様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２２ 日現在

機関番号：１０１０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１４９６９

研究課題名（和文）Pathogenomics and eco-epidemiology of Mycobacterium orygis, an emerging zoonotic tuberculosis organism

研究課題名（英文）Pathogenomics and eco-epidemiology of Mycobacterium orygis, an emerging zoonotic tuberculosis organism

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交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：我々が行ったMycobacterium orygisの比較全ゲノム解析により、この菌が南アジアの風土病起因菌として分布しており、南アジア近隣地域ではMycobacterium orygis関連結核の脅威があることが明らかになった。ネパールのサイから分離されたMycobacterium orygisのゲノム解析により、Mycobacterium orygisに特有の変異があることが判明した。そこで、簡易診断法として RD301領域をターゲットとしたMycobacterium orygis特異的PCR法を開発した。更にはネパールにおける人獣共通感染症結核の生態疫学を理解するために調査を実施した。

研究成果の学術的意義や社会的意義
The research identified that the Mycobacterium orygis is an important causative agent of zoonotic tuberculosis (TB) in south Asia region, and there is a threat from Mycobacterium orygis-associated TB in south Asia and beyond.

研究成果の概要（英文）：Our comparative whole genome analysis of Mycobacterium orygis revealed that it is endemically distributed in South Asia, and there is a threat from Mycobacterium orygis-associated tuberculosis in South Asia and beyond. We did genome analysis of Mycobacterium orygis that was isolated from rhinoceros in Nepal, and the genome analysis showed that it had Mycobacterium orygis specific mutation in anti-SigK factor, which is responsible for higher production of mpt70 and mpt83. We found that Mycobacterium orygis isolates have specific polymorphisms in gyrB, mmpL6, PPE55, Rv2042c, TbD1. We developed a PCR diagnostic method targeting RD301 region that can specifically detect Mycobacterium orygis by differentiating it from other members of Mycobacterium tuberculosis complex. We performed molecular epidemiological surveillance to understand tuberculosis eco-epidemiology across the human/livestock/wildlife interface in Nepal.

研究分野：Veterinary Medicine

キーワード：Tuberculosis　Mycobacterium orygis　Pathogenomics　Molecular epidemiology　Nepal

１．研究開始当初の背景

Mycobacterium orygis, initially referred to as oryx bacillus or the antelope clade of *M. tuberculosis complex* (MTBC), was recognized as a sub-species of *M. tuberculosis* complex that can cause tuberculosis (TB) in animals and humans in 2012. Among the few known isolates at that time, most of them had an epidemiological link to South Asia and South-East Asia. From our previous studies, we discovered that *M. orygis* was the cause of death of an endangered rhinoceros in Chitwan National Park (CNP). We also found that different strains of *M. orygis* were circulating in CNP, and speculated that an unknown maintenance host of *M. orygis* exists in wildlife or livestock in and around CNP. Similarly, from our work in Bangladesh, we found that diverse genetic variants of *M. orygis* were circulating in livestock and captured monkeys. Thus, based on our studies and available information regarding *M. orygis*, we have hypothesized that *M. orygis* is endemically distributed in South Asia. Our hypothesis on the epidemiological link of *M. orygis* to South Asia has now been supported by many recent studies as they reported several cases of *M. orygis* in people and animal of South Asian countries and in people of South Asian origin in the USA, UK, and Europe.

TB is caused by different members of MTBC, for example, human TB is mostly caused by *M. tuberculosis* and the common cause of animal TB is *M. bovis*. Although these MTBCs bacteria are genetically 99.9% similar, they have specific host pathogenicity. Even within *M. tuberculosis*, different strains have strong geographic links for disease and drug resistance. *M. orygis* is considered animals adapted MTBCs but still has caused many cases of human TB with South Asian epidemiological links. The reasons for this unique phenomenon are unclear. In this perspective, whole genomic analysis, and eco-epidemiological studies on *M. orygis* will provide a unique opportunity to further understand how *M. orygis* has evolved and caused TB.

２．研究の目的

The main purpose of this study is to better understand the pathogenomics and eco-epidemiology of *M. orygis* as a model to understand its geographic distribution and its unique genetic features. We also aim to understand molecular epidemiology of *M. orygis* and zoonotic tuberculosis across the human/wildlife/livestock interface in Nepal to understand its zoonosis.

３．研究の方法

We performed the next generation sequencing of *M. orygis* isolate using Illumina MiSeq technology. Library preparation was performed using Nextera XT DNA library Preparation kit. Publicly available *M. orygis* and representative sequences of other members of MTBCs from SRA database were downloaded and analyzed using different bioinformatics softwares. Phylogeographic analysis was performed using the single nucleotide polymorphisms among genomes to understand the molecular epidemiology of *M. orygis*. We performed molecular epidemiological surveillance across the human/wildlife/livestock interface in Nepal. The obtained *M. tuberculosis* complex strains were analyzed by PCR, loop-mediated isothermal amplification (LAMP), and genotyping methods.

４．研究成果

1. Comparative whole genome analysis of *Mycobacterium orygis*

Single nucleotide polymorphism (SNP) based phylogenetic analysis of *M. orygis* isolates confirmed that the *M. orygis* strains are epidemiologically related to South Asia (Figure 1). A pair-wise SNP distance matrix analysis showed that there was 0 - 460 SNP difference among the globally available *M. orygis* isolates. When this SNP difference is compared with *M. tuberculosis*, it is very less, suggesting that the strain diversity is significantly lower. This phenomenon further confirms that the geographic focus area of *M. orygis* is narrow, perhaps specific to South Asia. We found that there were 0 – 6 SNP difference among some isolates in different clusters in animals in India, suggesting a close transmission *M. orygis* in those animals (Figure 1). All the *M. orygis* isolates that were isolated outside of South Asia had at least greater than 100 SNP difference. This finding suggests that *M. orygis* in those countries were uniquely imported, and no transmission of *M. orygis* had happened in those countries. The closest relative of *M. orygis* strain from Nepal was the strain that was isolated from a human in New York in USA, and there was a difference of 197 SNPs between these isolates. Our study concludes that *M. orygis* is endemic in South Asia and is an important causative agent of zoonotic tuberculosis in the region. Taken together, there is a threat of *M. orygis*-associated tuberculosis in South Asia and beyond.

Figure 1. Whole genome-based phylogeny of *Mycobacterium orygis* and others strains of *Mycobacterium tuberculosis* complex.

2. Genomic signatures specific in *Mycobacterium orygis*

We did genome analysis of *M. orygis* that was isolated from rhinoceros in Nepal, and the genome analysis showed that it had *M. orygis* specific mutation (G698C) in anti-SigK factor (Rv044c), which is responsible for higher production of mpt70 and mpt83. We confirmed that *M. orygis* isolates had specific polymorphisms in *gyrB, mmpL6*, TBD1, *PPE55*, and Rv2042c (Table 1).

Table 1. *Mycobacterium orygis* specific SNPs

	gyrB				*mmpL6*	TbD1	*PPE55*		Rv2042c
	756	1113	1410	1450	551	171	2162	2163	38
M. tuberculosis	G	G	C	G	C	C	T	C	T
M. bovis	A	G	T	T	G	C	T	C	T
M. bovis BCG	A	G	T	T	G	C	T	C	T
M. orygis	**G**	**A**	**C**	**T**	**G**	**G**	**G**	**T**	**G**

3. Development of *Mycobacterium orygis* specific rapid molecular diagnostic method

We targeted several *M. orygis* specific genetic regions such as RD12, RD301, and RD315 for developing rapid detection methods employing PCR and loop-mediated isothermal amplification method. Finally, we developed a PCR detection method targeting RD301 region that can specifically detect *M. orygis* by differentiating it from other members of MTBCs (Figure 2).

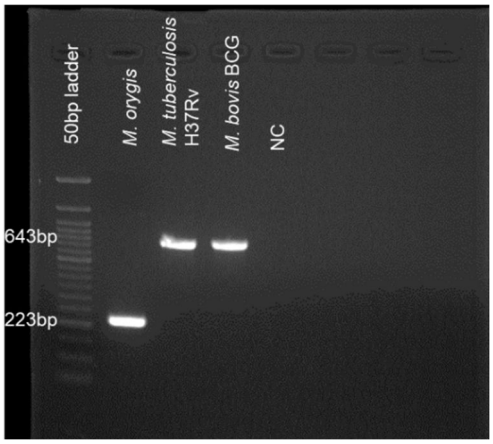

Figure 2. Development of RD301 based *M. orygis* specific PCR detection method.

4. Surveillance of zoonotic tuberculosis in Nepal

We performed surveillance of tuberculosis across the human/wildlife interface in Nepal. We developed a fecal mycobacterial assay that can be used to detect *M. tuberculosis* complex in wild animals by using PCR and LAMP methods. We were successful in detecting *M. tuberculosis* in elephants; however, we did not detect new strains of *M. orygis* during this study period. We will use the knowledge and skill developed during this study to continue our surveillance work on zoonotic tuberculosis in across the human/livestock/wildlife interface in Nepal and other countries.

５．主な発表論文等

〔雑誌論文〕　計15件（うち査読付論文　14件／うち国際共著　14件／うちオープンアクセス　12件）

１．著者名 Kapalamula Thoko Flav、Thapa Jeewan、Hayashida Kyoko、Chizimu Joseph、Tanomsridachchai Wimonrat、Nyenje Mirriam Ethel、Mkakosya Rajab、Nakajima Chie、Suzuki Yasuhiko	４．巻 35
２．論文標題 Direct detection of Mycobacterium bovis by a dry loop-mediated isothermal amplification assay in cattle samples collected during routine abattoir examination in Malawi	５．発行年 2023年
３．雑誌名 Journal of Veterinary Diagnostic Investigation	６．最初と最後の頁 307～310
掲載論文のDOI（デジタルオブジェクト識別子） 10.1177/10406387231164596	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Thapa Jeewan、Chizimu Joseph Yamweka、Kitamura Soyoka、Akapelwa Mwangala Lonah、Suwanthada Pondpan、Miura Nami、Toyting Jirachaya、Nishimura Tomoyasu、Hasegawa Naoki、Nishiuchi Yukiko、Gordon Stephen V.、Nakajima Chie、Suzuki Yasuhiko	４．巻 11
２．論文標題 Characterization of DNA Gyrase Activity and Elucidation of the Impact of Amino Acid Substitution in GyrA on Fluoroquinolone Resistance in Mycobacterium avium	５．発行年 2023年
３．雑誌名 Microbiology Spectrum	６．最初と最後の頁 e0508822
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/spectrum.05088-22	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Kapalamula Thoko Flav、Chizimu Joseph Yamweka、Akapelwa Mwangala Lonah、Barnes David Atomanyi、Toyting Jirachaya、Bwalya Precious、Basikolo Linda、Squarre David、Chambaro Herman M.、Gordon Stephen V.、Thapa Jeewan、Nakajima Chie、Suzuki Yasuhiko	４．巻 164
２．論文標題 Insight into the genetic diversity of Mycobacterium bovis isolated from cattle in Malawi	５．発行年 2023年
３．雑誌名 Research in Veterinary Science	６．最初と最後の頁 105030～105030
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.rvsc.2023.105030	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Damina Sadiq Mohammed、Barnes David Atomanyi、Inuwa Bitrus、Ularamu Gulak Hussaini、Bello Mohammed、Okaiyeto Olu Solomon、Kudi Ayuba Caleb、Thapa Jeewan、Nakajima Chie、Suzuki Yasuhiko	４．巻 45
２．論文標題 Molecular Characterisation of Mycobacterium bovis Isolates from Cattle Slaughtered in Adamawa and Gombe States, North-Eastern Nigeria	５．発行年 2023年
３．雑誌名 Current Issues in Molecular Biology	６．最初と最後の頁 6055～6066
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/cimb45070382	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Narongpun Pawarut、Chanchaithong Pattrarat、Yamagishi Junya、Thapa Jeewan、Nakajima Chie、Suzuki Yasuhiko	4．巻 12
2．論文標題 Whole-Genome Investigation of Zoonotic Transmission of Livestock-Associated Methicillin-Resistant Staphylococcus aureus Clonal Complex 398 Isolated from Pigs and Humans in Thailand	5．発行年 2023年
3．雑誌名 Antibiotics	6．最初と最後の頁 1745～1745
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/antibiotics12121745	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Toyting Jirachaya、Miura Nami、Utrarachkij Fuangfa、Tanomsridachchai Wimonrat、Belotindos Lawrence P.、Suwanthada Pondpan、Kapalamula Thoko Flav、Kongsoi Siriporn、Koide Kentaro、Kim Hyun、Thapa Jeewan、Nakajima Chie、Suzuki Yasuhiko	4．巻 11
2．論文標題 Exploration of the novel fluoroquinolones with high inhibitory effect against quinolone-resistant DNA gyrase of Salmonella Typhimurium	5．発行年 2023年
3．雑誌名 Microbiology Spectrum	6．最初と最後の頁 e0133023
掲載論文のDOI（デジタルオブジェクト識別子） 10.1128/spectrum.01330-23	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Suwanthada Pondpan、Kongsoi Siriporn、Miura Nami、Belotindos Lawrence P.、Piantham Chayada、Toyting Jirachaya、Akapelwa Mwangala L.、Pachanon Ruttana、Koide Kentaro、Kim Hyun、Thapa Jeewan、Nakajima Chie、Suzuki Yasuhiko	4．巻 29
2．論文標題 The Impact of Substitutions at Positions 1 and 8 of Fluoroquinolones on the Activity Against Mutant DNA Gyrases of Salmonella Typhimurium	5．発行年 2023年
3．雑誌名 Microbial Drug Resistance	6．最初と最後の頁 552～560
掲載論文のDOI（デジタルオブジェクト識別子） 10.1089/mdr.2023.0014	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Jeewan Thapa, Stephen V. Gordon, Chie Nakajima, Yasuhiko Suzuki	4．巻 3
2．論文標題 Threat from Mycobacterium orygis-associated tuberculosis in south Asia	5．発行年 2022年
3．雑誌名 The Lancet Microbe	6．最初と最後の頁 e641-e642
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/S2666-5247(22)00149-5	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Dipti Shrestha, Bhagwan Maharjan, Jeewan Thapa, Mwangala Lonah Akapelwa, Precious Bwalya, Joseph Yamweka Chizimu, Chie Nakajima, Yasuhiko Suzuki	4．巻 44
2．論文標題 Detection of Mutations in pncA in Mycobacterium tuberculosis Clinical Isolates from Nepal in Association with Pyrazinamide Resistance	5．発行年 2022年
3．雑誌名 Current Issues in Molecular Biology	6．最初と最後の頁 4132-4141
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/cimb44090283	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Thoko Flav Kapalamula, Francis Kawonga, Misheck Shawa, Joseph Chizimu, Jeewan Thapa, Mirriam Ethel Nyenje, Rajhab Sawasawa Mkakosya, Kyoko Hayashida, Stephen Gordon, Chie Nakajima, Musso Munyeme, Bernard M Hang'ombe, Yasuhiko Suzuki	4．巻 9
2．論文標題 Prevalence and risk factors of bovine tuberculosis in slaughtered cattle, Malawi	5．発行年 2023年
3．雑誌名 Heliyon	6．最初と最後の頁 e13647:1-7
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.heliyon.2023.e13647	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Jeewan Thapa, Susan K. Mikota, Kamal Prasad Gairhe, Sarad Paudel, Dinesh Kumar Singh, Ishwari Prasad Dhakal, Chie Nakajima, Yasuhiko Suzuki	4．巻 83
2．論文標題 Tuberculosis seroprevalence and comparison of hematology and biochemistry parameters between seropositive and seronegative captive Asian elephants of Nepal	5．発行年 2021年
3．雑誌名 Journal of Veterinary Medical Science	6．最初と最後の頁 1278-1283
掲載論文のDOI（デジタルオブジェクト識別子） 10.1292/jvms.21-0113	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Bhagwan Maharjan, Jeewan Thapa, Dhirendra Kumar Shah, Bhabana Shrestha, Korkut Avsar, Yasuhiko Suzuki, Chie Nakajima	4．巻 74
2．論文標題 Comparison of Xpert MTB/RIF to Microscopy and Culture for the Diagnosis of Tuberculosis in a Referral Laboratory in Nepal	5．発行年 2021年
3．雑誌名 Japanese Journal of Infectious Diseases	6．最初と最後の頁 517-521
掲載論文のDOI（デジタルオブジェクト識別子） 10.7883/yoken.JJID.2020.921	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Joseph Yamweka Chizimu, Eddie Samuneti Solo, Precious Bwalya, Thoko Flav Kapalamula, Mwangala Lohah Akapelwa, Partick Lungu, Dipti Shrestha, Yukari Fukushima, Victor Mukonka, Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki	4．巻 114
2．論文標題 Genetic Diversity and Transmission of Multidrug-Resistant Mycobacterium tuberculosis strains in Lusaka, Zambia	5．発行年 2022年
3．雑誌名 International Journal of Infectious Diseases	6．最初と最後の頁 142-150
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.ijid.2021.10.044	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Joseph Yamweka Chizimu, Eddie Samuneti Solo, Precious Bwalya, Wimonrat Tanomsridachchai, Herman Chambaro, Misheck Shawa, Thoko Flav Kapalamula, Partick Lungu, Yukari Fukushima, Victor Mukonka, Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki	4．巻 11
2．論文標題 Whole-Genome Sequencing Reveals Recent Transmission of Multidrug-Resistant Mycobacterium tuberculosis CAS1-Kili Strains in Lusaka, Zambia	5．発行年 2022年
3．雑誌名 Antibiotics (Basel)	6．最初と最後の頁 29
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/antibiotics11010029	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Precious Bwalya, Eddie S Solo, Joseph Y Chizimu, Dipti Shrestha, Grace Mbulo, Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki	4．巻 133
2．論文標題 Characterization of embB mutations involved in ethambutol resistance in multi-drug resistant Mycobacterium tuberculosis isolates in Zambia	5．発行年 2022年
3．雑誌名 Tuberculosis (Edinb)	6．最初と最後の頁 102184
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.tube.2022.102184	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計9件（うち招待講演　1件／うち国際学会　4件）

1．発表者名 Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki
2．発表標題 Threat from Mycobacterium orygis associated tuberculosis in South Asia
3．学会等名 The 16th Asian Society of Conservation Medicine（国際学会）
4．発表年 2023年

１．発表者名
Sasini Jayaweera, Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
WQ-3810: A novel fluorouinolone with a strong potential against fluoroquinolone-resistant Mycobacterium avium

３．学会等名
Sapporo summer Symposium for One Health (SaSSOH 2023)

４．発表年
2023年

１．発表者名
Arjun Pandit, Jeewan Thapa, Yasuhiko Suzuki, Chie Nakajima, Amir Sadaula, Shrestha Bijaya Kumar, Naresh Subedi, Susan Mikota, Michito Shimozuru, Bhawana Shrestha, Sarad Paudel, Baburam Lamichhane, Toshio Tsubota

２．発表標題
Surveillance of elephant tuberculosis in captive and wild elephants of Nepal

３．学会等名
Sapporo summer Symposium for One Health (SaSSOH 2023)

４．発表年
2023年

１．発表者名
Jeewan Thapa

２．発表標題
Mycobacterium orygis-associated tuberculosis

３．学会等名
U.S.-Japan Cooperative Medical Science Program, International Conference on Emerging Infectious Diseases（招待講演）

４．発表年
2022年

１．発表者名
Jeewan Thapa, Bhagwan Maharjan, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
Evaluation of rapid molecular diagnostic methods with microscopy and culture for the diagnosis of tuberculosis in Nepal

３．学会等名
ASM Microbe 2022（国際学会）

４．発表年
2022年

１．発表者名
Jeewan Thapa, Joseph Chizumu, Soyoka Kitamura, Mwangala Lonah Akapelwa, Pondpan Suwantada, Nami Miura, Jirachaya Toyting, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
Role of quinolone resistance-associated mutations in gyrA of Mycobacterium avium to the fluoroquinolone resistance

３．学会等名
第6回抗酸菌研究会

４．発表年
2022年

１．発表者名
Jeewan Thapa, Joseph Chizumu, Soyoka Kitamura, Mwangala Lonah Akapelwa, Pondpan Suwantada, Nami Miura, Jirachaya Toyting, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
Role of fluoroquinolone resistance-associated mutations in Mycobacterium avium gyrA to resistance

３．学会等名
96th Annual Meeting of Japanese Society of Bacteriology

４．発表年
2022年

１．発表者名
Jeewan Thapa, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
Mycobacterium orygis associated tuberculosis is an emerging threat for wildlife conservation in Nepal

３．学会等名
The 13th International Virtual Conference of Asian Society of Conservation Medicine (ASCM)（国際学会）

４．発表年
2021年

１．発表者名
Jeewan Thapa, Bhagwan Maharjan, Chie Nakajima, Yasuhiko Suzuki

２．発表標題
Development of methyl green dry loop-mediated isothermal amplification method for the simple detection of Mycobacterium tuberculosis in clinical samples

３．学会等名
World Microbe Forum 2021（国際学会）

４．発表年
2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関