WHITE OAK CONSOLIDATED ANIMAL PROGRAM
FOOD AND DRUG ADMINISTRATION

ANIMAL STUDY PROPOSAL

Animal Biosafety Level: ABSL2
as of 11/1/2024 at 01:41:41 PM

Protocol #	2009-19
Date submitted	
Approval Date	06/12/2024
Expiration Date	06/12/2027

A. ADMINISTRATIVE DATA

Protocol Title

Non-human Primate Model of Pertussis Infection and Transmission

Lead Scientist: (b) (6)

Center: CBER Office/Division/Lab: OVRR/ (b) (6)

Identify all individuals authorized to conduct procedures involving animals under this proposal:

Name	Center	O/D/L		Emergency Contact (at least 2)	
(b) (6)	CBER	OVRR/	(b) (6)	Yes	1st Phone: (b) (6) 2nd Phone: (b) (6) 3rd Phone: (b) (6)
(b) (6)	CBER	OVRR/	(b) (6)	Yes	1st Phone: (b) (6) 2nd Phone: (b) (6)
(b) (6)	CBER	OVRR	(b) (6)	No	
(b) (6)	CBER	OVRR/	(b) (6)	No	
(b) (6)	CBER	OVRR/	(b) (6)	No	

Note that all authorized personnel must enroll in the WO Animal Exposure Program and complete the online WOC ACUC Animal User Training course. The PI is responsible for ensuring that all authorized personnel are competent to perform the animal work outlined in this protocol.

B. ANIMAL REQUIREMENTS

Species and Stock or Strain:

Species	Stock or Strain
Primate	Baboon

Age/Weight: 4 months to 8 years
Sex: MF
Source(s): Southwest National Primate Research Center (SNPRC), MD Anderson Keeling Center and Mannheimer Foundation

Total Number of Animals (automatically populated from Section E): 48

C. TRANSPORTATION

Transportation of animals must conform to all institutional guidelines/policies and federal regulations.

Will live animals be removed from main vivarium or utilized in the ABSL3 satellite corridor? No

D. STUDY OBJECTIVES (understandable to a non-scientist)

Briefly explain in non-scientific terms the aim of the study, why the study is important to FDA's mission, and the benefit the study will have on society.

Pertussis is a highly contagious respiratory disease. Despite very high vaccination rates, the incidence of pertussis in the US has increased steadily over the last thirty years. We still do not know which types of immune responses are required to protect against pertussis infection. It is clear the currently licensed vaccines protect against disease however these vaccines do not prevent infection, carriage and transmission. The continued circulation of B. pertussis in the population results in exposure of the most vulnerable members of the population: unvaccinated individuals, infants too young to be vaccinated and individuals with waning immunity. New vaccines are required to respond to the increase in pertussis in the U.S.

Until recently, there has not been an animal model that reproduces pertussis disease seen in humans. The baboon model of pertussis closely mimics clinical pertussis in humans, including the development of paroxysmal cough and the demonstration of host-to-host transmission. The baboon model provides a powerful tool for studying pertussis disease and developing and testing vaccines and therapeutics. Data from our studies will allow us to develop improved vaccines by identifying the immune responses needed to protect from infection and identifying the proteins on the surface of the bacterial cell that make the best targets for new vaccines. Our ongoing goal is to use the baboon model of pertussis to improve our understanding of disease and vaccine-mediated immunity, and to test next generation pertussis vaccines.

This work will aid in the development and introduction of next generation pertussis vaccines that will provide better control of circulation of pertussis in the U.S. population reducing the number of pertussis cases in vulnerable individuals.'

The knowledge gained from these studies will allow the FDA to guide new pertussis vaccine development make more informed decisions regarding vaccine testing and approval.

E. RATIONALE FOR USE OF ANIMALS

① Briefly explain your rationale for animal use.

The development of any vaccine requires an understanding of the disease pathogenesis and the mechanisms of protection. These studies cannot be undertaken in humans due to the severity and duration of the disease and the associated risks to subjects and the risk of transmission to subject contacts. In vitro models cannot recapitulate the complex interactions between B. pertussis and host tissues in the airway or host immune responses. Therefore,

these studies require the use of an adequate animal model that accurately reflects human disease and immunity.

② Justify the appropriateness of the species selected. Additionally, please justify the use of single sex/gender animals if applicable, e.g., experiments utilizing all males or all females.

Animal studies have been used to study the pathogenesis of pertussis however only the baboon model developed under this protocol adequately recapitulates the full spectrum of the disease observed in humans. Significant effort has been made to reproduce human-like pertussis in rabbits, guinea pigs, rats and mice. Although disease in mice infected with B. pertussis resembles the human disease in several aspects: higher susceptibility of infant mice than adult mice to infection, similar period of pulmonary infection and manifestations of pertussis toxin action, such as hypoglycemia, leukocytosis and histamine sensitization, mice do not display cough and fail to transmit the disease to other mice. Another animal model for pertussis is the newborn piglet. Infected piglets at 1–4 weeks of age exhibit a wide range of clinical symptoms, including hypoglycemia, lymphocytosis, nasal discharge, breathing difficulties, non-paroxysmal cough, bronchopneumonia and weight retardation. However, infected piglets do not transmit disease. Rabbits become colonized with B. pertussis but the colonization is asymptomatic and transmission has never been observed. The baboon model of pertussis offers the best opportunity for the study of pertussis infection, clinical disease and transmission. Baboons are the only animals demonstrated to develop the characteristic symptoms of human pertussis: paroxysmal cough and mucus production. In addition, baboons develop lymphocytosis and transmit the disease, both important features of the disease in humans.

③Ⓐ Account for the total number of animals to be used by briefly outlining all treatment groups and the number of animals per group. A rationale for the latter must be provided, regardless of group size. For help in justifying group size, please refer to the Animal Study Proposal Guideline for Animal Numbers Justification. Detailed experiments and animal procedures should not be included in this section. These details should be provided in Sections F1 to F9. If breeding is included in this ASP, the total number of animals needed for breeding (including replacement breeders) as well as the total number of unusable pups will be populated in the table below from the information provided in Section F10.

A statistical analysis to justify numbers of animals is not practical because the magnitude of the difference in outcomes between groups is unknown. It is desirable to use as few baboons as possible to reach a scientifically valid conclusion. In most studies four to six animals will be used per group. Four animals is the smallest number likely to provide statistically significant results assuming a large difference between groups. Six animals per group provides a greater probability of success if the differences are smaller. The number of animals to be used in each project is outlined below:

EXPERIMENT ONE: EVALUATION OF VACCINES ADJUVANTED TO DRIVE TH1, TH2, AND/OR TH17 RESPONSES (ADJUVANT STUDY)
Immunogenicity studies in mice and baboons has enabled the identification of adjuvants that drive primarily TH1, primarily TH2 or primarily TH17 responses when formulated with the pertussis antigens PT, FIM and PRN. In this study, which was approved and initiated in the preceding three-year period, baboons vaccinated with these adjuvants will be challenged in order to determine the degree to which pertussis-specific TH1, TH2 or TH17 responses contribute to clearance of B. pertussis following exposure. This information will enable development of pertussis vaccines designed to prevent colonization and carriage of B.

pertussis following exposure.

Groups:
6 baboons vaccinated with PT, FIM and PRN + alum
(b) (4), (b) (5)
6 baboons vaccinated with PT, FIM and PRN +
6 baboons vaccinated with PT, FIM and PRN +
6 baboons vaccinated with PT, FIM and PRN +
6 baboons vaccinated with PT, FIM and PRN +
6 baboons vaccinated with PT, FIM and PRN +
6 unvaccinated baboons

A total of 42 baboons were required for this study

In the preceding three-year period, 23 animals were vaccinated twice, two months apart at the baboon breeding facility under ASP approved by the facility's IACUC prior to delivery to CBER. These 23 animals were vaccinated a third time following delivery to CBER. Blood was drawn from these 23 vaccinated animals and 3 unvaccinated control animals 7 and 14 days post-third vaccination to evaluate vaccine-induced T-cell responses. For the remaining 13 animals, vaccinations were performed three times, two months apart, following delivery to CBER. Blood was drawn from these 13 animals and 3 additional unvaccinated control animals 7 and 14 days post-third vaccination to evaluate vaccine-induced T-cell responses.

NOTES ON ANIMAL NUMBERS:
Due to the logistical difficulty of challenging and collecting samples from 42 animals, the animals in this study were divided into three cohorts. This study was approved and initiated in the previous three years. The first two cohorts, totaling 26 animals, were vaccinated and challenged prior to this renewal. Those 26 animals were returned to the breeding facility and are no longer at CBER. The third cohort is at CBER and will complete vaccination prior to renewal of this ASP. This cohort of 16 animals (13 vaccinated and 3 unvaccinated) will be challenged in the upcoming 6 months.

Therefore, to complete this study, 16 animals are required in this three-year study period. These 16 animals were approved and delivered to CBER in the previous three year period.

EXPERIMENT TWO: CONTINUATION OF EVALUATION OF BURKHOLDERIA PSEUDOMALLEI OUTER MEMBRANE VESICLES (OMVs) AS AN ADJUVANT FOR PERTUSSIS VACCINES.
In a previous study, we evaluated the ability of B. pseudomallei OMVs to adjuvant B. pertussis vaccines. We evaluated the OMV-adjuvanted acellular pertussis vaccine (OMV-aP) by both the IM and IN routes and compared them to the acellular vaccine and the whole cell vaccine. We found that the OMV-aP vaccine, delivered by the IN route, conferred better protection than the aP vaccine and protection comparable to the whole cell vaccine. This is the first example of an acellular pertussis vaccine providing better protection than the currently licensed aP vaccine in the baboon model. In this follow-up study, we will examine the reproducibility of that result and increase the statistical power. We will focus on the IN route of delivery for the OMV-aP vaccine and compare it to the licensed aP vaccine which represents the current standard of care.

6 unvaccinated baboons
6 baboons vaccinated with licensed aP vaccine (Daptacel®) by the IM route
6 baboons vaccinated with OMV-aP by the IN route

A total of 18 baboons will be required for this study.

NOTE: This study was approved and initiated in the previous three year period. These 18 animals are at CBER and have completed vaccination. These animals will be challenged in the upcoming year.

EXPERIMENT THREE: EVALUATION OF PROTECTION CONFERRED BY ANTI-PT HUMAN MONOCLONAL ANTIBODY (mAb).
In this pilot study we will evaluate the ability of the human monoclonal antibodies (mAbs) (b) (4), (b) (5) and (b) (4), (b) (5) to protect against disease in the baboon model. (b) (4), (b) (5) and (b) (4), (b) (5) recognize different epitopes on pertussis toxin and each has been shown to potently neutralize pertussis toxin in vitro. In this pilot study 4 animals will be administered a cocktail of (b) (4), (b) (5) and (b) (4), (b) (5) 24 hours prior to challenge with B. pertussis strain D420. The effectiveness with which the mAb cocktail prevents signs of disease will be evaluated. If the mAbs confer protection, it will support the hypothesis that antibodies against pertussis toxin are sufficient to confer protection and will support the further development of these mAbs as therapeutics for pertussis.
The four animals in the mAb group will be challenged at the same time as the animals in the final cohort of Study 1. This will allow the use of the unvaccinated control group from study one as the untreated control group for this study, thereby reducing the total number of animals required. Four animals will be required for this study.
NOTE: This study was approved and initiated in the previous three year period. These animals are at CBER and awaiting challenge.

REPLACEMENT OF B. BRONCHISEPTICA POSITIVE ANIMALS:
The 22 animals required for Experiments 2 and 3 were delivered to CBER in the previous three year period. 10 of the 22 animals tested positive for Bordetella bronchiseptica. Although asymptomatic infection with B. bronchiseptica is common in baboons, it is an exclusion criteria for our studies. These 10 animals will be returned to the breeding facility and replaced with 10 B. bronchiseptica-free animals.

Ⓑ Summarize the information in the text box above by completing the table below. Indicate in the far right columns the distribution of these animals by USDA Pain and Distress Category.

Experiment Number	Number of Groups	Number of Animals per Group	Number of Animals for This Experiment	Number of Times Performed	Total	Number of Animals by USDA Pain and Distress Category		
						C	D	E
1 (on site)	1	16	16	1	16	0	16	0
2 (on site)	3	6	18	1	18	0	18	0
3 (on site)	1	4	4	1	4	0	4	0
Replacement	1	10	10	1	10	0	10	0

TOTAL:	48	0	48	0

Ⓒ Animals are indicated in USDA Pain and Distress Category D or E. You must conduct a literature search for alternatives to procedures described herein which may cause more than momentary pain or distress. At least two literature databases must be queried. Identify the databases searched, the time period covered by the search, keywords used, and the dates the searches were performed. For additional guidance, please refer to the ASP form instructions.

Database Searched	Dates Covered in the Search	Keywords Related to the Painful Procedure Performed	Date Search was Performed
PubMed	April, 2014 to April, 2024	Bordetella, Vaccine, Model, Non-human primate, Baboon, Alternatives and Transmission	04/23/2024
AGRICOLA	April, 2014 to April, 2024	Bordetella, Vaccine, Model, Non-human Primate, Baboon, Alternatives and Transmission	04/23/2024

Ⓓ Animals are indicated in USDA Pain and Distress Category D or E. Briefly describe if alternatives to painful procedures exist and why they are not being used in the protocol. Consideration must be given to alternative procedures, refinement of current procedures, and reduction in the number of animals used. This information will accompany the Annual Report to the USDA and is subject to the Freedom of Information Act.

To investigate mechanisms of pathogenesis and the impact of vaccines on disease outcome, it is necessary to allow animals to progress through pertussis disease and as a result, animals experience typical pertussis signs of disease. Baboons typically tolerate the infection and return to good health following a cough illness lasting 2-4 weeks. Many of the animals in treatment groups do not experience signs of disease. All animals undergo minimally-invasive procedures including blood draws and nasal swabs and washes. These procedures are performed under anesthesia and there are no alternatives.

F. DESCRIPTION OF EXPERIMENTAL DESIGN & ANIMAL PROCEDURES

Explain the experimental design and specify all animal procedures. This description should allow the ACUC to understand the experimental course of an animal from its entry into the facility until the final disposition.

① Briefly outline the study design. Incorporate all previous amendments into the body of this section. Detailed justification of animal numbers should not be included in this section; these details should be provided in Section E3.

DELIVERY AND QUARANTINE:
Two nasopharyngeal washes will be performed and blood will be collected during quarantine to screen for B. bronchiseptica infection and to establish baseline values. These washes will be performed when

animals are sedated for performance of routine TB tests.

EXPERIMENT ONE: ADJUVANT STUDY
The 13 vaccinated animals in the final cohort will receive their third vaccination in July and will be bled for serology and cellular responses on days 7 and 14 post vaccination. Approximately one month following vaccination, the 13 vaccinated animals and three, unvaccinated control animals, will be challenged with B. pertussis. Following challenge, samples will be collected twice per week: Nasal wash samples will be collected to quantify bacterial colonization and mucosal antibodies. Nasal swabs will be collected to obtain host mucosal cells for analysis. Blood will be collected for CBCs, analysis of immune responses in peripheral mononuclear cells, and for serology. The animals will be followed until two-consecutive negative culture samples are collected or no longer than ten weeks.

EXPERIMENT TWO: OMV-aP STUDY
The 12 vaccinated animals in this study received their three vaccinations in the previous three year period.
The six unvaccinated control animals were determined to be B. bronchiseptica-positive upon quarantine entry testing. Those six animals will be returned to the breeding facility and six B. bronchiseptica-free animals will be delivered to replace them.

The 12 vaccinated animals and six unvaccinated control animals will be challenged with B. pertussis. Following challenge, samples will be collected twice per week: Nasal wash samples will be collected to quantify bacterial colonization and mucosal antibodies. Nasal swabs will be collected to obtain host mucosal cells for analysis. Blood will be collected for CBCs, analysis of immune responses in peripheral mononuclear cells, and for serology. The animals will be followed until two-consecutive negative culture samples are collected or no longer than ten weeks.

EXPERIMENT THREE: ANTI-PT HUMAN MONOCLONAL ANTIBODY STUDY.
The four animals were determined to be B. bronchiseptica-positive upon quarantine entry testing. Those four animals will be returned to the breeding facility and four B. bronchiseptica-free animals will be delivered to replace them.

The four animals will be infused with the human monoclonal antibodies (b) (4), (b) (5) and (b) (4), (b) (5). 24 hours following infusion the four animals will be challenged with B. pertussis and blood will be drawn to assess antibody levels. These four animals will be challenged along with the final cohort in study one in order to share the unvaccinated/untreated control group. Following challenge, samples will be collected on days 3, 7, and 10: Nasal wash samples will be collected to quantify bacterial colonization and mucosal antibodies. Nasal swabs will be collected to obtain host mucosal cells for analysis. Blood will be collected for CBCs, analysis of immune responses in peripheral mononuclear cells, and for serology. On day 15 the four infused animals will be euthanized and necropsies will be performed to evaluate the histopathology of the trachea, lungs, and draining lymph nodes to evaluate the level of protection from disease conferred by the mAB infusion.

POST-STUDY PROCEDURES
Nasopharyngeal washes and swabs and blood draws may be performed on baboons being held before studies, between studies, or after studies in order to obtain samples for immunological analysis. No more than one sample collection will be performed in any given week.

Animal tissues and organs taken post-mortem from animals on this protocol will be used by FDA researchers for in-vitro experiments. Researchers will be enrolled in the AEP and all OH&S policies around the handling of NHP issues will be followed.

Blood draws may be taken from animals on this protocol to provide cells and sera to FDA researchers for in-vitro experiments.

DVS Attending Veterinarians oversee all aspects of animal care and handling. DVS staff perform all procedures involving direct handling of animals assisted by research team members (listed in F2) and DVS staff perform daily care and feeding of animals and perform routine health checks.

② For each individual listed on this protocol, describe their roles and identify the procedures they will be responsible for on this protocol. In the far right column, briefly state their level of experience and whether the individual is proficient with each of the listed procedures. If the individual is not trained in either the procedures or species (or both), indicate who will provide the training.

All procedures listed in the protocol should be accounted for. Additionally, if training is required, supervised training should continue until proficiency can be determined.

Name	Roles/Procedures	Previous Experience/Related Training for Each Procedure
(b) (6)	Principal Investigator. Oversees conduct of studies. Assists with performance of pertussis challenges, bleeds, nasopharyngeal washes and other sample collections as needed.	15 years experience conducting pertussis challenges of NHPs under supervision of CBER primate staff and veterinarians.
(b) (6)	Assists with performance of pertussis challenges, bleeds, nasopharyngeal washes and other sample collections as needed.	12 years experience conducting pertussis challenges of NHPs under supervision of CBER primate staff and veterinarians
(b) (6)	Assists with performance of pertussis challenges, bleeds, nasopharyngeal washes and other sample collections as needed.	11 years experience conducting pertussis challenges of NHPs under supervision of CBER primate staff and veterinarians.
(b) (6)	Assists with performance of pertussis challenges, bleeds, nasopharyngeal washes and other sample collections as needed.	3 years experience conducting pertussis challenges of NHPs under supervision of CBER primate staff and veterinarians.
(b) (6)	Assists with performance of pertussis challenges, bleeds, nasopharyngeal washes and other sample collections as needed.	7 years performing studies in mouse models. 3 years conducting pertussis challenges of NHPs under supervision of CBER primate staff and veterinarians.

③ Describe in detail all injections, inoculations, and other experimental treatments (e.g., infectious agents, chemicals, drugs, adjuvants, injection or treatment sites, routes of administration, dosing schedule, etc.). In addition to completing this text box, please also list all substances (including vehicles/diluents) in the table below.

INFECTIONS:
Infection with B. pertussis: Baboons will be anesthetized with ketamine (5-10 mgs/kg) i.m. +/- Dexmedetomidine (0.01-0.05 mg/kg) i.m. and will be provided thermal support to combat hypothermia. Approximately 1 x 10^9 bacteria in 1 cc non-bacteriostatic saline will be instilled directly into the

trachea through an endotracheal tube (IT route). Following the inoculation through the endotracheal tube, the tube will be flushed with 1 cc non-bacteriostatic saline to ensure delivery of the entire inoculum. Approximately 1 x 10^9 in 1 cc will be instilled into the nares (0.5 cc delivered to each nares)(IN route). Dexmedetomidine will then be reversed with Antisedan, 1 mg/kg IM. The animal will be returned to its cage and monitored until fully recovered from the anesthesia.

VACCINATIONS:

Vaccinations with the U.S. licensed pediatric pertussis vaccine Daptacel or with experimental acellular (aP) vaccines will be administered by the IM route. 0.5 mls will be administered using a 22-28 gauge needle.

The OMV-aP vaccine will be administered by the IN route. O.5 mls will be administered using the MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex Medical, Salt Lake City, UT, USA). In addition, 0.5 mls will be instilled into the nares (0.5 cc delivered to each nares)(IN route)

INFUSION OF HUMAN MONOCLONAL ANTIBODIES (mAbs) (b) (4), (b) (5) and (b) (4), (b) (5):

Baboons will be anesthetized with ketamine (5-10 mgs/kg) i.m. +/-Dexmedetomidine (0.01-0.05 mg/kg) i.m. and will be provided thermal support to combat hypothermia. For allergic prophylaxis or treatment, diphenhydramine may be provided up to 5mg/kg PO/IM/IV q 6-8hrs as directed by DVS veterinarians. 10 mg/kg of (b) (4), (b) (5) and 10 mgs/kg mg of (b) (4), (b) (5) in sterile PBS will be infused intravenously over a period of 15 minutes using an infusion pump or time-controlled administration from syringe. A peripheral IV catheter will be placed to facilitate the infusion. The catheter will be flushed with sterile saline to ensure patency prior to initiating the infusion. Animals will be monitored for signs of anaphylaxis, excessive redness, heat, or swelling at the catheter placement site prior to reversal of sedation with Antisedan, 1 mg/kg IM. The animal will be returned to its cage and monitored until fully recovered from the anesthesia. Laboratory staff will continue to monitor the animals for 1 hour following recovery from sedation.

④ List all substances administered to live animals in the table below. Do not include anesthetics, analgesics, sedatives, or tranquilizers. Those should be listed in Section H.

Substance	Route	Concentration	Volume/ Quantity	Needle Type/Gauge	Dosage Interval	Number of Intervals	Pharm. Grade?
Acellular pertussis vaccine	Intramuscular or Intranasal	human dose	0.5 mls	22g-28g	6-8 weeks	2-3	Yes
Non-bacteriostatic saline	Intranasal	0.9% Sodium Chloride	10 mls	N/A	2 times per week	2 per week	Yes
B. pertussis strain D420	IT and IN	10^8 to 10^9 CFUs per ml	2 mls	N/A	N/A	1	No
OMV-aP	Intramuscular or Intranasal	100 micrograms/ml	0.25 mls	22g-28g	N/A	1	No
Azithromycin	oral	40mg/ml	20-40 mgs/kg	N/A	Daily	5	Yes

Cefazolin	IV or IM	100 mg/ml	20-25 mg/kg	20-28 gauge	N/A	1	Yes
Cefazolin	IM	100 mg/kg	20-25 mg/kg	20-28 gauge	Twice Daily	10-14	Yes
Cephalexin	oral	250 mg/pill	20-25 mg/kg	N/A	Twice Daily	10-14	Yes
Ceftiofur Crystalline Free Acid (CCFA)	Subcutaneous	100-200mg/ml	5 - 20mg/kg	20-28 gauge	Single Dose	N/A	Yes
PT, FIM and PRN + alum	IM	5 ug PT, 5 ug FIM, 5 ug PRN, 0.065% w/w alum	0.5 mls	22-28 guage	2 months	2	No
PT, FIM and PRN + (b) (4), (b) (5) (b) (4), (b) (5)	IM	5 ug PT, 5 ug FIM, 5 ug PRN, (b) (4), (b) (5)	0.5 mls	22-28 guage	2 months	2	No
PT, FIM and PRN + (b) (4), (b) (5) (b) (4), (b) (5)	IM	5 ug PT, 5 ug FIM, 5 ug PRN, (b) (4), (b) (5)	0.5 mls	22-28 guage	2 months	2	No
PT, FIM and PRN + (b) (4), (b) (5) (b) (4), (b) (5)	IM	5 ug PT, 5 ug FIM, 5 ug PRN, (b) (4), (b) (5)	0.5 mls	22-28 guage	2 months	2	No
PT, FIM and PRN + (b) (4), (b) (5) (b) (4), (b) (5)	IM	5 ug PT, 5 ug FIM, 5 ug PRN, (b) (4), (b) (5)	0.5 mls	22-28 guage	2 months	2	No
PT, FIM and PRN + (b) (4), (b) (5)	IM	5 ug PT, 5 ug FIM, 5 ug PRN, (b) (4), (b) (5)	0.5 mls	22-28 guage	2 months	2	No
OMV-aP Vaccine	IN	25 ug PT, 20 µg FHA, 5 ug FIM, 8 ug PRN, and 100 µg OMV	1 ml (2 x 0.5 mls)	N/A	6-8 weeks	2-3	No
mAb (b) (4), (b) (5) (b) (4), (b) (5)	IV	(b) (4), (b) (5)	10 mg/kg	20-22	N/A	1	No
PBS	IV	N/A	10 mls	20-22	N/A	1	No
mAB (b) (4), (b) (5) (b) (4), (b) (5)	IV	(b) (4), (b) (5)	10 mg/kg	20-22	N/A	1	No

Diphenhydramine	PO/IM/IV	50 mg/ml	Up to 5 mg/kg	*not entered*	0	Once per mAb Infusion	Yes

Use of non-pharmaceutical grade substances has been indicated. For each, describe its quality and purity and provide information on how it was prepared to ensure sterility and suitability for use in animals. If the substance is available in pharmaceutical-grade, please state this and justify your choice to use a non-pharmaceutical grade version.

B. pertussis strain D420 —	Pharmaceutical-grade is not available. A pure frozen inoculum is streaked on BG-plates. Following outgrowth on plates and confirmation of lack of contamination, the bacteria are resuspended in saline at the target concentration
OMV-aP —	OMV-aP vaccine will be formulated by combining pharmaceutical grade, licensed aP vaccine with GMP-produced OMV adjuvant.
PT, FIM and PRN + alum —	Pharmaceutical-grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. Adju-Phos (alum) is a research grade adjuvant obtained commercially. The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.
PT, FIM and PRN + (b) (4), (b) (5) —	Pharmaceutical-grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. (b) (4), (b) (5) (b) (4), (b) (5). The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.
PT, FIM and PRN + (b) (4), (b) (5) —	Pharmaceutical grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. (b) (4), (b) (5) (b) (4), (b) (5) The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.
PT, FIM and PRN + (b) (4), (b) (5) —	Pharmaceutical grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. (b) (4), (b) (5) (b) (4), (b) (5) The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.
PT, FIM and PRN + (b) (4), (b) (5) —	Pharmaceutical grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. (b) (4), (b) (5) (b) (4), (b) (5) The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.
PT, FIM and PRN + (b) (4), (b) (5) —	Pharmaceutical grade is not available. PT, FIM and PRN are obtained commercially without significant endotoxin content. (b) (4), (b) (5) (b) (4), (b) (5) The formulation is prepared in an aseptic environment and kept sterile before use. This experimental vaccine has been used in other baboon studies and is well tolerated.

OMV-aP Vaccine	—	PT, FHA, FIM and PRN are obtained commercially without significant endotoxin content. OMV adjuvant is GMP-produced.
mAb (b) (4), (b) (5)	—	Pharmaceutical grade is not available. (b) (4), (b) (5) Following purification testing was performed to verify purity, sterility, lack of endotoxin, and absence of other potential contaminants to ensure that the final product meets stringent safety standards. (b) (4), (b) (5) was tested for safety in a mouse model and found to be well-tolerated.
PBS	—	Pharmaceutical grade is not available. The (b) (4), (b) (5) is provided in PBS. The pH buffering capacity of PBS is required to ensure antibody stability.
mAB (b) (4), (b) (5)	—	Pharmaceutical grade is not available. (b) (4), (b) (5) Following purification testing was performed to verify purity, sterility, lack of endotoxin, and absence of other potential contaminants to ensure that the final product meets stringent safety standards. (b) (4), (b) (5) was tested for safety in a mouse model and found to be well-tolerated.

⑤ List in the table below all substances from the above table that may have been passaged through, exposed to, or derived from laboratory animals. Please also include other potential sources of adventitious agents used in this ASP but not included in the substances table above, such as cells, serum, antibodies, etc.

No adventitious entered

I certify that the tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens. Please submit all test results to the ACUC Coordinator prior to ACUC Committee Review.

Certified on 04/23/2024 at 09:01:20

⑥ Will blood be collected? Yes

If Yes, describe each blood withdrawal including total volume, frequency, intervals between multiple withdrawals, and withdrawal site. If blood is collected from anesthetized or euthanized animals, include this information.

Two bleeds will be performed during the quarantine period in order to collect pre-infection samples to establish baseline values and to screen for B. bronchiseptica exposure. In addition, digit, footpad or tail-pricks will be performed to obtain a drop of blood for glucose levels. These bleeds will be performed when animals are anesthetized for performance of routine TB tests. The total volume will not exceed 6 mls whole blood/kg/2 weeks. Following challenge baboons will be bled

two times per week for up to ten weeks. In addition, digit, footpad or tail-pricks will be performed to obtain a drop of blood for glucose levels. For the mAb study, a bleed will be performed at time of challenge to determine t = 0 antibody titers. The total volume of whole blood collected will not exceed 6 mls/kg/2 weeks for any animal.

If an animal is found to be positve for B. bronchiseptica exposure, the animal will be removed from the study and an amendment will be submitted to replace the animal.

In vaccination studies, in addition to the bleeds described above, bleeds will be performed immediately prior to each vaccination and on days 7 and 14 following vaccination. Baboons will be anesthetized no more frequently than twice weekly and the total volume of whole blood collected will not exceed 6 mls/kg/2 weeks.

For the antibody infusion study, in addition to the blood collections described, blood will be collected imediately prior to challenge in order to determine the antibody levels. The total volume of whole blood collected from these animals will not exceed 6 mls/kg/2 weeks.

Baboons being held prior to initiating studies or between studies or after studies may be bled in order to obtain sera or peripheral blood cells for in vitro immunological studies, blood chemistries and/or CBCs. In addition, digit, footpad or tail-pricks may be performed to obtain a drop of blood for glucose levels. No more than one bleed per week will be performed and The total volume of whole blood collected will not exceed 6 mls/kg/2 weeks.

For all blood withdrawals baboons will be anesthetized with ketamine (5-10 mg/kg)i.m. +/- Dexmedetomidine (0.01-0.05 mg/kg) i.m. and will be provided thermal support to combat hypothermia. Dexmedetomidine will then be reversed with Antisedan, 1 mg/kg IM.

⑦ Will animals be physically restrained longer than 5 minutes? No

⑧ Describe the animal identification methods that will be used for this study (e.g., ear tags, ear punch or notch, tattoos, collar, cage card, permanent marker, etc.).

Monkeys will be identified by tattoo.

⑨ Other procedures (e.g., tumor measurements, live imaging, oral gavage, etc).

POST-CHALLENGE SAMPLE COLLECTION:
Baboons will be anesthetized with ketamine (5-10 mg/kg)i.m. +/- Dexmedetomidine (0.01-0.05 mg/kg)i.m. and clinical samples will be collected on days 1, 3, and 7. The collection of three samples in the first week following challenge is necessary to capture data on the early host response to infection. Starting the second week, clinical samples will be collected two times per week for the duration of the study (up to ten weeks). Clinical samples will be collected as follows: On the schedule described above, blood will be collected via femoral vein, not to exceed 6 mls whole blood/kg/2 weeks. Whole blood will be evaluated for WBC counts within twenty-four hours and serum will be banked for evaluation of cytokines and antibody titers. Nasopharyngeal washes and/or swabs will be performed at each blood draw as follows: To perform washes, the animal will be placed on its side with the head over the edge of the table. The animal's snout will be wiped with an alcohol wipe. Five mls of non-bacteriostatic saline will be infused into the upper nares using a catheter. The wash through will be collected in a 50 ml sterile tube held under the lower nares. The animal will be turned over and the procedure repeated with five mls of non-bacteriostatic saline infused into the other nares. This method results in recovery of large volumes of wash but we've observed contamination of samples by this

method in some animals. When contamination is a recurring problem for an animal we will utilize an alternative method that yields a lower volume but reduces contamination. In this method a three ml syringe with a catheter tip is used to instill 2 mls of non-bacteriostatic saline into the nares. Following instillation the plunger is immediately retracted in order to pull as much saline as possible back into the stringe. The procedure is repeated in the other nares and the samples are combined. The samples will be evaluated for presence of B. pertussis by plating for colony-forming units. The remaining sample may be analysed for microbial gene expression or host-cell activity or will be banked for future analysis. Nasal swabs are performed using a nasal mucosal lining fluid sampling device (Nasosorption™). The Nasosorption device is a non-sterile, single-use device consisting of a synthetic absorptive matrix strip attached to an applicator handle for ease of nasal sampling. For sampling, it will be inserted into the nasal cavity of each nostril of the animals, to an approximate depth of 25-30 mm, and held for a period of 60+/-2 seconds in order to absorb the mucosal lining fluid from the lowest nasal turbinate. It will be performed prior to nasopharyngeal washes after the animals are sedated for procedures. Alternatively, a FLOQSwabs® 503CS01 - Flexible Mini Tip Size Nylon® Flocked Swab will be inserted into the nasal cavity of each nostril of the animals and used to swab the mucosal surface. These are minimally invasive procedures that are expected to be well-tolorated.

Temperature will be measured rectally when clinical samples are collected. Baboons that become colonized and display symptoms will be followed until the infection is cleared and white blood cell counts return to baseline, at which time baboons will be treated with Azithromycin or other appropriate antibiotic therapy as determined by the clinical veterinarian.

⑩ Does this research study have a breeding component? No

⑪ Describe significant symptomatology, if any, as a result of the experiments. Indicate frequency of and who is responsible for animal observations.

All study animals will be observed at least twice daily by DVS veterinary staff. Following challenge with pertussis the young baboons may exhibit one or more of the following symptoms: lethargy, inappetance, dehydration, unkempt hair coat, paroxysmal coughing spells, expectoration of phlegm, vomiting, fever, ocular and/or nasal discharge, sneezing, gagging, loss of body weight and mild to moderate debilitation. A veterinarian will be contacted immediately once animals begin to exhibit clinical signs. Clinical signs can be transient and typically peak around 1 week post inoculation/infection. In past studies and in rare cases, some animals recover, and then begin to decline again after about 3-5 days. These animals typically exhibit a reoccurrence of lethargy and inappetance, and will be monitored particularly closely. Those animals that produce significant amounts of phlegm will have their mouth, nose and throat gently cleared by DVS personnel each time the animal is anesthetized, and during recovery from anesthesia, as needed.

If deemed necessary by the DVS veterinarian, supportive care or treatment will be provided. This includes: parenteral administration of warmed fluids, supplemental nutrition/vitamins and/or analgesics such as ibuprofen or similar NSAIDs. At the veterinarian's discretion, supplemental heat, oxygen or IV glucose may be provided. The lab will be notified regarding supportive care provided.

Animals vaccinated with licensed acellular vaccines, or WHO-qualified whole-cell vaccines and convalescent animals, typically display no outwards signs of disease upon challenge. If signs of disease are observed, they are expected to be milder than disease signs observed in naive animals.

No adverse reactions are expected following infusion of mAb (b) (4), (b) (5) , howver animals will be monitored as described above and any reactions will be treated by DVS veterinary staff.

⑫ Describe the planned experimental endpoint (i.e., study duration length, animal age, maximum tumor size, etc.). Whenever possible animal studies should be designed to end prior to the onset of unrelieved pain or distress. Give the maximum length of time that animals will remain on study. Death as an expected endpoint must always be scientifically justified.

Baboons presenting symptoms will be followed until infections are cleared naturally and/or circulating white blood cell counts return to baseline. Allowing baboons to progress through the disease is necessary in these studies in order to characterize pertussis disease pathogenesis in primates and compare it to clinical pertussis in humans, characterize immune responses to infection and compare immune responses and protection in vaccinated, convalescent and non-immune animals. Our experience to date is that most young baboons tolerate the infection and return to normal activity following a cough illness that persists for two to three weeks and has a period of peak intensity that lasts approximately one week. All animals will be treated with Azithromycin at the termination of each study to ensure clearance of B. pertussis.

Following recovery from infection and clearance of B. pertussis from the airway, the baboons are typically in good health and will be made available for transfer according to the FDA AWC Placement Guidelines.

Alternatively, end-of-study baboons may be euthanized for data collection, such as collection of samples from deep lung fields, but only when scientifically necessary based on study results, with concurrence of DVS Veterinarians.

⑬ Describe the humane endpoint criteria or the set of predetermined physiological or behavioral signs that define the point at which an animal will be removed from the experiment. Humane endpoints should be identified that accurately predict or indicate pain and/or distress, imminent deterioration, or progression to death. Some examples of humane endpoints could be hunched posture, hypothermia, dehydration, weight loss of 15% or more, inability to rise or ambulate, 20 mm diameter tumor (mouse), presence of labored respiration, or ulcerated, necrotic or infected tumors. Keeping animals on study following the onset of these signs should be explained in the proposal and may necessitate a category E justification.

If clinical signs such as: paroxysmal coughing with excessive phlegm production; weight loss of >15%; vomiting; listlessness or lethargy; persistent hypoglycemia; moderate to severe dyspnea or cyanosis are noted so that the animal progresses from mild/moderate debilitation to severe debilitation, the DVS vet and PI will be immediately contacted. The veterinarian will make a decision on supportive care (outlined in symptomatology) or immediate euthanasia. Although not expected, the PI and a DVS veterinarian will be immediately contacted if an animal is found to be severely lethargic or unable to rise, and the animal will be immediately euthanized.

⑭ Describe any special housing and/or animal care required related to species or strain. List any restrictions from environmental enrichment, food and/or fluid restrictions (with length of time). Special housing or restrictions related to safety should be addressed in Section J.

Pairs of animals will be selected for enhanced aerosol monitoring. Those animals will be co-housed in the transmission containment unit for 14 days in order to enable characterization of airborne bacterial shedding. These studies are designed to answer important questions about how signs of disease contribute to shedding of bacteria from infected animals and airborne transmission of disease and will enable us to evaluate the impact of vaccination on shedding. The answers to these questions will improve our understanding of pertussis spread through the population and help with the design of

interventions to prevent pertussis transmission in the population. The animals included in the transmission/shedding studies will be between 7 and 14 months of age.

Is a socialization exemption required? No
Is a environmental enrichment exemption required? No

Provide a scientific justification if either of the above exemptions are required.

not applicable

G. SURGERY

Does this proposal involve survival surgery? No

Does this proposal involve nonsurvival surgery? No

H. ANESTHESIA, ANALGESIA, TRANQUILIZATION

Specify the anesthetics, analgesics, sedatives, or tranquilizers, including euthanasia agents (except CO2) that are to be used. Include the name of the agent(s), concentration, dosage, route, and frequency of administration.

Agent	Route of Administration	Concentration	Dosage	Frequency of Administration	Pharmaceutical Grade?
Ketamine	IM	100 mg/ml	5-10 mg/kg	up to 2x weekly for a maximum of ten weeks	Yes
Dexmedetomidine	IM	0.5 mg/ml	0.01-0.03 mg/kg	up to 2x weekly for a maximum of ten weeks	Yes
Meloxicam	SC	5 mg/mL	0.1-0.2 mg/kg	Once per surgery, option to administer once daily for up to 3 days post-operation	Yes

Atipamezole	IM	5 mg/mL	1 mg/kg	up to 2x weekly for a maximum of ten weeks	Yes
Pentobarbital/Phenytoin	IM	390mg/ml	85 mg/kg	Once	Yes

I. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS

Primary method(s) of euthanasia:

Sodium Pentobarbital or euthanasia solution

Secondary method(s) to ensure euthanasia:

Tissue/organ harvest

For each method of euthanasia to be used, provide a brief description for the method and how death will be confirmed. Indicate if anesthetics will be used to facilitate euthanasia and include agents and doses in Section H. Ensure that the description is congruent with the selections made.

The animal to be euthanized is first anesthetized with ketamine and dexmedetomidine per dosages in Section H of this protocol. 1 ml/4.5 kg euthasol is administered, IV once the animal is fully anesthetized. Animals are confirmed dead by ensuring absence of a heart beat upon chest auscultation, and absence of palpebral and corneal reflexes.

Briefly describe other alternate dispositions as applicable after consultation with the Attending Veterinarian (i.e., transfer to another ASP/institution, cryopreservation, adoption, retirement, etc.).

Animals may be placed according to the AWC Placement Guidelines

J. HAZARDOUS AGENTS

Use of biological material requires the approval of a Center Safety Officer. For all hazardous chemicals or drugs, please provide a copy of the Safety Data Sheet information to the ACUC Coordinator. Use of radioisotopes requires an approved Radiation Safety Protocol.

IBC Applications Yes IBC Application #(b) (6) Entry Type: Full Review Status: OHS Clearance
Title: Burkholderia OMV Vaccine Platforms

IBC Application # (b) (6) Entry Type: Full Review Status: Approved/Draft
Title: Bordetella pertussis

The information above comes from the FDA IBC Application System. Changes should be made there.

Animal Biosafety Level: ABSL2

Hazardous Chemicals or Drugs: No — Description: N/A
(e.g., gas anesthesia, solvents)

Radioisotopes: No — Description: N/A

List any special housing, equipment, animal care required because of hazardous agents used in the protocol, the immunological conditions of the animals, or special caging, water, feed, or waste disposal, etc. If animals are not allowed to have environmental enrichment, please state and give justification.

Cages housing B. pertussis-infected monkeys will be held within a high containment BioBubble. All animal bedding, carcasses and waste will be disposed of as MPW. Cage pans will be scraped and dumped into a liner/MPW bag containing dry bedding to adsorb liquid waste. The bag containing waste will be placed inside a second bag in an MPW box and the MPW box will be closed and labeled. Cage pans will be thoroughly sprayed with an appropriate disinfectant (i.e. MB-10 (Vimoba) made fresh every week) before leaving the animal holding room prior to transport to the cage wash.
Both entrances to the animal holding room housing the infected primates will be posted as "Biohazardous" including the name of the infectious agent, required PPE for entry, and emergency contact information. Animals in biobubbles that have completed study, and have completed the 5 day baytril treatment can have cages hosed down and waste washed to the room waste trough without disinfecting pans and bagging waste, following the procedure practiced in other standard NHP rooms.

Additional safety considerations for animals and animal handlers, caretakers, etc.:

Animal handlers will wear all required PPE for working with non-human primates. All individuals on the protocol must be enrolled in the FDA Respiratory Protection Program and have annual PAPR training. Animal handlers will be current for the tdap vaccine prior to entering the containment unit or handling infected or exposed monkeys.