem detection of infections with *Mycobacterium genavense* in birds is thus urgently needed.

About 1.8% of ocular lesions in birds have been described to be due to mycobacteria (Korbel et al., 1997). Therefore, in two aviaries in this study ophthalmologic examinations were conducted antemortem to get some information, whether these investigations might be useful in identifying infected birds within a flock. Only two of the seven birds, which had *Mycobacterium genavense* detected in their tissue samples, were found to have eye lesions. A budgerigar was suffering from a chorioretinitis, and such lesions have been described beforehand in avian mycobacteriosis (Korbel et al., 1997). One of the infected Eurasian goldfinches was shown to have a superficial corneal defect, but this kind of lesion is regarded as unspecific and not related to mycobacteriosis. Five infected birds did not show any eye

lesions and a cataract was diagnosed in another not-infected bird. The infection stages, whether early or late, of the birds included in this study are not known. Since many clinically healthy birds were involved, it might be a very early infection stage. At least the results from this limited number of birds included do not support the view, that ophthalmologic examination can substantially support antemortem detection of *Mycobacterium genavense* infections.

With regard to the zoonotical potential of *Mycobacterium genavense*, an infection risk arises from such flocks for human beings in close contact to these birds or to their environments. This public health concern, in private holdings, includes the owners themselves and people living in the same household but, in non-private holdings open to the public, might extend to zoo keepers, visitors, students, veterinarians and many others. Based on the results of our investigation, culling of flocks in close contact to humans seems to be justified in consideration of public health until reliable antemortem diagnostic tools are available. Nevertheless, such decisions are only sensible if the epidemiological settings allow an effective prevention of the pathogens' re-introduction.

Mycobacterium genavense-infections are more common in pet birds and more widespread within flocks than anticipated until now. There is evidence that birds with viral infections and therefore presumptively reduced immune status may be increasingly prone to infections with *Mycobacterium genavense*. To evaluate the zoonotic risk of captive passeriform and psittaciform bird flocks, there is an urgent need to improve antemortem diagnostics.

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Conflict of Interest

The authors declare that they have no competing interests.

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Table 1. Prevalence of *Mycobacterium genavense*, circovirus, polyomavirus and adenovirus in the examined aviaries (n, total number of birds in the aviary)

Aviary	Species		n	Portion of positive birds			
	Common Name	Scientific Name		*M. genavense*	Circovirus	Polyomavirus	Adenovirus
A	budgerigar	*Melopsittacus undulatus*	42	10%	0%	0%	7%
B	Eurasian goldfinch	*Carduelis carduelis*	10	50%	0%	40%	0%
C	weaver species	Ploceidae	20	85%	0%	0%	25%
C	Fischer's lovebird	*Agapornis fischeri*	8	0%	0%	0%	0%
D	zebra finch	*Taeniopygia guttata*	31	74%	3%	100%	0%
E	zebra finch	*Taeniopygia guttata*	23	91%	0%	96%	0%
F	budgerigar	*Melopsittacus undulatus*	36	3%	0%	0%	8%

Table 2. Number of birds with positive *M. genavense* DNA detection antemortem in faeces (3-day pools) or swabs and postmortem in tissues of birds from aviaries A-E (n, number of birds included in the antemortem-postmortem comparison)

Aviary	n	positive antemortem		positive postmortem	
A	35	0[a]	0%	2	6%
B	7	0[a]	0%	3	43%
D	21	6[b]	29%	16	76%
E	23	9[b]	39%	21	91%
total	**86**	**15**	**17%**	**42**	**49%**

[a] detection in faeces (3-day pool); [b] detection in cloacal swabs