Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _18 (11 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation with bovine viral diarrhea virus (BVDV). Pyrexia, lethargy, cough, nasal discharge, ocular discharge, and leukopenia clinical presentation have been described in the literature and severity of these signs are generally associated with virulence of the virus. These clinical signs are not reproducible in every infection and typically minimal to no clinical symptoms are observed.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Typical treatment would include anti-inflammatories but treatment with these drugs could augment the progression of disease and it is uncertain if these treatments would impact the depletion patterns. Therefore, treatments cannot be administered.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _40 (10 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

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may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Dairy calves and cows will be maintained in an on-site herd to allow study of paratuberculosis in subclinical and clinical stages of disease. It is critical that animals are housed as typical dairy cattle, undergoing stressors of gestation, parturition, and lactation in order to progress from subclinical to clinical disease. Severely clinical cows may present with a range of distinct features including: watery diarrhea, submandibulary edema, rapid weight loss, reduced appetite, dehydration, or recumbency (does not get up unless prompted).

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The use of drugs may change the natural course of disease progression and alter the interpretation of the results but in cases of unrelated ailments analgesics may be used with prior consultation of the clinical veterinarian and researcher.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _6 (6 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Ocular, intranasal, intravenous, intramuscular, or intratracheal challenge with SARS-CoV2. Previous work with bovine Coronaviruses did not result in any clinical disease when cattle were challenged. Calves might develop mild to moderate diarrhea or mild respiratory disease that may warrant after hours observation but is unlikely to warrant euthanasia. Criteria used to determine when a calf should be euthanized include inability to rise with gentle prodding and assistance.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The proposed studies require the evaluation of the pathogenesis of the disease which requires the disease to progress naturally, thus unaffected by drugs.

Column E Explanation

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _15 (15 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Cattle will be infected with a mastitis-causing pathogen (e.g. E. coli, S. uberis, or S. aureus) in one quarter of the mammary gland. Clinical signs may be possible fever, loss of milk production, loss of appetite, changes in milk consistency, and change in their good nature.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Treatment (antibiotics) cannot be given because this will not allow the disease to progress in an experiment. Use of pain drugs affect the immune system and should not be used unless animals have a rectal temperature of greater than 106F. Those animals will be immediately treated for fever as recommended by veterinarian or delegate. Animals that have a rectal temperature of greater than 104.5F for two consecutive observations will be treated for fever as recommended by veterinarian or delegate.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _12 (12 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Column E Explanation

Intratracheal inoculation with Mannheimia haemolytica and/or intratracheal injections of bNK2A peptide. The procedure is well tolerated by calves which exhibit a brief period of distress (under 10 min) with slight coughing and tachypnea. However, the intent with this procedure is to establish M. haemolytica pulmonary infection which can be associated with substantive clinical effects. Clinical effects of pulmonary infection with M. haemolytica include depression, fever, nasal discharge, tachypnea, respiratory insufficiency, and mortality. If the bNK2A peptide is effective, calves are expected to show mild respiratory symptoms due to challenge. Assessment of any clinical signs will be performed as appropriate in consultation with and under direction of a veterinarian.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The animals will be monitored for clinical endpoints which warrant euthanasia to alleviate pain/distress. However, on occasion the progression of clinical signs can progress so rapidly that even frequent monitoring may fail to detect an animal before it succumbs to disease. Anti-inflammatory drugs to alleviate inflammation or pain would necessarily alter signs of disease, reduce the ability to detect clinical endpoints and therefore, these treatments cannot be administered.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _20 (4 in Cat E)_____of animals in this study.

3. Species (common name)__Cattle_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intratracheal challenge with Mannheimia haemolytica. The procedure is well tolerated by calves which exhibit a brief period of distress with slight coughing and tachypnea. However, the intent with this procedures is to establish Mannheimia pulmonary infection which can be associated with substantive clinical effects. Clinical effects of Mannheimia pulmonary infection include depression, fever, nasal discharge, tachypnea, respiratory insufficiency, and mortality.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Column E Explanation

The calves will be carefully monitored for clinical endpoints which warrant euthanasia to alleviate pain/distress. However, on occasion the progression of clinical signs can progress so rapidly that even frequent monitoring may fail to detect an animal before it succumbs to disease. Drugs to alleviate inflammation or pain would necessarily alter signs of disease, reduce our ability to detect clinical endpoints, as well as potentially invalidate the study.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number:___42-G-0001_____

2. Number _ 41 (2 in Cat E)_____of animals in this study.

3. Species (common name)__Elk_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Calves could be born with interstitial pneumonia from brucellosis that causes mortality without demonstration of clinical signs as cows are challenged intraconjunctivally with Brucella when pregnant.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

For assessment of vaccine efficacy, it is essential to determine if offspring are viable at birth. No pain-relieving drugs would be beneficial in treating calves suffering from brucellosis and other ameliorative treatments could not be administered because they may influence the measurement of vaccine efficacy.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number: 42-G-0001

2. Number _26 (24 in Cat E)_____of animals in this study.

3. Species (common name) Ferret of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation of Influenza A virus. Only mild clinical signs are anticipated following infection with IAV such as lethargy, inappetance, fever, nasal and ocular discharge, coughing.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

We anticipate only mild clinical signs following infection with IAV. Since the use of drugs would mask the clinical signs of influenza illness, evaluation of clinical signs without the use of drugs is required to meet the project objectives of IAV pathogenesis, transmission, and host response in ferrets.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number:___42-G-0001_____

2. Number _136 (43 in Cat E)_____of animals in this study.

3. Species (common name)__Hamsters_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Hamsters may be inoculated with live leptospires using urine, concentrated urine, culture medium, liquid culture, or tissue homogenate in transport medium administered intraperitoneally. Hamsters inoculated with leptospira can develop outward clinical signs (observable hemorrhage, ruffled hair coat, weight loss, and isolation).

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The observation of clinical signs and their stage of advancement will be used to indicate euthanasia. Alleviating or relieving these signs will interfere with assessment. No alternatives to the painful procedure were identified which will allow evaluation of leptospiral virulence. Animals will be weighed once daily beginning on day of inoculation, weight loss of >20% or development of outward clinical signs (observable hemorrhage, ruffled hair coat and isolation, loss of interest in food and water) will warrant euthanasia. (Haake. Current Protocols in Microbiology, 2006 Supp. 2, 12E.2.1)

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number:___42-G-0001_____

2. Number _2867 (375 in Cat E)_____of animals in this study.

3. Species (common name)__Mouse_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intracranial or intraperitoneal inoculation of transmissible spongiform encephalopathy (TSE). Lack of hygienic behavior (urine stained or roughened haricot), ataxia, circling, or inability to rise are suggestive of clinical signs.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs can be used to alleviate pain and distress associated with treatments or concurrent illness. Some mice are expected to develop clinical signs of prion disease for which there is no treatment, thus all mice in the protocol are listed as category E.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number:___42-G-0001_____

2. Number _90 (57 in Cat E) _____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation with Porcine Reproductive and Respiratory Syndrome Virus; or a single porcine respiratory disease complex (PRDC) pathogen or their deletion mutants. Following challenge, it is anticipated that some pigs will develop fever, anorexia, lethargy, lameness, and/or sneezing/coughing. Some of the bacteria can cause severe systemic disease including septicemia and/or meningitis.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

To evaluate the pathogenicity of a virus or bacterial isolate/mutant, or the efficacy of intervention strategies such as vaccination, we will need to evaluate host response which precludes the use of drugs that might alleviate clinical signs thus masking the true pathogenic nature of the inoculum. If severe illness occurs animals will be euthanized.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _120 (3 in Cat E) _____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Pigs will be inoculated intranasally with Bordetella bronchiseptica. Pigs involved in studies examining secondary bacterial infection will be inoculated after B. bronchiseptica inoculation intranasally with Pasteurella multocida, Haemophilus parasuis, or Streptococcus suis. Pigs infected with Bordetella bronchiseptica and/or Pasteurella multocida may develop respiratory signs such as sneezing and coughing and could become lethargic. Pigs infected with Streptococcus suis or Haemophilus parasuis can develop systemic disease characterized by septicemia, meningitis, arthritis, or polyserositis that can result in lameness, lethargy, depression, dyspnea, or neurologic signs such as incoordination, tremors, or seizures.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   The purpose of the proposed work is to evaluate the pathogenicity/virulence of the isolates/mutants of Bordetella as well as their interaction with other pathogens. Typical antiinflammatory drugs may alter these interactions and thus will not be used. In the case of antibiotic studies we are hoping to ameliorate the disease in antibiotic treated groups but must have a non-treated group to compare the effectiveness of the treatment.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _235 (200 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal inoculation with influenza A viruses will use physical restraint of the pig and inoculum will be dripped into each nostril. Intratracheal inoculation with influenza A viruses will require anesthetizing the pig. Inoculum will be passed into the trachea via a small plastic tube. Only mild clinical signs are anticipated following infection with IAV such as lethargy, inappetence, fever, nasal and ocular discharge, coughing.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Only mild clinical signs are anticipated following infection with influenza A viruses. Since the use of drugs would mask the clinical signs of influenza illness, evaluation of clinical signs without the use of drugs is required to meet the project objectives of IAV pathogenesis, transmission, and host response in swine.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _12 (12 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal or oral challenge of swine enteric coronavirus (SECV). Challenge may induce mild to moderate diarrhea that would be recognized as a loose/liquid stool for several days. In pigs less than 10 lbs, challenge will induce a severe diarrhea (watery) and vomiting which would result in death for the most affected pigs.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Some of the proposed studies require the evaluation of the pathogenesis of the disease which requires the disease to progress naturally, thus unaffected by drugs. Some of the proposed studies require evaluation of protective immunity. If efficacious pain relieving drugs or other ameliorative treatments were used, they would mask clinical signs which would obscure the evaluation of protective immunity.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _181 (181 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal or oral challenge of pigs with Senecavirus A (SVA). Vesicular lesions can develop on one or more feet and snout beginning 48-72 hours post inoculation in pigs 3 weeks of age or older. The lesions can last for 4-5 days and then begin to heal. Some animals may develop a transitory mild lameness for 2-3 days. Other than the development of vesicular lesions, the pigs appear healthy. In neonatal pigs, a recent experiment resulted in morbidity (lethargy, diarrhea) and mortality after challenge with SVA. These clinical effects may be viral related or a combination of viral and bacterial disease after challenge.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Three part answer: 1) To study the pathogenesis of the SVA infection in swine, the host response has to be "normal," no alterations from extraneous treatments. 2) If drugs would be beneficial in reducing pain/distress, then they would have some effect on the pig's response which alters the pathogenesis of the virus. 3) To evaluate the potential efficacy of a vaccine or treatment, the natural expression of the disease is necessary.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _36 (32 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Intranasal challenge of pigs with porcine reproductive and respiratory syndrome virus (PRRSV). Clinical disease (lethargy, anorexia, diarrhea, dyspnea) will be defined as mild, moderate, or severe. Mild clinical disease would be recognized by pigs being less energetic, slight elevated body temperature, pigs huddled together. Moderate clinical disease would entail increased body

temperature, anorexia, increased respiration rate, lethargy. Severe clinical disease would be pyrexia, anorectic, dyspnea, and less responsive to human presence.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

It is necessary to have a challenge group that may get sick, for which no ameliorative treatments may exist to produce a natural host response to PRRSV infection. This would provide a comparative treatment group to evaluate the efficacy of the commercial vaccine, and for comparing vaccine concepts to challenge only group and to commercial vaccine group.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _2 (2 in Cat E)_____of animals in this study.

3. Species (common name)__Pigs_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Inoculate pigs oronasally with swine acute diarrhea syndrome virus (SADS virus) and some pigs may be exposed to SADS virus via direct contact. Challenge with virus in pigs over 50 lbs would results in mild clinical signs which would include mild diarrhea (a soft stool) and may see vomiting. Onset might be 24-48 hours post inoculation. Duration would be expected to be up to 7 days. Challenge in pigs 10-50 lbs may induce mild to moderate diarrhea that would be recognized as a loose/liquid stool for several days. Onset would be 1-2 days post challenge, duration may be 7-10 days. Challenge in pigs less than 10 lbs may induce a severe diarrhea (watery) and vomiting which would result in death for the most affected pigs.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Research objective is to produce infectious SADS virus to study the pathogenesis of SADS virus infection in swine. Swine are the only species known to be naturally infected with SADS virus and thus have to use swine to determine infectivity of reverse genetics derived virus. Once infectious virus is rescued, then can study the pathogenicity of this virus in pig model. If efficacious pain relieving drugs or other ameliorative treatments were used, they would mask clinical signs which would obscure the study of the pathogenesis of this swine virus.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number: 42-G-0001

2. Number _24 (15 in Cat E)_____of animals in this study.

3. Species (common name) Pigs of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetence, respiratory signs, GI distress, vomiting, and diarrhea.

Challenge with SARS-CoV-2 will be administered by one of the following routes (ocular, intranasal, intravenous, intramuscular, or intratracheal) and by contact to challenged animals. Previous work with SARS-CoV-1 did not result in any clinical disease when pigs were challenged. Porcine coronavirus infection in 4-6 week-old swine can range from no clinical signs to mild to moderate diarrhea that would be recognized as a loose/liquid stool for several days to mild respiratory disease.

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The proposed studies require the evaluation of the pathogenesis of the disease which requires the disease to progress naturally, thus unaffected by drugs.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _ 264 (220 in Cat E)_____of animals in this study.

3. Species (common name)__Turkeys_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Inoculation by oral gavage with Salmonella. Although typically considered commensal bacteria, Salmonella enterica may induce clinical changes in poultry. In adult poultry, disease is rarely seen even if they have bacteremia. In very young poults (up to two weeks old), Salmonella can cause disease (and potentially death). The disease symptoms may vary and include weakness, loss of appetite and poor growth. The animals may crowd close to heat sources and sit with drooping wings and their eyes closed. Watery diarrhea may also occur as a result of Salmonella inoculation.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

The validity of the experimental results requires that the Salmonella enterica outbreak isolates be allowed to colonize without the use of therapeutic drugs. Otherwise, the altered state of the host due to the use of antimicrobials may change the clinical response, potentially modifying the observed pathogenicity of the Salmonella isolates.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

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1. Registration Number: 42-G-0001

2. Number _112 (6 in Cat E)_____of animals in this study.

3. Species (common name) Sheep of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Oral, oronasal, or intracerebral inoculation of transmissible spongiform encephalopathy (TSE). Clinical (neurologic) signs may occur as a result of experimental inoculations months to years after the procedure which may include ataxia, tremor, prolonged recumbency, scratching/loss of wool, or unawareness of surroundings.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

Drugs will be used to alleviate pain and distress that occurs during procedures or due to intercurrent disease. Animals are requested in category E due to the potential that they will exhibit clinical signs as a result of being inoculated with a prion disease agent. Currently, there are no known treatments for prion disease.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PII information such as names (principle investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animal room numbers, grant information, veterinary care programs and the like. A Column E explanation must be written so as to be understood by a lay persons as well as scientists.

1. Registration Number:___42-G-0001_____

2. Number _37 (1 in Cat E)_____of animals in this study.

3. Species (common name)__White-tailed Deer_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Oronasal inoculation of Transmissible Spongiform Encephalopathy (TSE). No immediate clinical effects or changes are expected from this procedure. Clinical signs of weight loss, altered gait, tremor, or behavioral change could occur 18-60 months post inoculation.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   Drugs will be used to alleviate pain and distress that occurs during procedures or due to intercurrent disease. Animals are requested in category E due to the potential that they will exhibit clinical signs as a result of being inoculated with a prion disease agent. Currently, there are no known treatments for prion disease.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None

1. Registration Number:___42-G-0001_____

2. Number _6 (6 in Cat E)_____of animals in this study.

3. Species (common name)__White-tailed Deer_____of animals used in the study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which

may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappetance, respiratory signs, GI distress, vomiting, and diarrhea.

Challenged by intranasal instillation with SARS-CoV2. Deer receiving inoculum with virus may eventually develop clinical signs such as fever or lethargy.

5. Attach or include with the reason(s) for why anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

   The proposed studies require the evaluation of the pathogenesis of the disease which requires the disease to progress naturally, thus unaffected by drugs. Under the direction of veterinarian, euthanasia will occur after 24 hours of lethargy and no response to veterinary recommended treatment.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document. None