

NON-TUBERCULOUS MYCOBACTERIA

<u>IN PETS</u>

AUTHORS

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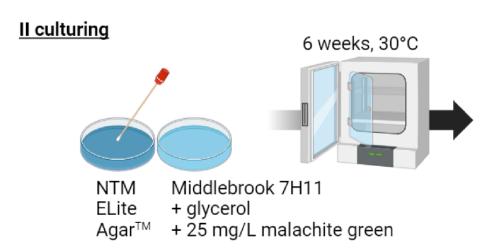
Non-tuberculous mycobacteria (NTM) are a group of *ca.* 240 bacteria species, most of which are free-living saprophytes. Most of them are environmental opportunistic pathogens of humans and animals. Animal-to-human transmission of the NTM-disease has not yet been proven, nonetheless, single cases of suspected zoonotic infections are being reported.

METHODOLOGY

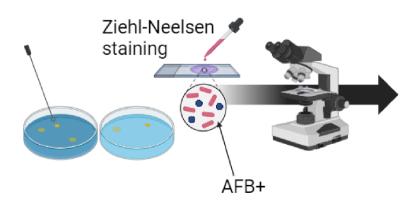
The workflow of the study is depicted on **Fig. 1**. A total of 210 samples were collected using sterile cotton swabs from pets in a year 2023. Furthermore, data on animals' medical history and habits were collected. Swabs of each specimen were cultured directly on two mycobacterial-growth promoting media (NTM Elite Agar, bioMérieux, Poland; Middlebrook 7H11, BD, Poland). After the incubation time, colonies suspected of being mycobacteria were stained and all acid-fast bacilli (AFB+) were selected. DNA was then isolated with a GenoLyse (Hain, Germany) kit and used for PCR of two mycobacterial molecular markers.

Strains were then identified to the species level by PCR-sequencing of *rpoB* and *hsp65* molecular markers, of which the results were BLAST-analysed. The identity of at least 98% to a type strain for both genes was considered a correct species assignment.

I swabs collection nose fur/skin at least DOG n=10 3 swabs n=29 N=22 from each pet ear n=56 anus n=49 transport up to 48h mouth n=54 other CAT n=12 N=35



III NTM selection



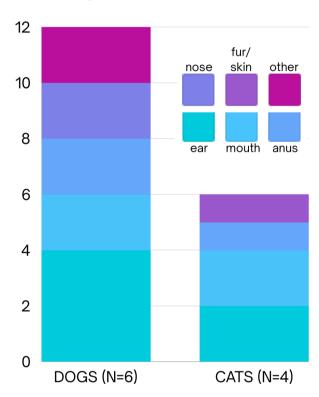
PCR-sequencing, BLAST rpoB hsp65 Acreated in BioRender.com bbo

Fig. 1. Experimental workflow. Four major steps are depicted: I - collection of samples, II - culturing and incubation, III - macromorphological observation of potential mycobacteria and micromorphological selection of AFB+ cultures, IV - DNA isolation, PCR and BLAST analysis of sequences of two molecular markers.

The aim of the study was to evaluate the distribution of NTM among pets.

RESULTS

Of 57 recruited animals, 10 (17.5%) were NTM-positive - 6/22 (27.3%) dogs and 4/35 (11.4%) cats, aged from 3 to 13 years. Distribution of NTM-positive swabs among cats and dogs is presented on Fig. 2. Overall, 18/210 (8.6%) specimen were NTM-positive: 6 ear, 4 mouth, 3 anus, 1 fur/skin, 2 nose and 2 other swabs. Distribution of NTM-positive specimen among each swab type is depicted on a graph below (Fig. 3).



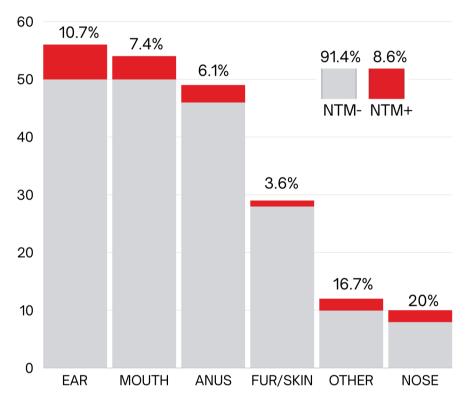


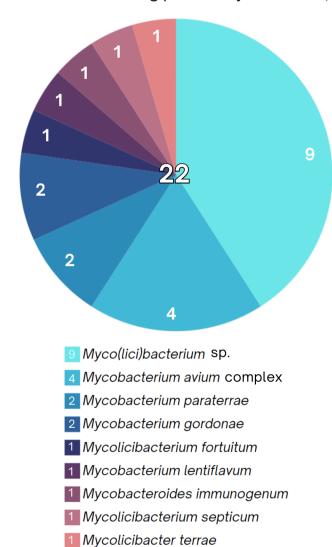
Fig. 2. Distribution of NTM+ samples among cats and dogs.

Fig. 3. Distribution of NTM-positive (red) and -negative (grey) specimen among all collected samples.

MOUTH

SKIN/FUR

Overall, **22 mycobacterial isolates** were retrieved from **18 NTM-positive swabs**. Number and distribution of identified species (**Fig. 4**) are depicted on (**Fig. 5**). All of NTM-positive animals were regularly going outside except for a cat that was kept strictly at home. One of four NTM-positive cats was treated with steroids for allergy. One of six NTM-positive dogs had a history of Lyme disease and reoccurring pulmonary infections, entailing prolonged antibiotics intake.



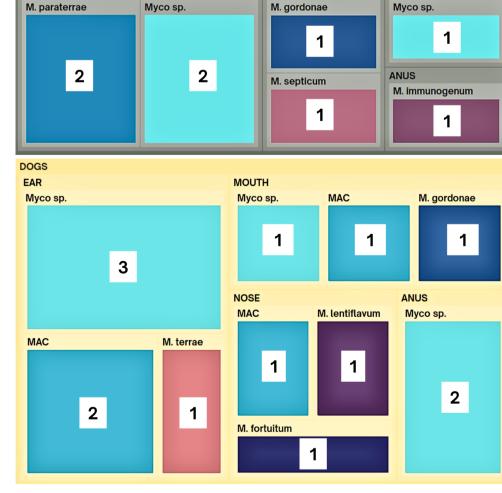


Fig. 5. Distribution of identified species among cats (grey) and dogs (yellow).

CONCLUSIONS

Fig. 4. Number of identified species.

- This ongoing study is the largest of its kind to date.
- NTM were more often isolated from **dogs** than cats.
- M. avium complex, M. paraterrae and M. gordonae were the dominant species.
- Further sequencing-based investigations are required to establish the species of 9 isolated NTM.