Sequestration and Destruction of Rinderpest Virus–Containing Material 10 Years after Eradication

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In 2021, the world marked 10 years free from rinderpest. The United Nations Food and Agriculture Organization and World Organisation for Animal Health have since made great strides in consolidating, sequencing, and destroying stocks of rinderpest virus–containing material, currently kept by only 14 known institutions. This progress must continue.

In 2011, ten years after the last confirmed outbreak, the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (WOAH, formerly OIE) jointly declared global freedom from rinderpest. Rinderpest, also known as cattle plague, is only the second infectious disease eradicated from the world, smallpox being the first. Over the 10 years since eradication, the main goal of the Rinderpest Post-Eradication Programme (https://www.woah.org/en/disease/rinderpest) has been to track and reduce global stocks of rinderpest virus–containing material (RVCM).

RVCM comprises field and laboratory strains of rinderpest virus; vaccine strains of rinderpest virus, including valid and expired vaccine stocks; tissues, serum, and other clinical material from infected or suspect animals; diagnostic material containing or encoding live virus; recombinant morbilliviruses (segmented or nonsegmented) containing unique rinderpest virus nucleic acid or amino acid sequences; and full-length genomic material, including from virus RNA and cDNA copies of virus RNA. Subgenomic fragments of morbillivirus nucleic acid not capable of incorporation into a replicating morbillivirus or morbillivirus-like viruses are not considered RVCM.

Accounting for remaining RVCM is critical to limit the risk for reintroducing the pathogen by intentional or inadvertent release from a laboratory (*1*). In support of this effort, in 2015, FAO and WOAH started the Sequence and Destroy project, which enabled whole-genome sequencing of rinderpest virus (RPV) isolates before their destruction. Participating institutes were expected to deposit the genome sequences into publicly accessible databases. In addition, FAO has provided hands-on assistance and remote support to destroy viral stocks in Africa and Asia and led organization of >5 global and regional advocacy meetings. During June–October 2021, a review was conducted to mark progress towards RVCM sequestration and destruction 10 years after eradication. We report the main findings of this review.

The Study

In 2011, a total of 150 countries were surveyed regarding their RVCM stocks (*2*). At that time, 35 countries (44 laboratories) reported keeping RVCM. In 2013, WOAH began annual surveys of institutes keeping RVCM. In 2013, a total of 23 countries reported keeping RVCM; 13 kept live virulent virus and 19 live-attenuated virus in the form of vaccine (n = 17) or seedstock (n = 17), and 9 countries kept both virulent virus and vaccine. Because FAO and WOAH worked with members to eliminate or transfer RVCM stocks, the number of countries keeping RVCM had decreased to 12 (14 institutes) as of 2021 (Figure). In addition, FAO/WOAH designated some of these institutes either

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category A or B or dual-category rinderpest-holding facilities (RHFs) (https://www.oie.int/en/disease/rinderpest/#ui-id-3). Category A RHFs are designated for storing RVCM, excluding vaccine stocks; category B RHFs are approved for storing only manufactured vaccines and materials for their production.

To confirm that no relevant findings unknown to WOAH had been published by research groups or laboratories, we reviewed the scientific literature to identify any publications about rinderpest virus research undertaken since 2011. A search of 21 databases identified 623 unique publications of which we evaluated 17 at the full-text level (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/9/22-0297-App1.pdf). The search identified no institutes conducting work with RVCM not already known to WOAH. Nine (53%) of 17 reviewed studies were conducted in facilities that are FAO/WOAH-designated RHFs; 4/17 were published in 2011. Besides genome sequencing data, the main finding from recent research was that vaccination of cattle with peste des petits ruminants virus (PPRV) does not provide protective immunity against RPV (*3*), leading to the decision to maintain and even expand global contingency stocks of RPV vaccine (Appendix Table2).

Members of the study team contacted a representative from each institute known by WOAH to keep RVCM as of August 2021 to arrange an interview to discuss current and historic RVCM stocks and laboratory biosecurity. Interviews were conducted remotely and accompanied by completion of a structured questionnaire. All institutes keeping RVCM, except for 1 located in the Middle East, responded to the request for an interview. Because of logistical difficulties and COVID-19–related challenges, interviews were not conducted with institutes in 2 countries in Europe. Therefore, during August 8–September 17, 2021, interviews proceeded with 11 of the 14 institutes known to keep RVCM. Several of the institute directors contacted were not familiar with the specific content of their RVCM stocks and indicated that these materials were simply in

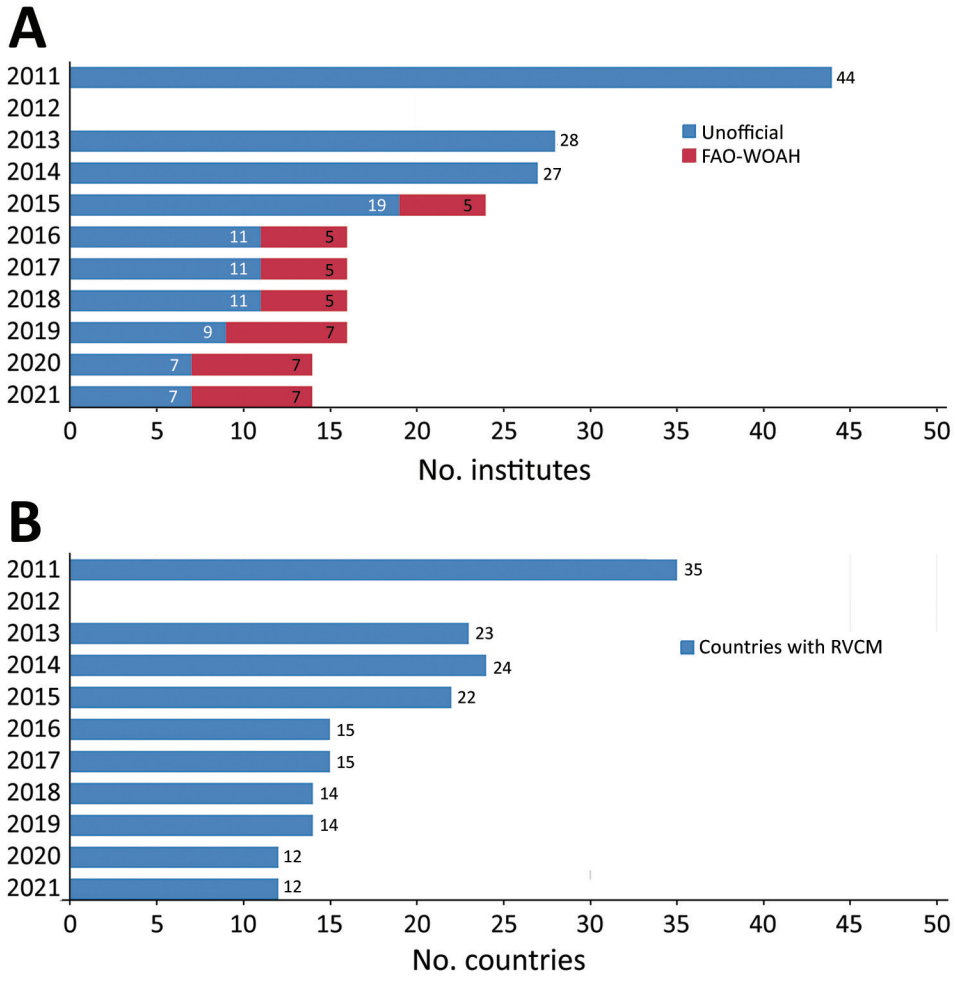

Figure. Number of institutes (A) and countries (B) keeping RVCM, by year, 2011 and 2013–2021. Data from 2011 are based on a single study, whereas data for 2013–2021 are based on WOAH country reports and institute director interviews (2021). FAO, Food and Agriculture Organization of the United Nations; RVCM, rinderpest virus–containing material; WOAH, World Organisation for Animal Health.

Table. Regional institutes with rinderpest virus–containing material, by biosafety level and type, for 2013 and 2021*

Category	Africa	Asia, Far East, and Oceania	Europe	Middle East	The Americas	World
Biologic safety level						
2	3/0	1/2	0/0	1/1	1/0	6/3
2+	0/0	0/1	0/0	0/0	0/0	0/1
3	4/1	9/3	3/3	0/0	2/1	18/8
3+	0/0	0/0	1/1	0/0	0/0	1/1
4	0/1	0/0	2/0	0/0	0/0	2/1
Unknown	1/0	0/0	0/0	0/0	0/0	1/0
Type						
A	0/0	0/1	0/1	0/0	0/1	0/3
B	0/0	0/1	0/0	0/0	0/0	0/1
A/B	0/1	0/1	0/1	0/0	0/0	0/3
Unofficial	8/1	10/3	6/2	1/1	3/0	28/7
Overall	8/2	10/6	6/4	1/1	3/1	28/14

*Values are given as 2013/2021 numbers.

storage, which is concerning because of the critical nature of these materials. At present, Africa is the only region actively attempting to consolidate its RVCM into a single facility.

According to Resolution 18, passed in 2011 during the 79th general session of the World Assembly of WOAH Delegates: "Rinderpest virus-containing material that is not in an approved BSL3 [Biosecurity Level 3] facility shall be destroyed by a validated process or transferred to an approved BSL3 facility." Biosecurity levels for institutes keeping RVCM during 2011–2021 ranged from BSL2 to BSL4 (Table) meaning some institutes still do not meet this requirement; continued efforts are therefore needed. Three category B RHFs have actively contributed to the global rinderpest vaccine reserve. One institute in Europe keeps a rinderpest RBOK (Muguga-modification of the Kabate-0-strain) vaccine seed bank sufficient to produce ≈800,000 doses. One institute in the WOAH Asia and the Pacific region biannually produces a total reserve of ≈772,000 doses of LA-AKO (master seed virus) strain vaccine. One institute in Africa has a historic reserve of ≈959,000 doses of RBOK vaccine. Three RHFs have participated in sequence and destroy projects, and 2 more have initiated the approval process for sequence and destroy projects from the FAO/WOAH rinderpest secretariat.

During recent genomic analysis of PPRV isolates held at an FAO/WOAH–designated RHF, 1 sample was found to contain a sequence that aligned with RPV in addition to PPRV sequences. A traceback investigation found that this sample, obtained from the field by another institute in the early 1970s, appears to have been destroyed. All materials derived from the original stock before the contaminated sample was identified were uncontaminated, but those derived from the contaminated stock were RPV-contaminated, so contamination appears to have occurred inside the institute, from an unknown source, but likely during a period in the late 1990s when both RPV and PPRV were being manipulated concurrently at the institute. All contaminated samples were destroyed. After examining all other samples being manipulated during the same period, the institute concluded that no others were contaminated. Records indicated that the institute had not shared this sample with other facilities and that it would thereafter screen all PPRV samples by PCR for RPV before sharing them. No contaminated samples had escaped containment and all processes to secure stocks appeared to be working well. Risks associated with remaining global stocks are being evaluated and will be presented in a future publication.

Conclusions

We document discovery of RPV-contaminated PPRV samples; our findings suggest that because of risk for cross-contamination, other laboratories should take precautions with samples manipulated alongside RPV, especially PPRV. Although progress is being made in consolidating RVCM stocks, 2 of 6 nonapproved institutes known to keep RVCM stockpiles have indicated no plans to destroy or transfer them to an FAO/WOAH RHF. Therefore, in spite of the progress, much work remains. Current FAO/WOAH strategy is to continue removing RVCM from nonapproved laboratories and advocating for reduced RVCM stocks in FAO/WOAH-designated RHFs. Ultimately, the only remaining RCVM materials should be manufactured vaccines and materials for vaccine production and diagnostics.

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Appendix

Appendix Table 1. Search terms used in the literature review to identify publications related to rinderpest virus research undertaken since 2011, among 21 databases searched

Search terms	Results
Global Health	
1910–2021 week 26	
exp Rinderpest morbillivirus/ or exp rinderpest/ or rinderpest*.ti,ab	432
Limited to 2011–2022	81
EMBASE	
OVID interface	
1974–2021 June 30	
exp Rinderpest virus/ or exp rinderpest/ or exp Cattle plague virus/ or rinderpest*.ti,ab.	829
Limited to 2011–2022	192
CAB Abstracts	
OVID interface	
1910–2021 Week 26	
exp Rinderpest morbillivirus/ or exp rinderpest/ or rinderpest*.ti,ab.	4,340
Limited to 2011–2022	274
International Pharmaceutical Abstracts	
OVID interface	
1970-June 2021	
Rinderpest*.ti,ab.	1
JBI EBP	
OVID interface	
current to June 23, 2021	
Rinderpest*.ti,ab.	1
Northern Light Life Sciences Conference Abstracts	
OVID interface	
2010–2021 Week 25	
Rinderpest*.ti,ab.	6
Limited to 2011–2022	5
MEDLINE	
OVID interface	
OVID interface	
OVID interface exp Rinderpest virus/ or exp Rinderpest/ or rinderpest*.ti,ab.	1,186
Limited to 2011–2022	162
CINAHL Complete	
EBSCOhost interface	
rinderpest* in Title OR rinderpest* in Abstract OR rinderpest* as a word in subject heading	14
Limited to 01/01/2011–12/31/2021	7
Web of Science Collection	
Databases searched simultaneously via Web of Science interface	
Science Citation Index- 1900-present	
Social Science Citation Index 1900-present	
Arts & Humanities Citation Index 1975-present	
Conference Proceedings Citation Index-Science 1990-present	
Conference Proceedings Citation Index-Social Science & Humanities 1900-present	
Book Citation Index-Science 2005-present	
Book Citation Index-Social Science & Humanities 2005-present	
Emerging Sources Citation Index 2005-present	
Current Chemical Reactions 1985-present	
Index Chemicus 1993-present	
TI = (rinderpest*) OR AB = (rinderpest*) OR TS = (rinderpest)	

Search terms	Results
Limited to 2011–2021	383
ABI INFORM ProQuest interface ab(rinderpest*) or ti(rinderpest*) or su(rinderpest*) Limited to 2011–2021	32
Academic Search Ultimate EBSCOhost interface Title = rinderpest* OR Subject Terms = rinderpest* OR Abstract = rinderpest* Limited to 01/01/2011–12/31/2021	176
NewsBank NewsBank interface Rinderpest in Headline OR rinderpest in Lead/First paragraph Limited to 2011–2021	274

Appendix Table 2. Summary of studies involving rinderpest virus published between 2011 and 2021.

Study category	Summary	Lab location	Reference
Vaccine development/ Immunity	The crystal structure of MHC 1 N*01801 complexed to rinderpest derived peptide IPA was evaluated and analysis revealed that the MHC groove can assume different conformations to bind with the rinderpest viral peptide.	Beijing, China	*1*
	RPV was cultured by utilizing a vaccine strain of the virus and it was mutated based on potential C1 monoclonal antibody binding sites. After characterizing C1 binding sites, it was discovered that the deletion or mutation of these sites resulted in C1 not being able to bind to RPV.	Pirbright, United Kingdom *	*2*
	Cattle were vaccinated with either wild-type or two established PPRV vaccine strains to determine the degree of protection to which individuals vaccinated for PPR have against RPV. Only animals vaccinated with the wild-type PPRV were protected from RPV challenge. These individuals were also able to neutralize RPV-pseudotyped vesicular stomatitis virus.	Pirbright, United Kingdom*,‡	*3*
Diagnostics	The goal of this study was to develop a one-step multiplex reverse transcription PCR assay for the simultaneous detection of Rift Valley Fever Virus, Bluetongue Virus, RPV, and PPRV. Dual- Priming Oligonucleotide was used to develop the diagnostic test for the four viruses.	Anyang, Republic of Korea	*4*
	This study used a replication-defective vesicular stomatitis virus based pseudotyping system to measure neutralizing antibodies against RPV and PPR. This system does not require the use of live infectious viral materials and thus mitigates the risk of accidental exposure. Analysis revealed that individuals vaccinated for RPV also are protected against PPR infection. Individuals that were vaccinated against PPR had lower antibody titers than those who were naturally infected and in individuals infected with either PPR or RPV neutralizing responses were highest against the homologous virus. This indicates that retrospective analysis of serologic samples can be used to determine the pathogen to which an infected individual was exposed.	Pirbright, United Kingdom*,‡	*5*
	This study focuses on the development of a multiplex RT-PCR and automated microarray assay for the simultaneous detection of eight important cattle viruses: vesicular stomatitis virus, bluetongue virus, bovine viral diarrhea virus type 1 and 2, malignant catarrhal fever virus, bovine herpesvirus-1, parapox virus complex, and RPV.	Pirbright, United Kingdom*,‡	*6*
	Because of the request to destroy all RPV samples following eradication a new diagnostic method must be developed that does not rely on RPV as a positive material. Newcastle Disease with small RNA inserts based on RPV or PPV was used as a positive control for extraction, reverse transcription, and amplification.	Lelystad, Netherlands†	*7*
Enzyme activity	The V proteins of RPV, measles virus, PPR, and canine distemper were compared to determine which had the ability to block type 1 and type 2 interferon action. Analysis revealed that the V proteins of each morbillivirus could block type 1 interferon action but they had varying abilities to block type 2 interferon action which is correlated with the co-precipitation of STAT1 with the V protein. Further analysis revealed that all morbillivirus V proteins form a complex with Tyk2 and Jak2, two interferon-receptor-associated kinases.	Pirbright, UK*	*8*
	The enzymatic role of RPV V protein was investigated to determine how it blocks interferon signaling. Analysis revealed that the morbillivirus V proteins have at least three functions that inhibit interferon signaling, the binding of STAT1 also seen with P and W proteins) which enables the blockade of type 2 interferon signaling, the binding of STAT1 which requires the Vs domain and	Pirbright, United Kingdom*	*9*

Study category	Summary	Lab location	Reference
	part of the W domain, and the association with interferon receptor-associated kinases which also requires the Vs domain.		
	Partially purified recombinant RNA polymerase complex of RPV was used to show in vitro methylation of capped mRNA. Analysis revealed that the catalytic module for cap 0 methyl transferase activity is located in domain 3 of the L protein whereas domain 2 stabilizes the enzyme and increases catalytic efficiency. This provides support for the modular nation of the RPV L protein.	Bangalore, India§	*10*
	E. coli was used to express the RTPase domain of RPV to investigate the RTPase activity of L protein. Analysis revealed that L protein exhibits RTPase and NTPase activities and that it has a two-metal mechanism similar to the RTPase domain of other viruses.	Bangalore, India§	*11*
	E. coli was used to express the RTPase domain of RPV to investigate its enzymatic abilities. Analysis revealed that the L protein of RPV has RNA-dependent RNA polymerase, RTPase, Guanylyltransferase (GTase), and Methyltransferase activity in addition to pyrophosphatase (Ppase) and tripolyphosphatase (PPPase) activity.	Bangalore, India§	*12*
Genome sequencing	The B and L strains of RPV were sequenced to investigate host range and virulence factors. The stock B strain is pathogenic to cattle whereas the L strain is pathogenic to rabbits but not cattle and buffalo. Analysis revealed that differences in pathogenicity to cattle is caused by nt/aa substitution in P/C/V genes.	Tokyo, Japan*	*13*
	The LATC06 strain of RPV was sequenced and compared to other rinderpest viral strains. Analysis revealed that the functions of the LATC06 (Korea) and LA (Japan) strains of RPV are similar with regards to immunodominance in humoral immunity.	Anyang, Korea	*14*
	The genomes of three strains of RPV, L72, LA77, and LA96, were sequenced and analyzed to investigate their genetic variability. Analysis revealed that genetic variability occurs within the vaccine virus strain and that amino acid sequence similarity between Fusan and other strains was the lowest within the P, C, and V proteins. This indicated that the difference in pathogenicity of different strains may be Because of the V protein.	Anyang, Korea	*15*
	The LA-AKO strain of the RPV vaccine was sequenced. Analysis revealed that the bulk vaccine comprises mixed viral populations with minor mutations at the nucleotide level.	Ibaraki, Japan*,‡	*16*
	In preparation for the destruction of all RPV samples, the full genome sequence was determined of each distinct RPV sample housed at Pirbright. Analysis revealed that the African isolates form a single disparate clade as opposed to two separate clades and that the clade containing viruses developed in Korea were more similar to African viruses than Asian viruses.	Pirbright, United Kingdom*,‡	*17*

*Conducted in association with a current FAO-WOAH designated RHF
†Presented research conducted before 2011
‡Supported by the FAO-WOAH Joint Advisory Committee for Rinderpest.
§Rinderpest virus containing material (RVCM) was not used in these studies.

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