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Project Title
The Non-neonatal Pig as a Model for Cryptosporidiosis

Project Number
354

Principal Investigator
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Objectives
In phase 1 of this study, experiments were conducted to determine the feasibility of using pigs as a model for cryptosporidiosis and to identify a regimen of immunosuppressive drugs that could be used in non-neonatal pigs inoculated with *C. parvum* oocysts to achieve the desired clinical signs of infection (diarrhea). In phase 2, development and validation of the non-neonatal pig model included identifying optimum parameters for immunosuppression and infection.

Background
Public awareness of clinical infection by the waterborne parasitic pathogen *Cryptosporidium parvum* (cryptosporidiosis) has remained high since the 1993 massive outbreak in Milwaukee, Wisconsin that resulted in more than 400,000 human illnesses and over 100 deaths. An animal model is necessary to approximate the clinical manifestation of human cryptosporidiosis. The pig is an appropriate model because it is one of the few animals that is susceptible to infection with some human isolates of this protozoan.

Highlights
Conventionally raised outbred non-neonatal pigs (Yorkshire, Hampshire, or crosses between these two swine breeds) served as a relatively inexpensive surrogate human pathogenicity animal model for cryptosporidiosis. Infections with *C. parvum* (at least 28 days postinoculation) will develop and progress to diarrhea in non-neonatal pigs when immunosuppression with FK-506 (Tacrolimus® - given orally at a dosage level of 1.4 mg/kg/day) and oocyst inoculation (10^6 oocysts/pig) are initiated at 14 days of age. Two separate animal isolates (genotype 2) of *C. parvum* were used in this study. The human isolate (genotype 1) that was tested failed to infect the pigs.

Approach
After determining that swine were an appropriate surrogate human pathogenicity animal model for cryptosporidiosis (Phase 1), data were collected on various immunosuppressive agents and regimens when administered to non-neonatal pigs of different age groups. In phase 2, time was spent fully developing and validating the non-neonatal pig model for cryptosporidiosis.
Experiments were conducted to optimize the dosage level of the immunosuppressive agent FK-506 to be used in non-neonatal pigs, determine when FK-506 should first be orally administered to these pigs such that clinical signs of cryptosporidiosis would result, discern how long infections can be maintained in immunosuppressed pigs, identify the minimum number of oocysts that will produce chronic infections with accompanying diarrhea in immunosuppressed pigs, and conduct susceptibility and dose-response studies in immunosuppressed pigs.

Results/Findings

Oral administration of FK-506 (Tacrolimus®) will sufficiently immunosuppress conventionally raised outbred non-neonatal pigs inoculated with *C. parvum* oocysts to achieve the desired clinical signs of infection (diarrhea). Acceptable levels and clinical signs of infection will develop in 14 day-old non-neonatal pigs if immunosuppression with FK-506 (1.4 mg/kg/day) is initiated on the same day as oocyst inoculation (10⁶ oocysts/pig). Immunosuppressed pigs can be infected with less than 10⁶ oocysts/pig. However, the prepatent period is extended beyond three days, clinical signs of infection are less severe, and tissue colonization by the parasite is reduced at 14 days postinoculation. Two animal isolates of *C. parvum* (genotype 2 - termed Iowa and TAMU) were used in this study. The ID₅₀ value for the TAMU isolate was reasoned to be less than 10 oocysts for immunosuppressed non-neonatal pigs. Compared to the Iowa isolate (bovine origin), the TAMU isolate (equine origin) was associated with looser stools, produced longer bouts of diarrhea, and had a lower ID₅₀. Similar findings have been reported from an adult human volunteer study when the Iowa and TAMU isolates of *C. parvum* were compared. The human isolate (genotype 1 – termed NEMC1) tested in this study failed to infect the pigs.

Impact

The surrogate human pathogenicity animal model for cryptosporidiosis we have developed using non-neonatal pigs can be used to generate scientific data on the biology of *C. parvum* infections in humans. Some of this data will ultimately be useful to the American Water Works Association by aiding utilities and public health officials in providing guidance to immunosuppressed and other susceptible human populations. For example, the swine model can be used to assess the safety of finished drinking water for humans by quantitating an infective dosage, establishing viability, and determining the infectivity of cryptosporidial oocysts obtained from various environmental sources and animal species.

Participating Utilities

None