### Laboratory Information

| Name of disease (or topic) for which you are a designated WOAH Reference Laboratory: | Paratuberculosis |
| Address of laboratory: | Via Strada della Faggiola, 1 |
| Tel.: | +390523523491 |
| E-mail address: | matteo.ricchi@izsler.it |
| Website: | www.izsler.it |
| Name (including Title) of Head of Laboratory (Responsible Official): | Dr. Frazzi Piero |
| Name (including Title and Position) of WOAH Reference Expert: | Dr. Ricchi Matteo |
| Which of the following defines your laboratory?
Check all that apply: | Governmental |

### TOR1: DIAGNOSTIC METHODS

1. Did your laboratory perform diagnostic tests for the specified disease/topic for purposes such as disease diagnosis, screening of animals for export, surveillance, etc.? (Not for quality control, proficiency testing or staff training)

   Yes

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Indicated in WOAH Manual (Yes/No)</th>
<th>Total number of test performed last year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect diagnostic tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38974</td>
<td>yes</td>
<td>28544</td>
</tr>
<tr>
<td>Direct diagnostic tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>yes</td>
<td>16</td>
</tr>
<tr>
<td>501</td>
<td>yes</td>
<td>501</td>
</tr>
</tbody>
</table>
**TOR2: REFERENCE MATERIAL**

2. Did your laboratory produce or supply imported standard reference reagents officially recognised by WOAH?
   No

3. Did your laboratory supply standard reference reagents (nonWOAH-approved) and/or other diagnostic reagents to WOAH Members?
   No

4. Did your laboratory produce vaccines?
   No

5. Did your laboratory supply vaccines to WOAH Members?
   No

**TOR3: NEW PROCEDURES**

6. Did your laboratory develop new diagnostic methods for the designated pathogen or disease?
   Yes

7. Did your laboratory validate diagnostic methods according to WOAH Standards for the designated pathogen or disease?
   Yes

<table>
<thead>
<tr>
<th>NAME OF THE NEW TEST OR DIAGNOSTIC METHOD DEVELOPED</th>
<th>DESCRIPTION AND REFERENCES (PUBLICATION, WEBSITE, ETC.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultural assay for isolation of Mycobacterium avium subsp. paratuberculosis from faeces</td>
<td>Method and validation protocol and report (in italian) can be requested addressing to: Servizio Assicurazione Qualità - Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna. Via Bianchi n. 9 - 25124 Brescia, Italy. Method code MP01/207</td>
</tr>
</tbody>
</table>

8. Did your laboratory develop new vaccines for the designated pathogen or disease?
   No

9. Did your laboratory validate vaccines according to WOAH Standards for the designated pathogen or disease?
   No

**TOR4: DIAGNOSTIC TESTING FACILITIES**
10. Did your laboratory carry out diagnostic testing for other WOAH Members?

Yes

<table>
<thead>
<tr>
<th>NAME OF WOAH MEMBER COUNTRY SEEKING ASSISTANCE</th>
<th>DATE</th>
<th>WHICH DIAGNOSTIC TEST USED</th>
<th>NO. SAMPLES RECEIVED FOR PROVISION OF DIAGNOSTIC SUPPORT</th>
<th>NO. SAMPLES RECEIVED FOR PROVISION OF CONFIRMATORY DIAGNOSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CROATIA</td>
<td>2022-12-02</td>
<td>ELISA</td>
<td>10430</td>
<td>0</td>
</tr>
</tbody>
</table>

11. Did your laboratory provide expert advice in technical consultancies on the request of an WOAH Member?

Yes

<table>
<thead>
<tr>
<th>NAME OF THE WOAH MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY</th>
<th>PURPOSE</th>
<th>HOW THE ADVICE WAS PROVIDED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Please, following the text of my email: Dear Dr. Saimre, Please find some reflections and suggestions about the questions raised in your email relative to the managing and control of paratuberculosis in zoos. I have personally followed a specific case in Italy where an outbreak occurred in an Italian zoo in captive Scimitar-Horned Oryxes (for your convenience I have included a copy of the paper in attach, Pigoli et al., 2020). Alas, as already mentioned by Dr. Alonso, the managing of the disease is time and cost demanding and many efforts are required. Indeed, to the best of my knowledge, there not official guidelines for the managing and control of paratuberculosis in zoos, however, one of most interesting documents, cited also in a recent review (see later), is that edited by the &quot;White Oak Conservation Center&quot; Florida, USA (see file in attach at the present email). This document reported all the most important factors required in order to manage the disease. Mind that in general, the control of the disease is carried out considering three stages/areas, which should be pursued in parallel: 1. Implementation of a diagnostic strategy and managing of the...</td>
<td></td>
</tr>
</tbody>
</table>
diagnostic test results; 2. Managing of the risk factors; 3. Managing of positive animals. For the first stage, please find in attach the above-mentioned review (Roller et al., 2020) where all the assays currently available for the detection of paratuberculosis in zoo animals, their appropriateness and their own limits are discussed. In this regard, you should always keep in mind that the available assays for paratuberculosis show poor sensitivity, although strongly dependent on the stage of the disease. In general, the more advanced is the status of disease, the higher is the sensitivity associated to each test. About the direct tests, the assays aimed at detecting the presence of Mycobacterium avium subsp. paratuberculosis (MAP), the culture, which is still considered the gold standard, is being replaced by PCR as “in vivo” test. The PCR shows a sensitivity similar to the cultural assay but is much quicker. In the document by the “White Oak Conservation Center”, you will find also some considerations about the importance of establishing and maintaining a diagnostic test protocol, some possible protocols of sampling suggesting the percentage of animal that should be tested according to the degree of freedom confidence desired and the minimal and optimal frequencies of testing (at least once per year, but preferably two or three times per year, depending on the prevalence, the resources available and the assays employed). In the same document, the risk factors that should be considered, such as the animals’ movements, management techniques and environmental sources of risk,
are also examined. Finally, in order to avoid the spread of the disease through direct or indirect contacts (i.e. fomites), positive animals should be isolated from the negative ones. Further considerations should be done in order to manage the outbreak, like the percentage and species of positive animals, the predictive values of the test employed, how the animals are housed (individually housed or sharing the same areas) and the importance of the animals (how easily they can be replaced). Moreover, before the reintroduction of animals or new animals in an area that has previously housed paratuberculosis infected animals, it should be well kept in mind how MAP proved to be extremely resistant in the environment, being able to maintain its viability even after many months. For more details about the ability of MAP to survive to different environmental conditions, I have included, for your convenience, a specific paper dedicated to this aspect (Whittington et al., 2004).

Hope these suggestions will be useful for you, in case of further questions, please don’t hesitate.

Sincerely, PhD Matteo Ricchi
WOAH Expert at the WOAH Reference Laboratory for Paratuberculosis Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna Sede territoriale di Piacenza Via Strada della faggiola, 1 29027 Podenzano (PC), Italia Tel: +390523524253 fax: +390523523491

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOAH Members other than the own?
No
TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?
No

15. Did your laboratory disseminate epidemiological data that had been processed and analysed?
Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

Data about the seroprevalence and molecular subtyping of field isolates recovered upon specific request

16. What method of dissemination of information is most often used by your laboratory? (Indicate in the appropriate box the number by category and list the details in the box)

a) Articles published in peer-reviewed journals:

3

b) International conferences:

4
3. “Validation of a IS900-qPCR assay for the detection of paratuberculosis in faeces according to the OIE - Principles and methods of validation of diagnostic assays for infectious disease”. A.Filippi, G.Galletti, N.Arrigoni, C.Garbarino, M.Ricchi (poster). 15th International Colloquium on Paratuberculosis, Dublin, Ireland, 14th-18th June 2022

C) National conferences:

3

d) Other (Provide website address or link to appropriate information):

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

17. Did your laboratory provide scientific and technical training to laboratory personnel from other WOAH Members?

No

TOR8: QUALITY ASSURANCE

18. Does your laboratory have a Quality Management System?

Yes

<table>
<thead>
<tr>
<th>Quality management system adopted</th>
<th>Certificate scan (PDF, JPG, PNG format)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 17025</td>
<td>Certificate of accreditation (pdf)</td>
</tr>
</tbody>
</table>

19. Is your quality management system accredited?

Yes

<table>
<thead>
<tr>
<th>Test for which your laboratory is accredited</th>
<th>Accreditation body</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR from faece, tissue and milk</td>
<td>ACCREDIA</td>
</tr>
<tr>
<td>Cultural method from faeces</td>
<td>ACCREDIA</td>
</tr>
<tr>
<td>Cultural method from milk</td>
<td>ACCREDIA</td>
</tr>
<tr>
<td>ELISA method from blood and milk</td>
<td>ACCREDIA</td>
</tr>
</tbody>
</table>

20. Does your laboratory maintain a “biorisk management system” for the pathogen and the disease concerned?

Yes

The laboratory works according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 1.1.4 and WHO Laboratory biosafety manual.

TOR9: SCIENTIFIC MEETINGS

21. Did your laboratory organise scientific meetings related to the pathogen in question on behalf of WOAH?

22. Did your laboratory participate in scientific meetings related to the pathogen in question on behalf of WOAH?
23. Did your laboratory exchange information with other WOAH Reference Laboratories designated for the same pathogen or disease? 
No  
24. Are you a member of a network of WOAH Reference Laboratories designated for the same pathogen? 
No  
25. Did you organise or participate in inter-laboratory proficiency tests with WOAH Reference Laboratories designated for the same pathogen? 
No  
26. Did your laboratory collaborate with other WOAH Reference Laboratories for the same disease on scientific research projects for the diagnosis or control of the pathogen of interest? 
No

**TOR11: OTHER INTERLABORATORY PROFICIENCY TESTING**

27. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than WOAH Reference Laboratories for the same pathogen? 
Yes

<table>
<thead>
<tr>
<th>Purpose for inter-laboratory test comparisons1</th>
<th>Role of your reference laboratory (organizer/participant)</th>
<th>No. participating laboratories</th>
<th>Region(s) of participating WOAH Member Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian national proficiency test for the detection of antibodies against paratuberculosis</td>
<td>Organizer</td>
<td>38</td>
<td>Europe</td>
</tr>
<tr>
<td>Proficiency test for the detection of antibodies against paratuberculosis from sera</td>
<td>Partecipant</td>
<td>50</td>
<td>Europe</td>
</tr>
<tr>
<td>Proficiency test for the detection of Mycobacterium avium subsp. paratuberculosis from lyophilized faces. Cultural and PCR assay</td>
<td>Partecipant</td>
<td>7</td>
<td>Europe</td>
</tr>
<tr>
<td>Proficiency test for the detection of antibodies against paratuberculosis from milk</td>
<td>Partecipant</td>
<td>36</td>
<td>Europe</td>
</tr>
</tbody>
</table>

**TOR12: EXPERT CONSULTANTS**

28. Did your laboratory place expert consultants at the disposal of WOAH? 
No  
29. Additional comments regarding your report: 
No