Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 61
3. Species animals used in this study, pigs.
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.

Piglets infected with the virus are expected to experience watery diarrhea, and occasional vomiting particularly in unvaccinated groups which may lead to transient dehydration. The infection in pigs is self-limiting and diarrhea stops within 3-5 days.

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)

Due to the importance of observing diarrhea, which is a key marker for the studies of the vaccine protective efficacy in this protocol, the piglets will not be administered any antibiotics, anti-inflammatories or electrolytes in order to alleviate the symptoms of virus infection. In particular, antibiotics are not indicated for treatment of the pigs because some of the research involves a microbiota or probiotic component, in which case antibiotic treatment would invalidate results. Hence, the normal course of infection in piglets of both unvaccinated and vaccinated groups have to be allowed in this study in order to evaluate the protection efficacy of the experimental vaccines tested against virus diarrhea.

6. What, if any, federal regulation requires 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.

Agency _____ CFR

Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 10
3. Species animals used in this study, pigs.
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.

   Piglets infected with the virus develop transient, mild diarrhea with spontaneous recovery in 1-3 days

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)

Due to the importance of observing diarrhea, which is a key marker for the studies of the vaccine protective efficacy in this protocol, the piglets will not be administered any antibiotics, anti-inflammatories or electrolytes in order to alleviate the symptoms of virus infection. In particular, antibiotics are not indicated for treatment of the pigs because some of the research involves a microbiota or probiotic component, in which case antibiotic treatment would invalidate results. Hence, the normal course of infection in piglets of both unvaccinated and vaccinated groups have to be allowed in this study in order to evaluate the protection efficacy of the experimental vaccines tested against virus diarrhea.

6. What, if any, federal regulation requires 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.

   Agency _____ CFR

Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 88
3. Species animals used in this study, bats.
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.

   Bats infected with the organism are expected to experience abnormal daytime activity and potential wing damage.

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)

   Studies of "pathogen virulence" require animals to become infected with a pathogen and quantify the metrics of virulence from the host. In most systems using animals, analgesia and anesthesia were not used because virulence metrics must be measured from infected hosts. To reduce distress, bats experimental infections are housed at conditions similar to hibernacula. Bats that are able to remain torpid will experience the least distress.

6. What, if any, federal regulation requires 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.

   Agency _____ CFR

Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 12
3. Species animals used in this study, calves.
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.

Calves infected with the organism are expected to experience a degree of diarrhea, dehydration and inappetence.

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)

Without these clinical signs being exhibited by the calves to a degree, data concerning the clinical course of disease would be unavailable. However, the calves are not allowed to experience pain/distress to the level of not consuming at least 50% of their meals/day OR recumbency, without appropriate interventions including NSAIDS for pain and intravenous fluids with sodium bicarbonate for dehydration and metabolic acidosis.

In commercial settings, calf mortality due to diarrhea averages around 7%, but can be as high as 10-15%, especially in the face of co-infection. Unlike an agricultural setting, our research calves will have access to 24-hour veterinary care, IV catheters to sustain hydration, and IV pain medication. Simply stated, they are likely to experience the same degree of illness, without risk of co-infection, and will receive far more extensive medical care. Also, once calves are infected with the organism and clear the infection, they develop sterile immunity, meaning they can never again be infected with C. parvum. Therefore, we are returning calves that are healthier in comparison to the general population. The knowledge gained from this controlled investigation, could ameliorate the symptoms of this painful and debilitating disease, that will improve the welfare and production of future dairy calves. Furthermore, since this organism is zoonotic, the knowledge gained here as the potential to positively impact human health as well, through the development of pharmaceuticals for use in livestock and human populations.

6. What, if any, federal regulation requires 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.

Agency _____ CFR

Column E Explanation

1. Registration Number: 52-R-0012
2. Number of animals categorized as column E used in this study. 32
3. Species animals used in this study, calves.
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.

Calves infected with the organism are expected to experience a degree of diarrhea, dehydration and inappetence.

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)

Without these clinical signs being exhibited by the calves to a degree, data concerning the clinical course of disease would be unavailable. However, the calves are not allowed to experience pain/distress to the level of not consuming at least 50% of their meals/day OR recumbency, without appropriate interventions including NSAIDS for pain and intravenous fluids with sodium bicarbonate for dehydration and metabolic acidosis.

In commercial settings, calf mortality due to diarrhea averages around 7%, but can be as high as 10-15%, especially in the face of co-infection. Unlike an agricultural setting, our research calves will have access to 24-hour veterinary care, IV catheters to sustain hydration, and IV pain medication. Simply stated, they are likely to experience the same degree of illness, without risk of co-infection, and will receive far more extensive medical care. Also, once calves are infected with the organism and clear the infection, they develop sterile immunity, meaning they can never again be infected with C. parvum. Therefore, we are returning calves that are healthier in comparison to the general population. The knowledge gained from this controlled investigation, could ameliorate the symptoms of this painful and debilitating disease, that will improve the welfare and production of future dairy calves. Furthermore, since this organism is zoonotic, the knowledge gained here as the potential to positively impact human health as well, through the development of pharmaceuticals for use in livestock and human populations.

6. What, if any, federal regulation requires 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.

Agency _____ CFR