# WOAH Reference Laboratory Reports Activities 2022

### Activities in 2022

### This report has been submitted : 8 mars 2023 11:14

### Laboratory Information

Name of disease (or topic) for which you are a designated WOAH Reference Laboratory:	Bovine babesiosis
Address of laboratory:	National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-13, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
Tel.:	+81155495649
E-mail address:	yokoyama@obihiro.ac.jp
Website:	https://www.obihiro.ac.jp/facility/protozoa/en/oie-reference-centres
Name (including Title) of Head of Laboratory (Responsible Official):	Prof. Naoaki Yokoyama
Name (including Title and Position) of WOAH Reference Expert:	Prof. Naoaki Yokoyama
Which of the following defines your laboratory? Check all that apply:	Academic institution

### **TOR1: DIAGNOSTIC METHODS**

Yes

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)

Diagnostic Test	Indicated in WOAH Manual (Yes/No)	Total number of test performed last year	
Indirect diagnostic tests		Nationally	Internationally
Babesia bovis IFAT	YES	0	293

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Babesia bigemina IFAT	YES	0	293
Babesia bovis ELISA	YES	0	293
Babesia bigemina ELISA	YES	0	293
Babesia ovata IFAT	NO	81	0
Direct diagnostic tests		Nationally	Internationally
Babesia bovis PCR	YES	0	453
Babesia bovis PCR Babesia bigemina PCR	YES	0	453 453
Babesia bovis PCR Babesia bigemina PCR Babesia naoakii PCR	YES YES NO	0 0 0	453 453 453

### **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?

Yes

TYPE OF REAGENT AVAILABLE	RELATED DIAGNOSTIC TEST	PRODUCED/ PROVIDE	AMOUNT SUPPLIED NATIONALLY (ML, MG)	AMOUNT SUPPLIED INTERNATIONALLY (ML, MG)	NO. OF RECIPIENT WOAH MEMBER COUNTRIES	COUNTRY OF RECIPIENTS
Babesia bovis IFAT slides	IFAT	Produced and provided	0	Nos. 40	1	America
Babesia bigemina IFAT slides	IFAT	Produced and provided	0	Nos. 40	1	America
Babesia bovis DNA	PCR	Produced and provided	0	0.01 MG	2	Africa America
Babesia bigemina DNA	PCR	Produced and provided	0	0.01 MG	2	Africa America

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

NAME OF THE NEW TEST OR DIAGNOSTIC METHOD DEVELOPED	DESCRIPTION AND REFERENCES (PUBLICATION, WEBSITE, ETC.)
	Ganzinelli S, Byaruhanga C, Primo ME, Lukanji Z, Sibeko K, Matjila
	T, Neves L, Benitez D, Enkhbaatar B, Nugraha AB, Igarashi I, Florin-
Cytochrome b gene-based nested PCR assays for Babesia bovis and	Christensen M, Schnittger L. 2022. International interlaboratory

Babesia bigemina va a	ralidation of a nested PCR for molecular detection of Babesia bovis and Babesia bigemina, causative agents of bovine babesiosis. Vet. Parasitol. 304, 109686.
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7. Did your laboratory validate diagnostic methods according to WOAH Standards for the designated pathogen or disease?

No

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

NAME OF WOAH MEMBER COUNTRY SEEKING ASSISTANCE	DATE	WHICH DIAGNOSTIC TEST USED	NO. SAMPLES RECEIVED FOR PROVISION OF DIAGNOSTIC SUPPORT	NO. SAMPLES RECEIVED FOR PROVISION OF CONFIRMATORY DIAGNOSES
MEXICO	2022-11-16	IFAT and ELISA	94	0
MONGOLIA	2022-10-27	IFAT and ELISA	199	0
JAPAN	2022-05-23	PCR	50	0
JAPAN	2022-07-26	PCR	100	0
JAPAN	2022-07-27	PCR	99	0
JAPAN	2022-07-29	PCR and IFAT	81	0
JAPAN	2022-08-01	PCR	49	0
JAPAN	2022-08-03	PCR	100	0
JAPAN	2022-08-09	PCR	99	0
JAPAN	2022-09-08	PCR	78	0
JAPAN	2022-11-14	PCR	132	0

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

Yes

TECHNICAE CONSULTANCE	PURPOSE	PROVIDED
MONGOLIA	Designing an epidemiological study for identifying tick vectors transmitting bovine Babesia species	In person
SRI LANKA	Designing an epidemiological study for surveying bovine clinical babesiosis, and methodology for the isolation and in vitro cultivation of bovine Babesia species	In person and electronic consultation

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KYRGYZSTAN	Designing epidemiological studies for surveying bovine clinical babesiosis, detecting carrier animals, and identifying tick vectors transmitting bovine Babesia species	In person and electronic consultation
KENYA	Designing an epidemiological survey to identify the Babesia species infecting cattle	In person
PARAGUAY	Surveillance and control of bovine babesiosis	Electronic consultation
MALAWI	Surveillance and control of bovine babesiosis	Electronic consultation
JAPAN	Diagnosis, surveillance, and control of bovine babesiosis	In person and electronic consultation

### **TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES**

#### 12. Did your laboratory participate in international scientific studies in collaboration with WOAH Members other than the own?

Yes

Title of the study	Duration	PURPOSE OF THE STUDY	PARTNERS (INSTITUTIONS)	WOAH MEMBER COUNTRIES INVOLVED OTHER THAN YOUR COUNTRY
Prevalence, causative agents, and presentation of clinical babesiosis in cattle in Sri Lanka	4 years	To determine the prevalence, causative Babesia species, and characteristics of clinical bovine babesiosis in Sri Lanka	Veterinary Research Institute	SRI LANKA
Epidemiology, isolation, and in vitro cultivation of bovine Babesia species in Thailand	3 years	To determine the current status of bovine Babesia infections and to cultivate local isolates in Thailand	Faculty of Veterinary Medicine, Chiang Mai University	THAILAND
Molecular epidemiology of bovine Babesia species in yaks, Bactrian camels, and small ruminants in Mongolia	4 years	To identify the non-cattle hosts for bovine Babesia species	Institute of Veterinary Medicine, Mongolian University of Life Sciences	MONGOLIA
Epidemiological survey of bovine babesiosis in Kyrgyzstan	4 years	To determine the current status of bovine babesiosis in Kyrgyzstan	Kyrgyz Research Institute of veterinary named after A Duisheev	KYRGYZSTAN
Epidemiological survey of bovine babesiosis in Paraguay	3 years	To identify the Babesia species infecting cattle in Paraguay	Vice Ministerio de Ganaderia	PARAGUAY

### TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?

Yes

#### THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED

We surveyed Bactrian camels in Mongolia and dromedary camels in Egypt for Babesia bovis, Babesia bigemina and Babesia naoakii.

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:

The data from our epidemiological surveys in Mongolia and Egypt were published in peer-reviewed international scientific journals (see the list of publication in 16a)

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:

#### 8

1. Salman D, Sivakumar T, Otgonsuren D, Mahmoud ME, Elmahallawy EK, Khalphallah A, Kounour AMEY, Bayomi SA, Igarashi M, Yokoyama N. 2022. Molecular survey of Babesia, Theileria, Trypanosoma, and Anaplasma infections in camels (Camelus dromedaries) in Egypt. Parasitol. Int. 90, 102618.

2. Otgonsuren D, Sivakumar T, Amgalanbaatar T, Enkhtaivan B, Narantsatsral S, Davaasuren B, Zoljargal M, Munkhgerel D, Davkharbayar B, Batmagnai E, Tuvshintulga B, Ahedor B, Myagmarsuren P, Battur B, Battsetseg B, Yokoyama N. 2022. Molecular survey of bovine Babesia species in Bactrian camels (Camelus bactrianus) in Mongolia. Ticks Tick Borne Dis. 13, 101871.

3. Ganzinelli S, Byaruhanga C, Primo ME, Lukanji Z, Sibeko K, Matjila T, Neves L, Benitez D, Enkhbaatar B, Nugraha AB, Igarashi I, Florin-Christensen M, Schnittger L. 2022. International interlaboratory validation of a nested PCR for molecular detection of Babesia bovis and Babesia bigemina, causative agents of bovine babesiosis. Vet. Parasitol. 304, 109686.

4. Mercado-Uriostegui MA, Castro-Sánchez LA, Batiha GE, Valdez-Espinoza UM, Falcón-Neri A, Ramos-Aragon JA, Hernández-Ortiz R, Kawazu SI, Igarashi I, Mosqueda J. 2022. The GP-45 protein, a highly variable antigen from Babesia bigemina, contains conserved B-cell epitopes in geographically distant isolates. Pathogens 11, 591.

5. Rittipornlertrak A, Nambooppha B, Muenthaisong A, Apinda N, Koonyosying P, Srisawat W, Chomjit P, Sangkakam K, Punyapornwithaya V, Tiwananthagorn S, Yokoyama N, Sthitmatee N. 2022. Immunization of cattle with recombinant structural ectodomains I and II of Babesia bovis apical membrane antigen 1 [BbAMA-1(I/II)] induces strong Th1 immune response. Front Vet Sci. 9, 917389.

6. Sivakumar T, Tuvshintulga B, Otgonsuren D, Batmagnai E, Ahedor B, Kothalawala H, Vimalakumar SC, Silva SSP, Yamagishi J, Yokoyama N. 2022. Phylogenetic analyses of the mitochondrial, plastid, and nuclear genes of Babesia sp. Mymensingh and its naming as Babesia naoakii n. sp. Parasit. Vectors 15, 299.

7. Hakimi H, Yamagishi J, Kawazu SI, Asada M. 2022. Advances in understanding red blood cell modifications by Babesia. PLoS Pathog. 18, e1010770.

8. Ji S, Ceylan O, Ma Z, Galon EM, Zafar I, Li H, Hasegawa Y, Sevinc M, Masatani T, Iguchi A, Kawase O, Umemiya-Shirafuji R, Asada M, Sevinc F, Xuan X. 2022. Protozoan and rickettsial pathogens in ticks collected from infested cattle from turkey. Pathogens. 11, 500.

b) International conferences:

c) National conferences:

#### 1

Mori N, Sivakumar T, Mizutani Y, Matsui S, Takahiro K, Shirafuji R, Inokuma H, Yokoyama. N. There are two types of large bovine piroplasma (bovine Babesia) in Japan. Cattle clinical parasite research group Hokkaido research meeting, Japan, October 28, 2022.

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

#### 1

https://www.obihiro.ac.jp/facility/protozoa/en/oie-reference-centres

### TOR7: SCIENTIFIC AND TECHNICAL TRAINING

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

Yes

a) Technical visit : 2

b) Seminars : 82

c) Hands-on training courses: 0

d) Internships (>1 month) 0

Type of technical training provided (a, b, c or d)	Country of origin of the expert(s) provided with training	No. participants from the corresponding country
A	SWEDEN	1
A	MEXICO	1
В	MONGOLIA	2
В	PARAGUAY	1
В	CONGO	2
В	THAILAND	2
В	BANGLADESH	1
В	SRI LANKA	4
В	KYRGYZSTAN	1
В	CHINA	1
В	EGYPT	1
В	TANZANIA	1
В	JAPAN	66

### TOR8: QUALITY ASSURANCE

#### 18. Does your laboratory have a Quality Management System?

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Quality management system adopted	Certificate scan (PDF, JPG, PNG format)	
ISO17025:2017	PDF	iso-Eng.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
PCR for Babesia bovis	Perry Johnson laboratory Accrediation, Inc. (PJLA)
PCR for Babesia bigemina	Perry Johnson laboratory Accrediation, Inc. (PJLA)

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

#### Yes

In accordance with the applicable laws, our university has regulations to ensure the safety when conducting experiments with pathogens, animals, and gene editing. The expert committees regularly review and update these regulations. The expert committees on biorisk management review and approve research plans involving animals, pathogens, and gene manipulation only after a thorough review. All laboratories are routinely examined to ensure that all experiments are carried out safely. All laboratories and animal facilities, including the RL for bovine babesiosis, are run at the BSL2 standard.

### **TOR9: SCIENTIFIC MEETINGS**

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

No

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

No

## TOR10: NETWORK WITH WOAH REFERENCE LABORATORIES

23. Did your laboratory exchange information with other WOAH Reference Laboratories designated for the same pathogen or disease? Yes

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?

Yes

PURPOSE OF THE PROFICIENCY TESTS: 1	ROLE OF YOUR REFERENCE LABORATORY (ORGANISER/ PARTICIPANT)	NO. PARTICIPANTS	PARTICIPATING WOAH REF. LABS/ ORGANISING WOAH REF. LAB.
Validating an ELISA	Participant	2	Organizing WOAH reference lab: WOAH reference laboratory for bovine babesiosis, Autonomous University of Querétaro, Mexico

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?

Yes						
TITLE OF THE PROJECT OR CONTRACT	SCOPE	NAME(S) OF RELEVANT WOAH REFERENCE LABORATORIES				
Development of ELISA for the serodiagnosis of Babesia bovis and Babesia bigemina	To develop an ELISA capable of detecting antibodies to both Babesia bovis and Babesia bigemina in cattle	WOAH reference laboratory for bovine babesiosis, Autonomous University of Querétaro, Mexico				

### 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					
Purpose for inter-laboratory test comparisons1	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Region(s) of participating WOAH Member Countries		
Validating nested PCR assays for Babesia bovis and Babesia bigemina	Participant	3	Africa America Asia and Pacific		

### **TOR12: EXPERT CONSULTANTS**

28. Did your laboratory place expert consultants at the disposal of WOAH?

No

29. Additional comments regarding your report:

No