Diagnostic TB-screening of great apes in Basel Zoo

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1 Introduction

Because of the renovation of the ape house in Basel Zoo, all animals had to be moved out in April 2010, including seven Sumatra orangutans (*Pongo pygmaeus*), eight lowland gorillas (*Gorilla g. gorilla)*, and ten chimpanzees (*Pan troglodytes*).

All gorillas were premedicated with oral 10 - 20 mg/animal BID zuclopenthixol (Clopixol Tropfen 2 %, 20 mg/ml, Lundbeck AG, Opfikon, Switzerland) starting three days before transportation. General anesthesia was induced in all three species with the same protocol using a combination of 5 mg/kg ketamine (ketamine dry powder 1 g (Ketavet®, Pfizer) mixed with 5.0 ml sterile saline solution = 200 mg/ml ketamine) and 0.05 mg/kg medetomidine (zalopine, 10 mg/ml, Orion, Dr. E. Graeub AG, Bern, Switzerland). The dose was calculated on estimated weights and applied by blowpipe intramuscularly. Anesthesia was monitored by an experienced anesthesiologist with the aid of pulsoximetry and electrocardiography. An intravenous access was immediately placed. In a few cases, anesthesia had to be maintained using face masks with isoflurane in oxygen or propofol boluses intravenously. The procedures lasted around one hour, and anesthesia was reversed after crating with 5.0 mg atipamezole per mg medetomidine intramuscularly (Antisedan, 5 mg/ml, Dr. E. Graeub AG, Bern, Switzerland). Further transportation was only performed, when the animal was at least able to sit.

The occasion of having all great apes of Basel Zoo under general anesthesia was used for intensive clinical examination and sampling including clinical screening for TB complex *mycobacteria*. All procedures including sampling were performed under zoo conditions in rooms of the great ape house with the exception of one orangutan which was intubated, maintained on isoflurane in oxygen, and transported to a private institute for computer tomography. The purpose of this study was to run different mostly non-validated indirect TB-tests and thorax ultrasound in great apes for further evaluation and discussion.

2 Methods for diagnostic TB-screening

Comparative intrapalpebral tuberculin tests were performed using 0.1 ml tuberculin PPD (= 2000 IU) RT 23 SSI (2 TE/0.1 ml) (Statens Serum Institute, Copenhagen, Denmark, distributed by Pro Vaccine AG, Baar, Switzerland) in the left eyelid and 0.1 ml bovituber PPD (= 2000 IU) (Synbiotics Europe, Lyon, France) in the right eyelid. The reaction was visually inspected daily and determined after 72 hours.

Two different commercial interferon gamma tests were used (Primagam®, Prionics, USA, Inc, La Vista, NE and QuantiFERON Gold®, Celestis, Australia). For the Primagam® test heparinized blood was sent to the laboratory on the same day. For the QuantiFERON Gold® test whole blood was incubated for lymphocytestimulation in the zoo at 37° Celsius for 16-24 hours, then centrifugated, and a plasma sample was sent to the laboratory.

To detect antibodies of *M. tuberculosis* in the blood sera, the Prima-TB STAT-PAK® (ChemBio Diagnostic Systems, Inc, Medford, NY) was used. The results were visually inspected after 20 minutes, and photographs were taken for documentation.

Pharyngeal swabs were taken and submitted for rpoB-PCR examination (Kim et al., 1999). The rpoB-gene encodes the β-subunit of the RNA polymerase. The primer detects the 342 bp DNA fragment and confirms the presence of the genus *Mycobacterium*. Further identification down to the species level can be achieved by sequence or restriction analysis of fingerprints. This was the only direct test used in this study.

Ventro-dorsal thorax ultrasound was performed by placing the anesthetized animal in a sitting upright position on a fenced cage door with the aid of mountain climbing gear to fix the arms. The detector cassette was also fixed at the door (Fig. 1).

No thorax X-rays were taken from two chimpanzee mothers with babies on their chests. The X-rays were interpreted by two independent board-certified radiologists (1 DM and 1 DVM).

3 Results

All animals were apparently healthy with the exception of age-related teeth and/or joint problems. Four gorillas were found to be affected by hepatic alveolar echinococcosis confirmed sonographically, by ELISA, and Western Blot. Oral herpes-like-lesions were present in a young male chimpanzee and were associated with *Pan troglodytes* lymphocryptovirus 1.

Results of diagnostic TB-testing are summarized for the different ape species in tables 1-3.

4 Discussion

Diagnostic TB screening can easily be performed during general anesthesia of great apes under zoo field conditions. However, a well prepared protocol and staff is needed and laboratories have to be contacted beforehand. In this study, a variety of results was obtained for indirect tests and thorax X-ray interpretations, while the only direct test performed (PCR pharyngeal swabs) was negative for all tested animals. Interpretation of this combined testing is challenging, and neither the presence nor absence of TB complex *mycobacteria* in the tested great apes could be conclusively determined.

We observed positive intrapalbebral *M. bovis* tuberculin tests in one orangutan (after 96 hours), one questionable minor reaction in one gorilla, and in four chimpanzees. All animals were tested negative for *M. tuberculosis* tuberculin. Cell-mediated immunologic tests like the intradermal test are known to have limitations and they can produce false-positive or false-negative reactions. Some species like orangutans, tapirs, bongo antelopes, and reindeer have an increased likelihood of nonspecific reactions (Miller, 2008). Therefore, further testing is required including skin tests for *M. avium* to rule out cross-reactivity.

One chimpanzee was tested positive in the Quantiferon-TB Gold® assay but was negative in the Primagam® test. This test result may be explained by differences in sample processing, lymphocyte stimulation or different cut-off levels used by the different laboratories.

The PrimaTB StatPak® test was positive for one orangutan and one gorilla. This fast test detects antibodies of *M. tuberculosis* with a combination of selected antigens. It uses lateral flow technology and was evaluated in 422 nonhuman primates (rhesus monkey, cynomolgus monkey, African green monkey) (Lyashchenko et al. 2007). The sensitivity was 90 % and the specifity 99 %. Best results were achieved in combination with the skin test. However, this test has not been validated for great apes and does not detect *M. bovis*. A currently commercially not available multiantigen print immunoassay (MAPIA) will be carried out on these samples and may provide further results.

Interestingly, interpretation of thorax x-rays showed no accordance at all between a human and veterinary radiologist. In human TB cases, lung lesions are often associated with calcifications that are easy to detect on radiographs. This feature is rare or absent in animals. However, both radiologists did not see clear evidence of typcial TB lesions. Both examinersmentioned other

differential diagnoses in their interpretations. The veterinary radiologist criticized the absence of an additional latero-lateral x-ray that would have been helpful for better interpretation.

Using the combination of the different screening tests, there was only one chimpanzee, which showed positive results in more than one test (positive *M. bovis* skin test, positive Quantiferon-TB Gold®, questionable positive x-ray interpretation of the human radiologist) (table 3). This individual has a history of close contact to humans because of historical animal trade activities. Therefore, this animal needs careful observation and further testing.

All the great apes will be moved back to the ape house in spring 2011 with the need of another general anesthesia. This will give us the opportunity to repeat and perform further tests. It is planned to perform more direct testing of excretions, body fluids and tissues (gastric lavage, tracheo-bronchial lavage, faeces, tissue with lesions) using acid-fast staining, culture and molecular assays in combination. Amplified *M. tuberculosis* direct test (MTD) and multiplex PCR may be used to distinguish pathogenic infections from atypical infections that may cause positive test results. Any other ideas from the community of primate and zoo veterinarians as well as physicians and bacteriologists are very welcome and should be addressed to the corresponding author.

If screening great ape collections for TB, one has to consider consequences of positive results before testing is performed, including those for the individual animal, the personnel, the authorities, the studbook and associated animal transfer affairs, the public, the media, and further capacities.

5 Conclusion

There is no *ante mortem* TB test for the great apes, which is 100 % reliable. Multiple test modalities have to be performed, although in this study even the combination of six tests did not bring a satisfying reliability. It has to be considered that most tests are not validated for great apes. Certain zoo species including orangutans are known to have an increased likelihood of nonspecific reactions in the skin test. Interpretation of thorax x-rays by different radiologists also revealed inconclusive results.

From the results of this study, we carefully assume that we do not have *M. tuberculosis* in the tested animals. However, we are not sure about the presence of *M. bovis* or atypical or non-tuberculous *mycobacteria*. We will therefore repeat all tests on a scientific basis and perform a triple combination of skin tests including *M. tuberculosis*, *M. bovis*, and *M. avium* tuberculin to

detect cross-reactions. The next protocol will also include more direct testing of excretions, lavage fluids and tissues, using acid-fast staining, culture, and molecular assays in combination.

Table 1. Diagnostic TB screening of Sumatra orangutans in Basel Zoo.

1.6 Orangutan 1.0 = male 0.1 = female	**0.1 Sexta**	**1.0 Schubbi**	**0.1 Ogan**	**0.1 Ziadah**	**0.1 Farida**	**0.1 Elsy**	**0.1 Kasih**
Skin Test (72h) *M. tuberculosis*	-	-	-	-	-	-	-
Skin Test (72h) *M. bovis*	- + (96h)	-	-	-	-	-	-
Quantiferon-TB Gold®	-	n.d.	-	n.d.	-	-	-
Primagam®	-	-	-	-	-	-	-
Prima TB StatPak®	-	+	-	-	-	-	-
X-ray Thorax Human MD	- CT	-	-	-	+ DD granuloma	-	-*
X-ray Thorax DVM	n.d.	(+) osteoma?	-	-	-	-	-

*other pathology
n.d. = test not done
CT = computer tomography

Table 2. Diagnostic TB screening of lowland gorillas in Basel Zoo.

2.6 Gorillas 1.0 = male 0.1 = female	**1.0 Kisoro**	**0.1 Faddama**	**0.1 Goma**	**0.1 Zungu**	**0.1 Joas**	**0.1 Quarta**	**0.1 Wima**	**0.1 Chelewa**
Skin Test (72h) *M. tuberculosis*	-	-	-	-	-	-	-	-
Skin Test (72h) *M. bovis*	(+)	-	-	-	-	-	-	-
Quantiferon-TB Gold®	-	-	-	-	-	-	-	-
Primagam®	-	-	-	-	-	-	-	-
Prima TB StatPak®	-	+	-	-	-	-	-	-
rpoB-PCR Pharnyx swap	-	-	-	-	-	-	-	-
X-ray Thorax Human MD	- AE	- (AE)	-	- AE	-	-* AE	n.d. AE†	n.d.
X-ray Thorax DVM	-	+ DD granuloma or lung cyst	-	-	-	-* AE	n.d.	n.d.

*other pathology
n.d. = test not done
AE = confirmed alveolar echinococcosis
† = animal died

Table 3. Diagnostic TB screening of chimpanzees in Basel Zoo.

3.5 chimpanzee 1.0 = male 0.1 = female	**0.1 Zamana**	**0.1 Xindra**	**0.1 Quamisha**	**0.1 Jacky**	**0.1 Benga**	**1.0 Wakili**	**1.0 Eros**	**1.0 Colebe**
Skin Test (72h) *M. tuberculosis*	-	-	-	-	-	-	-	-
Skin Test (72h) *M. bovis*	-	+	-	+	(+)	(+)	-	-
Quantiferon-TB Gold®	-	-	-	+	-	-	-	-
Primagam®	-	-	-	-	-	-	-	-
Prima TB StatPak®	-	-	-	-	-	-	-	-
rpoB-PCR Pharynx swap	-	-	-	-	-	-	-	-
X-ray Thorax Human MD	-	n.d.	-	-	n.d.	-	-*	-
X-ray Thorax DVM	-	n.d.	-	(+)	n.d.	-	-	-

*other pathology
n.d. = test not done, baby on the chest

Figure 1. Ventrodorsal thorax x-ray of a male anesthetized orangutan. The animal is fixed in a sitting and upright position on a cage door to obtain optimal results.

Acknowledgments

We thank many persons, without their indispensable help this study would not have been possible: Bernard Mengiardi, Renate Vögtli, Martina Wicki, Thomas Bodmer, Max Wittenbrink, Gabriela Hürlimann, Pia Krawinkel, Chiara Adami, Nina Zimmermann, Suzanne Aebi, Jakob Huber, Bruno Seiffert, Thomas Jermann and all involved keepers and craftsmen of the Basel Zoo.

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