

WOAH Collaborative Centre Reports Activities 2023

Activities in 2023

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Centre Information

Title of WOA Collaborating Centre	Surveillance and Control of Animal Protozoan Diseases
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Name (including Title and Position) of Head of the Collaborating Centre (WOAH Contact Point):	Prof. Naoaki Yokoyama, DVM, PhD
Name of the writer:	Prof. Naoaki Yokoyama

TOR1 AND 2: SERVICES PROVIDED

1. Activities as a centre of research, expertise, standardisation and dissemination of techniques within the remit of the mandate given by WOA

Category	Title of activity	Scope
		<p>1. The following compounds were evaluated as anti-protozoal agents: MMV665941, Norfloxacin, Ofloxacin, imidazo[1,2-a] pyridines, a combination of Diminazene aceturate and imidocarb dipropionate, resveratrol, idarubicin hydrochloride, vorinostat, Methanolic extract of Moringa oleifera Leaves, and FLLL-32. The following compounds were investigated as trypanocidal agents:</p> <p>Phytochemicals from Scutellaria scordifolia and Crossostephium chinense, synthetic nitrofurantoin-triazole hybrids, ciprofloxacin analogs, ascofuranone, curcumin and its nanoparticle, Nitrofuryl- and Nitrothienylazines. The following compounds were investigated as antiplasmodial agents: Phebestin, phytochemicals from Crossostephium chinense. Compounds from Crossostephium chinense were investigated for their cytotoxic activities against Leishmania major. 2. Regulation of reactive oxygen species and lipid peroxidation was investigated as a novel disease treatment strategy. 3. Advances in the development of animal models, their limitations, and future improvements for understanding immunology and Pathogenesis of babesiosis have</p>

<p>Disease control (true)</p>	<p>1. Identification and evaluation of anti-protozoan compounds as potential chemotherapeutic agents 2. Novel therapeutic approach 3. Understanding immunology and pathogenesis of protozoan diseases 4. Investigation of ultrastructure of protozoa 5. Elucidation of molecular mechanisms involved in protozoan invasion into host cells and development 6. Genome sequencing 7. Biology of and protozoa development in vectors and their control</p>	<p>been reviewed. Placental damage associated with neosporosis was investigated using mouse model. Histopathological and immunohistochemical changes associated Epididymitis caused by Trypanosoma equiperdum in a mouse model. Role of chemokine receptor CXCR3 in the immunological response against Neospora caninum was investigated in mouse model. The role of anti-erythrocyte and anti-platelet antibodies in the development of hemolytic anemia and thrombocytopenia in mice infected with Plasmodium spp. and Babesia spp. Variations in serum metabolomic profiles in mice infected with Babesia microti were investigated. Protective effect of immunity induced by Babesia microti against Plasmodium berghei was investigated in mouse model. Retinal ferroptosis was investigated as a critical mechanism for the induction of retinochoroiditis during ocular toxoplasmosis. 4. The distribution of glycerophospholipids phosphatidylserine and phosphatidylethanolamine in individual leaflets of cellular membranes of Toxoplasma gondii was determined. 5. The microneme adhesive repeat domain-containing region of the BBOV_III011730 was characterized as a dispensable domain for the blood stage growth of Babesia bovis. 6. Genomes of the following species were sequenced, annotated, and compared with the closely-related species to facilitate devising diagnostic and control methods for protozoan diseases: Babesia gibsoni, Babesia caballi, Babesia ovis, and Haemaphysalis longicornis. 7. Secreted ferritin (FER2) was localized in the embryos of Haemaphysalis longicornis. Sex-specific proteins in the intestinal tissue of Haemaphysalis qinghaiensis were identified using proteomics analysis. Secretion and circulation of extracellular vesicles in Rhipicephalus haemaphysaloides and Hyalomma asiaticum ticks were investigated. An antigen in Haemaphysalis longicornis homologous to ATAQ in Rhipicephalus microplus was investigated as a potential anti-tick vaccine candidate. RNA activation in ticks was investigated. Free Plasmodium oocysts were produced in Anopheles mosquitoes by injecting them with ookinetes.</p>
<p>Epidemiology, surveillance, risk assessment, (true)</p>	<p>1. Surveillance of protozoan parasites 2. Vector surveillance</p>	<p>1. Theileria equi and Babesia caballi infections were investigated in horses in Paraguay. Seasonal variation and factors affecting Trypanosoma theileri Infection in wild sika deer in Hokkaido were determined. Prevalence of Trypanosoma theileri and Trypanosoma evansi was determined in water buffalo, cattle, goats, and horses in the Philippines. Species of Babesia and Theileria infecting cattle in Bangladesh, Kyrgyzstan, Thailand, the Philippines, and Nepal, gayals and goats in Bangladesh, and goats and sheep in Turkey were identified. Bovine Babesia species infecting yaks in Mongolia were identified. The prevalence of Theileria annulata was determined in cattle in Turkey. 2. Myzomyia and Pyretophorus series of Anopheles mosquitoes were investigated as potential vectors of Plasmodium caprae and subjected to molecular characterization.</p>
<p>Training, capacity building (true)</p>	<p>Training on diagnosis, surveillance, and control of</p>	<p>Scientists from Myanmar, Mongolia, South Africa, Philippines, India, Sri Lanka, Vietnam, Kyrgyzstan, China, Bangladesh, Thailand, Ghana, Kenya, Malawi,</p>

	animal protozoan diseases	Japan, Paraguay etc. were trained on diagnosis, surveillance, and control of animal protozoan diseases via technical visits, seminars, and internships.
Diagnosis, biotechnology and laboratory (true)	1. Development and evaluation of diagnostic assays 2. Development of transgenic protozoa	1. A novel loop-mediated isothermal amplification (LAMP) with improved thermostability, sensitivity and alternative visualization methods was developed for Babesia bovis. An ELISA was developed for the serodiagnosis of Babesia caballi. A sandwich ELISA based on secreted antigen-1 was developed for detecting Babesia ovis infection in sheep. A PCR assay for the specific detection of Babesia caballi and genotype-specific PCR assays for Theileria equi were developed. 2. A stable transfection and gene targeting system was developed for Babesia divergens.
Vaccines (true)	1. Evaluation of vaccine delivery systems 2. Characterization of protozoan antigens as vaccine candidates	1. An ionizable lipid material with a vitamin E scaffold was characterized as an mRNA vaccine platform for efficient cytotoxic t cell responses. 2. Three members of the multidomain adhesion Ccp family in Babesia gibsoni were characterized as candidate antigens for transmission-blocking vaccines. The spherical body protein 4 in Babesia bigemina was characterized as a vaccine candidate.
Evolution (true)	Elucidation of evolutionary relationship	Phylogenetic positions of bat Polychromophilus and ungulate Plasmodium species were determined based on mitochondrial genome sequences.

TOR3: HARMONISATION OF STANDARDS

2. Proposal or development of any procedure that will facilitate harmonisation of international regulations applicable to the main focus area for which you were designated

Proposal title	Scope/Content	Applicable area
Validation of diagnostic assays	The following diagnostic assays were developed and validated: a loop-mediated isothermal amplification (LAMP) for Babesia bovis, an ELISA Babesia caballi, a sandwich ELISA Babesia ovis, A PCR assay for Babesia caballi, and genotype-specific PCR assays for Theileria equi.	health management

3. In exercising your activities, have you identified any regulatory research needs* relevant for WOA?H?

No

4. Did your Collaborating Centre maintain a network with other WOA?H Collaborating Centres (CC), Reference Laboratories (RL), or organisations designated for the same specialty, to coordinate scientific and technical studies?

Yes

Name of WOA?H CC/RL/other organisation(s)	Location	Region of networking Centre	Purpose
1) RL for Dourine, ANSES, France, 2) RL for Surra, Institute of Tropical Medicine Antwerp, Belgium, 3) RL for Surra, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary	France Belgium Japan	Asia and Pasific Europe	To create awareness on NTTAT as high impact neglected veterinary diseases, develop tools to enhance the surveillance capacity, foster collaborative research, and fill gaps in knowledge on disease

Medicine, Japan, and 4) RL for trypanosomes (tsetse-transmitted), CIRAD-IRD, France		epidemiology, pathogenesis, drug efficacy, vaccines, modes of transmission, reservoir hosts, and vector control.
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TOR4 AND 5: NETWORKING AND COLLABORATION

5. Did your Collaborating Centre maintain a network with other WOA Collaborating Centres, Reference laboratories, or organisations in other disciplines, to coordinate scientific and technical studies?

Yes

Name of WOA CC/RL/other organisation(s)	Location	Region of networking Centre	Purpose
Department of Epidemiology of Microbial Diseases, Yale School of Public Health	USA	Americas	To review the currently available animal models for babesiosis and their limitations.
Faculty of Veterinary Science, Chulalongkorn University	THAILAND	Asia and Pasific	1) To investigate Anopheles mosquitoes acting as potential vectors of Plasmodium caprae and 2) to develop loop-mediated isothermal amplification (LAMP) to detect Babesia bovis infection in cattle.
State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University	CHINA	Asia and Pasific	To analyse sex-specific proteins in the intestinal tissue of Haemaphysalis qinghaiensis using proteomics.
School of Engineering and Applied Sciences, National University of Mongolia	MONGOLIA	Asia and Pasific	To identify the phytochemicals in Scutellaria scordiifolia and to investigate their trypanocidal activity.
Centro de Diagnostico Veterinario, San Lorenzo	PARAGUAY	Americas	To detect Theileria equi and Babesia caballi in horses in Paraguay and determine their genotypes.
WOAH RL for bovine babesiosis, Facultad de Ciencias Naturales, Universidad Autonoma de Queretaro	MEXICO	Americas	To characterize spherical body protein 4 in Babesia bigemina as a vaccine candidate.
Institute of Molecular Parasitology and Protozoan Diseases at Main Campus and College of Veterinary Medicine at Barili Campus, Cebu Technological University	PHILIPPINES	Asia and Pasific	To identify the piroplasm and Trypanosoma species infecting livestock in the Philippines.
Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University	BANGLADESH	Asia and Pasific	To identify the piroplasm species infecting livestock in Bangladesh.

College of Veterinary Medicine, Huazhong Agricultural University	CHINA	Asia and Pasific	To sequence and annotate the genome of Babesia gibsoni.
Unit for Environmental Sciences and Management, North-West University	SOUTH AFRICA	Africa	To investigate anti-trypanosomal activity of synthetic nitrofurantoin-triazole hybrids, and to identify the tick vectors transmitting Rickettsia africae.
Centre of Excellence for Pharmaceutical Sciences, North-West University	SOUTH AFRICA	Africa	To investigate trypanocidal activities of ciprofloxacin analogs and Nitrofuryl- and Nitrothienylazines.
Kyrgyz Research Institute of Veterinary Named After A. Duisheev	KYRGYZSTAN	Asia and Pasific	To identify the vector-borne pathogens infecting cattle in Kyrgyzstan.
Institute of Science and Technology, Tribhuvan University	NEPAL	Asia and Pasific	To identify the species of Babesia and Theileria infecting cattle in Nepal.
Faculty of Veterinary Medicine, University of Selcuk	TURKEY	Europe	To sequence and annotate the genome of Babesia ovis and analyse the diversity of its multigene families, and to identify piroplasm species infesting small ruminants in Turkey.
Institute of Veterinary Medicine, Mongolian University of Life Sciences	MONGOLIA	Asia and Pasific	To identify bovine Babesia species infecting yaks in Mongolia.
Faculty of Veterinary Medicine, Kasetsart University	THAILAND	Asia and Pasific	To detect tick-borne pathogens infecting dairy cattle in Thailand and their association with reduced haematocrit.
Department of Zoology and Entomology, University of the Free State	SOUTH AFRICA	Africa	To investigate the efficacy of curcumin and curcumin Nanoparticle as trypanocidal agents.
Faculty of Veterinary Science, Chulalongkorn University	THAILAND	Asia and Pasific	To investigate the phylogenetic position of ungulate Plasmodium.
Veterinary Research Institute	SRI LANKA	Asia and Pasific	To determine the genotypes of Theileria equi infecting donkeys in Sri Lanka.

Faculty of Science, University of South Bohemia in Ceske Budejovice	CZECHIA	Europe	To establish a stable transfection and gene targeting system in Babesia divergens.
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TOR6: EXPERT CONSULTANTS

6. Did your Collaborating Centre place expert consultants at the disposal of WOA?H?

No

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

7. Did your Collaborating Centre provide advice/services to requests from Members in your main focus area?

Yes

Provision of confirmatory diagnostic services

Horse samples from USA, UK, New Zealand, and China were tested for equine piroplasmosis. Samples from animals were tested for neosporosis.

Supply of diagnostic and research materials

Theileria equi and Babesia caballi IFAT slides were supplied to institutions in India, Singapore, China, Japan, Australia, UK, Ireland, France, The Netherlands, and Argentina. Babesia divergens slides were provided to an institution in Austria. Theileria equi and Babesia caballi DNA samples were provided to institutions in India, Singapore, Japan, Kyrgyzstan, Malawi, Germany, and Argentina. DNA samples of bovine Babesia, Theileria, and Anaplasma species were provided to institutions in Mongolia, Paraguay, Argentina, and Kyrgyzstan. Microscopic slides of Trypanosoma parasites were provided to institutions in Iran and South Africa. Institutions in Japan were provided with tick colonies.

Expert advice on the diagnosis of protozoan diseases and vectors

Expert advice on the diagnosis of animal protozoan diseases was provided to institutions in India, Singapore, China, Sri Lanka, Japan, The Philippines, Kyrgyzstan, Australia, Morocco, Malawi, South Africa, Turkey, The Netherlands, UK, Chile, USA, and Argentina. Advice on disease control measures were provided to institutions in China, Japan, Uganda, USA and Indonesia. Advice on regulations related to surra (Trypanosoma evansi) and equine piroplasmosis during transnational movement of animals was provided to veterinarians in Japan and USA and South Africa, respectively. Advice on vector identification and surveillance was provided to an institution in Mongolia. Expert advice on tick distribution, breeding, research, and pathogen detection in ticks was provided to institutions in Japan. Advice on disease surveillance was provided to institutions in Sri Lanka, Kyrgyzstan, and Malawi.

8. Did your Collaborating Centre provide scientific and technical training, within the remit of the mandate given by WOA, to personnel from WOA Members?

Yes

a) Technical visit : 9

b) Seminars : 1208

c) Hands-on training courses: 60

d) Internships (>1 month) : 2

TYPE OF TECHNICAL TRAINING PROVIDED (A, B, C OR D)	CONTENT	COUNTRY OF ORIGIN OF THE EXPERT(S) PROVIDED WITH TRAINING	NO. PARTICIPANTS FROM THE CORRESPONDING COUNTRY
A	Training on genotyping of Theileria equi	INDIA	1
A	Training on PCR detection of bovine and equine piroplasmosis	KYRGYZSTAN	2
A	Training on screening trypanocidal drugs in vitro	SOUTH AFRICA	2
A	Training on production of IFAT slides for equine piroplasms	JAPAN	2

A	Training on PCR-based survey of bovine Babesia species	PARAGUAY	1
A	Training on molecular survey and genotyping of equine piroplasms	MALAWI	1
B	Education on management of bovine babesiosis	CHINA	84
B	Education on surveillance of animal protozoan diseases and their vectors	MONGOLIA	28
B	Education on diagnosis and control of equine piroplasmosis	NEPAL	3
B	Education on surveillance and in vitro cultivation of bovine and equine piroplasms	SRI LANKA	25
B	Education on diagnosis and control of equine piroplasmosis	BANGLADESH	1
B	Education on genotyping of and in vitro cultivation of Theileria equi	INDIA	4
B	Education on management of bovine babesiosis	PHILIPPINES	1
B	Education on diagnosis and control of equine piroplasmosis	THAILAND	4
B	Education on diagnosis and control of equine piroplasmosis	MYANMAR	2
B	Education on drug screening assays for protozoan parasites	INDONESIA	2
B	Education on the surveillance and control of equine piroplasmosis	KYRGYZSTAN	19
B	Education on diagnosis, surveillance, and control of protozoan parasites and their vectors.	JAPAN	968
B	Education on effective management strategies for bovine babesiosis	UGANDA	40

B	Education on the limitations of equine piroplasmosis diagnostics	GHANA	3
B	Education on managing equine piroplasmosis	MALAWI	1
B	Education on diagnosis and treatment of trypanosomosis	SOUTH AFRICA	8
B	Education on managing bovine theileriosis through surveillance	KENYA	2
B	Education on risk factors of bovine babesiosis	PARAGUAY	13
C	Hands-on training on surveillance of protozoan diseases and their vectors	MONGOLIA	38
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	THAILAND	4
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	MYANMAR	2
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	CHINA	2
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	PHILIPPINES	1
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	NEPAL	3
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	JAPAN	8
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	BANGLADESH	1
C	Hands-on training with WOA- recommended diagnostic assays for equine piroplasmosis	GHANA	1

D	Education and training on drug sensitivity testing for Trypanosoma parasites	SOUTH AFRICA	2
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TOR8: SCIENTIFIC MEETINGS

9. Did your Collaborating Centre organise or participate in the organisation of scientific meetings related to your main focus area on behalf of WOA?H

No

TOR9: DATA AND INFORMATION DISSEMINATION

10. Publication and dissemination of any information within the remit of the mandate given by WOA?H that may be useful to Members of WOA?H

a) Articles published in peer-reviewed journals:

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1. Mohamed Abdo Rizk, Shima Abd El-Salam El-Sayed, Ikuo Igarashi, *In vivo activity and atom pair fingerprint analysis of MMV665941 against the apicomplexan parasite Babesia microti, the causative agent of babesiosis in humans and rodents. Pathogens and Global Health. 2023 May; 117(3): 315-321.*
2. Abhinav Kumar, Aditya Kabra, Ikuo Igarashi, Peter J Krause, *Animal models of the immunology and pathogenesis of human babesiosis. Trends in Parasitology. 2023 Jan; 39(1): 38-52.*
3. Mohamed Abdo Rizk, Shima Abd El-Salam El-Sayed, Ikuo Igarashi, *In Vitro Inhibitory Effects and Bioinformatic Analysis of Norfloxacin and Ofloxacin on Piroplasm. Acta Parasitologica. 2023 Mar; 68(1): 213-222.*
4. Zeyu Tang, Hang Li, Suzhu Xie, Shaowei Zhao, Shuang Zhang, Hao Wang, Nanli Li, Xuancheng Zhang, Fanglin Zhao, Lijun Jia, *A preliminary study on placental damage associated to experimental neosporosis in BALB/c mice. Parasitology Research. 2023 Mar; 122(3): 781-788.*
5. Anh Hoang Lan Nguyen, Sittiporn Pattaradilokrat, Winai Kaewlamun, Osamu Kaneko, Masahito Asada, Morakot Kaewthamasorn, Myzomyia and Pyrethrophorus series of Anopheles mosquitoes acting as probable vectors of the goat malaria parasite Plasmodium caprae in Thailand. *Scientific Reports. 2023 Jan; 13(1): 145.*
6. Mohamed Abdo Rizk, Shima Abd El-Salam El-Sayed, El-Sayed El-Alfy, Ikuo Igarashi, *Imidazo[1,2-a]pyridine: a Highly Potent Therapeutic Agent Clears Piroplasm Infection In Vitro. Acta Parasitologica. 2023 Mar; 68(1): 249-256.*
7. Yusuke Tanaka, Keisuke Suganuma, Kenichi Watanabe, Yoshiyasu Kobayashi, *Epididymitis in mice experimentally infected with Trypanosoma equiperdum: a histopathological and immunohistochemical study. Journal of Comparative Pathology. 2023 Feb; 201: 1-9.*
8. Jose Ma M Angeles, Atcharaphan Wanlop, Minh-Anh Dang-Trinh, Masashi Kirinoki, Shin-Ichiro Kawazu, Aya Yajima, *Evaluation of Crude and Recombinant Antigens of Schistosoma japonicum for the Detection of Schistosoma mekongi Human Infection. Diagnostics (Basel). 2023 Jan; 13(2): 184.*
9. Hanan H Abdelbaky, Shuichiro Mitsuhashi, Kenichi Watanabe, Nanako Ushio, Miku Miyakawa, Hidefumi Furuoka, Yoshifumi Nishikawa, *Involvement of chemokine receptor CXCR3 in the defense mechanism against Neospora caninum infection in C57BL/6 mice. Frontiers in Microbiology. 2023 Jan; 13: 1045106.*
10. Anh Hoang Lan Nguyen, Yudhi Ratna Nugraheni, Trang Thuy Nguyen, Aung Aung, Duriyang Narapakdeesakul, Winai Kaewlamun, Masahito Asada, Morakot Kaewthamasorn, *Molecular characterization of anopheline mosquitoes from the goat malaria-endemic areas of Thailand. Medical and Veterinary Entomology. 2023 Jun; 37(2): 381-395.*
11. Emmanuel Pacia Hernandez, Kei Shimazaki, Hiroko Niihara, Rika Umemiya-Shirafuji, Koza Fujisaki, Tetsuya Tanaka, *Localization of secreted ferritin (FER2) in the embryos of the tick Haemaphysalis longicornis. Parasites & Vectors. 2023 Jan; 16(1): 42.*
12. Apinya Arnuphappasert, Yudhi Ratna Nugraheni, Aung Aung, Masahito Asada, Morakot Kaewthamasorn, *Detection of Babesia bovis using loop-mediated isothermal amplification (LAMP) with improved thermostability, sensitivity and alternative visualization methods. Scientific Reports. 2023 Feb; 13(1): 1838.*
13. Hejia Ma, Eloiza May Galon, Yanjun Lao, Ming Kang, Xuenan Xuan, Jixu Li, Yali Sun, *De novo assembled transcriptomics assisted label-free quantitative proteomics analysis reveals sex-specific proteins in the intestinal tissue of Haemaphysalis qinghaiensis. Infection Genetics and Evolution. 2023 Apr; 109: 105409.*
14. Mototada Shichiri, Hiroshi Suzuki, Yuji Isegawa, Hiroshi Tamai, *Application of regulation of reactive oxygen species and lipid peroxidation to disease treatment. Journal of Clinical Biochemistry and Nutrition. 2023 Jan; 72(1): 13-22.*
15. Stipan Nurbyek, Buyanmandakh Buyankhishig, Keisuke Suganuma, Yoshinobu Ishikawa, Mika Kutsuma, Marie Abe, Kenroh Sasaki, Bekh-Ochir Davaapurev, Javzan Batkhuu, Toshihiro Murata, *Phytochemical investigation of Scutellaria scordifolia and its trypanocidal activity. Phytochemistry. 2023 May; 209: 113615.*
16. Believe Ahedor, Thillaampalam Sivakumar, Maria Fátima Rodríguez Valinotti, Davaajav Otgonsuren, Naoaki Yokoyama, Tomás J Acosta, *PCR detection of Theileria equi and Babesia caballi in apparently healthy horses in Paraguay. Veterinary Parasitology: Regional Studies and Reports. 2023 Apr; 39: 100835.*
17. Hang Li, Shengwei Ji, Eloiza May Galon, Iqra Zafar, Zhuowei Ma, Thom Do, Moaz M Amer, Yihong Ma, Junya Yamagishi, Mingming Liu, Xuenan Xuan, *Identification of three members of the multidomain adhesion CCp family in Babesia gibsoni. Acta Tropica. 2023 May; 241: 106890.*
18. Mo Zhou, Lianfeng Li, Keisuke Suganuma, *Editorial: Epidemic status and prevention of swine infectious diseases. Frontiers in Veterinary Science. 2023 Mar; 10: 1169644.*
19. Juan Mosqueda, Diego Josimar Hernandez-Silva, Massaro W Ueti, Adolfo Cruz-Reséndiz, Ricardo Marquez-Cervantez, Uriel Mauricio Valdez-Espinoza, Minh-Anh Dang-Trinh, Thu-Thuy Nguyen, Minerva Camacho-Nuez, Miguel Angel Mercado-Uriostegui, Gabriela Aguilar-Tipacamú, Juan Alberto Ramos-Aragon, Ruben Hernandez-Ortiz, Shin-Ichiro Kawazu, Ikuo Igarashi, *Spherical Body Protein 4 from Babesia bigemina: A Novel Gene That Contains Conserved B-Cell Epitopes and Induces Cross-Reactive Neutralizing Antibodies in Babesia ovata. Pathogens. 2023 Mar; 12(3): 495.*
20. Mohamed Abdo Rizk, Shima Abd El-Salam El-Sayed, Ikuo Igarashi, *Diminazene aceturate and imidocarb dipropionate-based combination therapy for babesiosis - A new paradigm. Ticks and Tick-borne Diseases. 2023 Jul; 14(4): 102145.*
21. Zhengmao Xu, Yanan Wang, Meng Sun, Yongzhi Zhou, Jie Cao, Houshuang Zhang, Xuenan Xuan, Jinlin Zhou, *Proteomic analysis of extracellular vesicles from tick hemolymph and uptake of extracellular vesicles by salivary glands and ovary cells. Parasites & Vectors. 2023 Apr; 16(1): 125.*
22. Shima Abd El-Salam El-Sayed, Mohamed Abdo Rizk, Hanadi B Baghdadi, Aaron Edmond Ringo, Gantuya Sambuu, Arifin Budiman Nugraha, Ikuo Igarashi, *Development of a promising antigenic cocktail for the global detection of Babesia caballi in horse by ELISA. PLoS One. 2023 Apr; 18(4): e0284535.*
23. Paul Franck Adjou Moumouni, Souichirou Naomasa, Bumduuren Tuvshintulga, Nariko Sato, Kiyoshi Okado, Weiqing Zheng, Seung-Hun Lee, Juan Mosqueda, Hiroshi Suzuki, Xuenan Xuan, Rika Umemiya-Shirafuji, *Identification and Characterization of Rhipicephalus microplus ATAQ Homolog from Haemaphysalis longicornis Ticks and Its Immunogenic Potential as an Anti-Tick Vaccine Candidate Molecule. Microorganisms. 2023 Mar; 11(4): 822.*
24. Mo Zhou, Jun Xie, Osamu Kawase, Yoshifumi Nishikawa, Shengwei Ji, Shanyuan Zhu, Shinuo Cao, Xuenan Xuan, *Characterization of anti-erythrocyte and anti-platelet*

- antibodies in hemolytic anemia and thrombocytopenia induced by *Plasmodium* spp. and *Babesia* spp. infection in mice. *Frontiers in Cellular and Infection Microbiology*. 2023 Apr; 13: 1143138.
25. Liang Shen, Chunhua Wang, Ruilin Wang, Xue Hu, Shiyang Liao, Wentong Liu, Aoling Du, Shengwei Ji, Eloiza May Galon, Hang Li, Xuenan Xuan, Juan Xiao, Mingming Liu, Serum metabolomic profiles in BALB/c mice induced by *Babesia microti* infection. *Frontiers in Cellular and Infection Microbiology*. 2023 Apr; 13: 1179967.
26. Yujun Hong, Keisuke Suganuma, Yuma Ohari, Mitsunori Kayano, Kenji Nakazaki, Shinya Fukumoto, Shin-Ichiro Kawazu, Noboru Inoue, Seasonal Variation and Factors Affecting *Trypanosoma theileri* Infection in Wild Sika Deer (*Ezo Sika Deer Cervus nippon yesoensis*) in Eastern Hokkaido. *Animals (Basel)*. 2023 May; 13(10): 1707.
27. Kofi Dadzie Kwofie, Emmanuel Pacia Hernandez, Anisuzzaman, Hayato Kawada, Yuki Koike, Sana Sasaki, Takahiro Inoue, Kei Jimbo, Fusako Mikami, Danielle Ladzekpo, Rika Umemiya-Shirafuji, Kayoko Yamaji, Tetsuya Tanaka, Makoto Matsubayashi, Md Abdul Alim, Samuel Kweku Dadzie, Shiroh Iwanaga, Naotoshi Tsuji, Takeshi Hatta, RNA activation in ticks. *Scientific Reports*. 2023 Jun; 13(1): 9341.
28. Nanang R Ariefita, Baldoj Pagmadulam, Masaki Hatano, Noriko Ikeda, Kunio Isshiki, Kazuaki Matoba, Masayuki Igarashi, Coh-Ichi Nihei, Yoshifumi Nishikawa, Antiplasmodial Activity Evaluation of a Bestatin-Related Aminopeptidase Inhibitor, Phebestin. *Antimicrobial Agents and Chemotherapy*. 2023 Jul; 67(7): e0160622.
29. Bumduuren Tuvshintulga, Azirwan Guswanto, Arifin Budiman Nugraha, Thillaiampalam Sivakumar, Rika Umemiya-Shirafuji, Naoaki Yokoyama, Disruption of a DNA fragment that encodes the microneme adhesive repeat domain-containing region of the BBOV_III011730 does not affect the blood stage growth of *Babesia bovis* in vitro. *Molecular and Biochemical Parasitology*. 2023 Jun; 255: 111576.
30. Shima Abd El-Salam El-Sayed, El-Sayed El-Alfy, Mohamed Z Sayed-Ahmed, Uday Kumar Mohanta, Saad S Alqahtani, Nawazish Alam, Sarfaraz Ahmad, Md Sajid Ali, Ikuo Igarashi, Mohamed Abdo Rizk, Evaluating the inhibitory effect of resveratrol on the multiplication of several *Babesia* species and *Theileria equi* on in vitro cultures, and *Babesia microti* in mice. *Frontiers in Pharmacology*. 2023 May; 14: 1192999.
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b) International conferences:

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1. Uday Kumar Mohanta, Rika Umemiya-Shirafuji, Xuenan Xuan. Molecular characterization of tick-borne selected pathogens in ticks and free-roaming dogs in Dhaka city, Bangladesh. 69th Joint Meeting of the Japanese Society of Parasitology and the Japanese Society of Medical Entomology and Zoology, Northern Japan Branch, Tohoku, Japan, October 14, 2023.
2. Thom Do, Bui Khanh Linh, Tawin Inpankaew, Eloiza May Galon, Ta Phuong Anh, Tran Khanh Trang, Tanjila Hasan, Shengwei Ji, Zhuowei Ma, Li Hang, Moaz M. Amer, Yihong Ma, Uday Kumar Mohanta, Iqra Zafar, Shima Abd El-Salam El-Sayed, Xuenan Xuan. Molecular detection, risk factors, and phylogenetic characteristics of canine tick-borne pathogens in northern Vietnam. 69th Joint Meeting of the Japanese Society of Parasitology and the Japanese Society of Medical Entomology and Zoology, Northern Japan Branch, Tohoku, Japan, October 14, 2023.
3. Zhuowei Ma, Ceylan Onur, Sevinc Ferda, Xuenan Xuan. Molecular Identification of Piroplasmids in Ticks from Infested Small Ruminants in Konya Province, Turkey. 69th Joint Meeting of the Japanese Society of Parasitology and the Japanese Society of Medical Entomology and Zoology, Northern Japan Branch, Tohoku, Japan, October 14, 2023.
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8. Hang L, Mingming Liu, Xuenan Xuan. Efficacy of cipargamin against *Babesia* parasites through targeted inhibition of the ATP4 ion pump. 69th Joint Meeting of the Japanese Society of Parasitology and the Japanese Society of Medical Entomology and Zoology, Northern Japan Branch, Tohoku, Japan, October 14, 2023.
9. Helena D. Janse van Rensburg, David D. N'Da, Keisuke Sukanuma. In vitro and in vivo trypanocidal efficacy of nitrofuryl- and nitrothienylazines. 69th Joint Meeting of the Japanese Society of Parasitology and the Japanese Society of Medical Entomology and Zoology, Northern Japan Branch, Tohoku, Japan, October 14, 2023.

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

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<https://www.obihiro.ac.jp/facility/protozoa/en/woah-reference-centres>

11. What have you done in the past year to advance your area of focus, e.g. updated technology?

We expanded our international collaborative networks and developed novel diagnostic tools for protozoan diseases. To accelerate research on animal protozoan diseases, we improved human resources in several WOA member countries by training young scientists. We have also initiated a drug discovery project aimed at developing novel therapeutics for managing protozoan diseases globally.

12. Additional comments regarding your report:

None