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 _8 (8 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.

Aerosol challenge with bovine respiratory syncytial virus (BRSV). Clinical signs may include oculonasal discharge, dyspnea, coughing, elevated body temperature.

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)

Because these studies are designed to examine the efficacy of a novel vaccine to protect against virulent BRSV challenge, delivery of pain relieving drugs could alter the outcome of the experimental challenge, by modulation of disease progression

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 _39 (9 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.

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1. Registration Number:___42-G-0001_____

2. Number _16 (12 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

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

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

Challenged intratonsilarly with virulent Mycobacterium bovis. Lesions will mostly develop in lymph nodes of the head, specifically the medial retropharyngeal lymph node. Outward clinical signs of disease have not been seen previously and are not anticipated.

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)

   To evaluate vaccine effectiveness, the primary variable being measured is the immune response of both vaccinates and non-vaccinates to experimental infection with the virulent organism. Unfortunately, there are no correlates of tuberculosis vaccine protection that can be measured antemortem. Vaccine success or failure is measured by the presence and severity of disease observed at postmortem exam. Altering the animals' immune response by modulating immune factors that result in pain through the use of analgesics will obfuscate results rendering the experiment meaningless.

7. 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

Column E Explanation

1. Registration Number:___42-G-0001_____

2. Number _21 (3 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 anethetics, 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

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1. Registration Number:___42-G-0001_____

2. Number _140 (110 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.

Intraperitoneal challenge of bovine respiratory disease complex causing bacteria and intravenous or intraperitoneal administration of NK-lysin-derived peptides. Mice are expected to develop septicemia following H. somni challenge along with severe clinical signs such as ruffled fur, hunched, eyes closed, reduced activity, cold ears, reluctant to move when prodded and moribund.

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)

Typical treatment to control BRDC would include antibiotics such as Excenel, Nuflor, and Baytril. However, treatment of mice with these antibiotics will affect the efficacy of NK-lysin peptides on BRDC causing bacterial pathogens. Therefore any antibiotic cannot be administered and mice will be euthanized immediately when they show severe clinical signs of septicemia.

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 _3184 (612 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

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1. Registration Number: 42-G-0001

2. Number _25 (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 _34 (11 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

Column E Explanation

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 anethetics, 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 _18 (1 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.

Oral, nasal or intracranial inoculation of Transmissible Spongiform Encephalopathy (TSE). Clinical signs or neurologic disease (lack of awareness of surroundings, incoordination, muscle tremors) are possible after approximately 18 months or more of incubation.

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 given to alleviate pain/distress for any non-TSE intercurrent disease. Since there are no known treatments for clinical neurologic signs associated with the development of TSE's, any animals that may develop neurologic signs have been requested in 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 _2 (2 in Cat E)_____of animals in this study.

3. Species (common name)__Guinea 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.

Guinea pigs may be inoculated with leptospira using urine, concentrated urine,culture medium, liquid culture, or tissue homogenate in transport medium administered intraperitoneally. Guinea pigs 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 _11 (2 in Cat E)_____of animals in this study.

3. Species (common name)__Goats_____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 or intracerebral inoculation of transmissible spongiform encephalopathy (TSE). Clinical signs may occur as a result of the inoculation of prion agents after approximately 6 months to 6 years of incubation. These clinical signs may include: loss of muscle tone and severe weight loss/wasting, muscle fasciculations, head tremor, uncoordinated gate, difficulty/inability to rise, and/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)

Any of the animals in this experiment that are inoculated with prion agents could develop clinical signs. There are no known treatments for prion disease, so all animals are listed in category E. Treatments will be given for any intercurrent diseases or injuries.

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)__Goats_____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.

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1. Registration Number:___42-G-0001_____

2. Number _28 (25 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

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 _62 (48 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 anethetics, analgesics and tranquillizers could not be used. (For Federally mandated testing, see Item 6 below)

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. 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.

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 _184 (172 in Cat E)_____of animals in this study.

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

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

Pigs may be directly inoculated with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) by the intranasal (IN) and/or intramuscular (IM) route or by transmission after being placed into direct contact with pigs that were directly inoculated. PRRSV is a viral pathogen of pigs and may cause fever, weight loss, lethargy/depression, and/or anorexia. Death is not an expected outcome of this study. Pigs may show signs/symptoms associated with secondary bacterial infection (lameness, neurological disease and/or coughing/sneezing).

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 purpose of the proposed work is to evaluate the interaction between disease agents as well as host immune response following infection and immune stimulation. It's possible that anti-inflammatory drugs will alter these interactions and thus will not be used. Antibiotics may be administered if deemed an appropriate course of action by the veterinarian.

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 _381 (351 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

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1. Registration Number:___42-G-0001_____

2. Number _102 (48 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 anethetics, 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 _99 (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.

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 _97 (11 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

Intratracheal inoculation with M. haemolytica followed by intratracheal administration of peptide injections approximately 1-6 hrs after M. haemolytica challenge and thereafter up to 18 hour intervals between injections. The procedure is well tolerated by goats which exhibit a brief period of distress with slight coughing and tachypnea due to the volume of fluid administered. If the peptide is effective, some goats are expected to show mild respiratory symptoms due to 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)

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

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 _ 248 (192 in Cat E) of animals in this study.

3. Species (common name) Chickens 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 chicks (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 aim of this study is to determine the antimicrobial resistance (AMR) genes that are enriched in the turkey/chicken gut microbiome due to veterinary feed directive (VFD)-allowed therapeutic or growth promoting antimicrobial and antimicrobial alternative compounds, identify the taxonomic hosts of those resistance genes throughout the microbiome, and determine which AMR genes can be horizontally transferred to Salmonella and/or Campylobacter while in the animal. The use of antimicrobials to treat any clinical signs detected may directly confound interpreting the efficacy results against the different inocula.

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