CONTROL ID: 2022070

TITLE: Outbreak and Elimination of PRRSV from Switzerland in 2012/2013
- Collaboration of Swiss Animal Health Authorities and QIAGEN Leipzig

ABSTRACT BODY:
Narrative: We describe an outbreak of Porcine Reproductive and Respiratory Syndrome (PRRS) in Switzerland, introduced into a Swiss PRRSV negative population by boar semen and how a good collaboration of the Swiss authorities together with a reliable supplier of high quality veterinary diagnostics helped to eradicate the disease in only seven weeks.
The Swiss pig population is 1.5 million and free of PRRS. Import of live pigs into Switzerland is only permitted after quarantine. Previous to 2013 up to 32,000 doses of boar semen per year were imported without restrictions.
Boar facilities tested approximately 10% of boars every 4 weeks using ELISA and PCR from serum. In addition, boars delivering semen for Switzerland were tested every 2 weeks in between by PCR from semen samples.
On November 27th 2012, PRRSV was detected in a German boar facility delivering boar semen to Switzerland. One blood sample and semen sample from two boars were PRRSV positive. The outbreak was confirmed by the detection of PRRSV in up to 90% of the boars in one boar-stable. The Swiss authorities were informed by the German boar station on November 28th. By that time 26 Swiss farms had received boar semen from the infected boar facility in the last 2 weeks, 5 of those farms received semen from the two boars which were initially tested as PRRSV positive. All 26 farms and all 61 contact farms were put on quarantine on November 29th.
The sudden, overnight requirement of PRRSV PCR for 87 farms was handled by the Swiss Institute of Virology and Immunology (IVI) in collaboration with QIAGEN Leipzig. In total, over 15,000 tests (ELISA and PCR) were conducted in a seven week time frame, where in over 7200 PCR. Samples were collected by veterinarians, vet students, and employees of the Swiss Pig Health Service. The testing was conducted in 3 Swiss laboratories. The costs for sample collection, diagnostic reagents, and testing service were estimated at € 1 Mill.
On January 11th, the PRRS free status of Switzerland was confirmed and restrictions lifted. The following new regulations, for importing boar semen into Switzerland, are now implemented by the Swiss authorities:
The foreign boar station must have EU approval and be free of Aujeszky. Testing for PRRSV must be performed on blood and semen samples by PRRSV PCR and ELISA. Swiss farms using fresh semen are not permitted to sell pigs for 4 weeks. This ban is lifted if blood samples from such farms are tested PRRSV negative 4 weeks after using such semen samples. Frozen semen can be used on Swiss Farms only three months after collection. During this monthly examination for PRRSV must be done on the original boar station.
Virotype PRRSV PCR kit is officially approved by the German and Swiss authorities for PRRS control. The PCR assay allowed to eradicate the 2012 PRRS outbreak and will be used to reliably prevent PRRSV from entering Switzerland.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2015940

TITLE: Genotype Prevalence and Mixed-Genotype Infections Revealed by Group A *Rotavirus* Full-Genome Sequencing in Cattle and Swine

ABSTRACT BODY:
Narrative: *Rotavirus* infections cause severe gastroenteritis in both humans and animals and are responsible for approximately 600,000 human deaths per year worldwide. *Rotavirus* groups A, B, and C are known to infect mammals and cause losses in production animals. Rotaviruses are members of the Reovirus family and have a double-stranded RNA genome comprising 11 segments approximately 680-3,500 bp in length. Kansas State University Veterinary Diagnostic Laboratory (KSVDL) processed more than 300 porcine and bovine fecal samples of suspected rotavirus infection for whole genome sequencing. By employing the single primer amplification technique (SPAT) combined with next-generation sequencing on the Illumina MiSeq, we obtained 240 full-length or near full-length genome sequences. In cattle, 100% (n=85) of the fecal samples sequenced were identified as group A rotavirus, while in swine, 37% (n=57) of the samples yielded group A sequences, 59% (n=91) were group C and 18% (n=28) were group B strains, with some mixed-group infections. In cattle, six VP4 and six VP7 genotypes were identified, with P[5]-G6 being most prevalent (present in 75% [n=63] of samples). In swine, six VP4 and seven VP7 genotypes were identified, with P[7]-G9 and P[23]-G4 being the most common genotypes (present in 26% [n=15] and 25% [n=14] of group A samples, respectively). Two alleles were identified for each of the other nine segments, with one exception in cattle (3 alleles for NSP1) and two exceptions in swine (3 alleles each for NSP1 and NSP3). Genotypes were considerably species-specific. However, a small number of porcine samples with bovine genotypes were identified, strongly suggesting cross-species transmission. Fourteen percent (n=21) of the porcine samples yielded full or near-full genome sequences of more than one rotavirus (8% [n=13] groups A and C, 4% [6] groups A and B, 1% [1] groups B and C, and 1% [1] groups A, B, and C). Several bovine and porcine samples also contained sequences for more than one genotype for a particular gene segment. The VP7 segment was the most common mixed genotype, with 9 porcine and 9 bovine samples yielding multiple VP7 genotypes. Similar to other studies, our results confirmed the co-circulation of diverse rotaviruses in farm animals and probable frequent interspecies infection. Whole genome sequencing is a valuable tool for determining the genome diversity and epidemiology of *Rotavirus* infections in pigs and cattle.

CURRENT CATEGORY/DISCIPLINE: Epidemiology | Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022717

TITLE: Behavioral Aspects of Swine Oral Fluid Sample Collection

ABSTRACT BODY:
Narrative: The use of swine oral fluid specimens in research, diagnostics, and surveillance has been the focus of recent investigations. The majority of this work has focused on the detection of pathogen-specific antibody or nucleic acid in the oral fluid matrix (1, 2). In contrast, the behavioral aspects of oral fluid collection have been under-researched and many questions remain. The general focus of the present study was on the process of oral fluid sample collection, with the specific aim of evaluating the effect of the location and number of

ropes provided in the pen on pig oral fluid sampling behavior. The ultimate goal of this line of research is to determine the number of ropes to be placed as a function of the number of pigs in the pen. The specific questions addressed in this study were: (1) The effect of the number of ropes in the pen on oral fluid sampling behavior; (2) the effect of the location of the rope(s) within the pen on sampling behavior.
Sixty 5-week-old pigs were divided into two groups of 30 (15 gilts, 15 barrows) and placed in two pens of identical size and structure. For 9 days pigs were acclimated to the pen and familiarized to oral fluid collection using 2 cm (3/4 inch) 3-strand 100% cotton rope. During acclimatization and throughout the study, oral fluids were collected at approximately 7:00 in the morning. The process of harvesting oral fluids and quantifying the sample is described in detail elsewhere (1). Ropes for collecting oral fluids were hung at the corners of the pens. Four cameras synchronously took pictures at 2 second intervals throughout the 20 minute sampling period. Pictures were used to quantify pig behavior. "Rope contact" was defined as a picture showing a pig's mouth closed around the rope. Observations were taken at both the group and individual level. That is, the whole pen (30 pigs per pen), and a subset of pigs (10 ear-tagged pigs in each pen). The tagged pigs were chosen at random and marked with colored ear tags. Analysis of data showed that the total volume of oral fluid, the number of pigs contacting rope(s), and the total time that pigs chewed rope(s) increased as more ropes were provided. Pigs showed a clear preference rope placed in certain locations, but the basis for this preference was undeterminable. Overall, these data imply that the collection of oral fluid samples can be improved by understanding the behavioral aspects of the process.

References:
1. Kittawornrat A, et al.: 2014, Vet Micro 168:331-339.
2. Mur L, et al.: 2013, Vet Micro 165:135-139.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2020481

TITLE: Studies of the Emerging Pestivirus Species *Pronghorn Virus*; Antigenic Cross Reactivity with other Pestiviruses, Recent Detection in Wildlife and Clinical Presentation in Goats and Deer

ABSTRACT BODY:

Narrative: The Pestivirus genus of the family *Flaviviridae*, is composed of a group of antigenically related positive strand RNA viruses. Recognized species of the genus include *bovine viral diarrhea virus* types 1 and 2, *classical swine fever virus* and *border disease virus*. Putative species include *Bungowannah virus, giraffe virus, HoBi-like virus and pronghorn virus (PHV). PHV was first isolated from an immature, blind pronghorn antelope. Phylogenetic analysis shows that PHV is more distant from the recognized species of pestivirus than the recognized species are to each other. In the study reported here, antigenic comparison, using hyperimmune goat serum, demonstrated that the antigenic cross reactivity between PHV and the recognized pestivirus species is low. For over a decade, no other detection of PHV was reported. However, in a PCR-based survey of samples collected in the state of Nevada from wildlife in the last three years, PHV was detected in mule deer, big horn sheep and mountain goat samples. Clinical presentation following infection of goats with PHV*

included mild, short-term pyrexia and a decrease in circulating lymphocytes. Infection of white tailed deer resulted in death in 2 out of 6 animals inoculated. Pre-existing antibodies, against BVDV in deer, did not prevent replication of PHV or decreases in circulating lymphocytes. PHV was not transmitted to penmates following infection of either goats or white tailed deer.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2016075

TITLE: The Prevalence and Epidemiology of *Equine Rhinitis A Virus* and *Equine Rhinitis B Virus* Urine Shedding in Horses.

ABSTRACT BODY:

Narrative: *Equine Rhinitis A Virus* (ERAV) has been considered to be a common respiratory viral infection in horses worldwide. Primary viral replication takes place in the pharyngeal region and spread from horse to horse through nasal secretions. The purpose of this study was to determine the prevalence of *Equine Rhinitis A Virus* and *Equine Rhinitis B Virus* (ERBV) in post-race urine samples from horses in three distinct geographic regions in the US using specific RT-PCR. Two-hundred sixty-eight post-race urine samples were collected from normal Thoroughbred and Quarter Horses from California, Pennsylvania, and Florida and submitted to IDEXX laboratories for detection of ERAV and ERBV by specific RT-PCR. Urine samples were stored at 40C for less than 3 months. The mean particles/mL in urine was determined for all *Equine Rhinitis Virus* positive samples. One-hundred eighteen urine samples were submitted from California. All 118 urine samples were ERBV negative, whereas 33 of 118 (27.9%) were ERAV positive. The mean ERAV particles/mL in urine was 8,866,620. One-hundred urine samples were submitted from Pennsylvania horses. There were 23 of 100 (23%) RT-PCR ERAV positive compared to 0 of 100 urine samples ERBV positive. The mean ERAV particles/mL in urine was 403,811. Fifty urine samples were submitted from Florida horses. Eleven of 50 (22%) were ERAV positive, whereas 0 of 50 were ERBV positive. The mean ERAV particles/mL in urine was 2,549,642. To confirm the presence of live-virus urine shedding, rabbit-kidney-13 (RK-13) cells were exposed to four separate filtered RT-PCR ERAV positive urine samples. Samples were incubated and examined every 24 hours for cytopathic effect (CPE). Cells were considered negative if CPE was not observed after 3 passages. Two of 4 samples had evidence of CPE and further investigated by electron microscopy. Briefly, the two isolates were concentrated by ultracentrifugation, supernatants were discarded, and the pellets fixed in 200uL of 0.1% gluteraldehyde in PBS. Conventional electron microscopy was performed on the preparation. Virus particles consistent with the Apthovirus genus of the *Picornaviridae* family were observed and electron micrographs were taken. Overall, 67 of 268 (25%) urine samples were positive by RT-PCR for ERAV, whereas all 268 urine samples were ERBV negative. Horses from California (27.9%) had the highest percent ERAV positive, followed by horses from Pennsylvania (23%) and Florida (22%). Urine shedding of live ERAV was established in 2 horses by propagation in RK-13 cells and confirmed by electron microscopy. Natural infection by ERAV in this population of horses resulted in viral RNA (mean ERAV particles/mL - 3,940,024) shed in urine. The mechanism of ERAV urine viral shedding requires further investigation along with the impact of urine viral shedding on ERAV transmission in horses.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021362

TITLE: Dual Infections with *Canine Parvovirus* Type 2 (CPV-2) and *Dog Circovirus* (DCV) in Michigan Dogs

ABSTRACT BODY:

Narrative: A Michigan dog breeding facility experienced outbreaks of bloody diarrhea in March 2013 and February 2014. Despite a history of vaccination, fatalities occurred in a number of both adult dogs and puppies. One dog from the 2013 outbreak and two dogs from the 2014 outbreak were submitted to the Diagnostic Center for Population and Animal Health (DCPAH) for necropsy.

Both gross and histologic findings were consistent with CPV-2 infection. Histologically, the affected segments of the small intestine of the dogs submitted had a marked loss and necrosis of mucosal crypts. There was marked lymphoid follicle depletion in the spleen and Peyer's patches. Numerous mesenteric lymph nodes had marked sinusoidal histiocytosis and small focal areas of granulomatous inflammation. Real-time PCR testing confirmed the presence of very high levels of CPV-2 DNA in affected tissues. Immunohistochemistry demonstrated the presence of abundant amounts of CPV-2 antigen within crypt epithelial cells in the affected segments of the small intestine, but only few positive cells in lymphoid follicles in spleen, lymph nodes and Peyer's patches.

In September 2013, archived tissue DNA from the CPV-2 infected dog submitted from the March 2013 outbreak was retrospectively tested for DCV. A previously described real-time PCR for DCV detected very high levels of the virus, revealing a case of dual infections with DCV and CPV-2. Subsequently, the two dogs from the 2014 outbreak were also determined to be dually infected with both viruses. An in situ hybridization assay for DCV, newly developed at DCPAH, detected abundant amounts of viral nucleic acid in all lymphoid tissues, most commonly in the cytoplasm of histiocytic cells. Large amounts of DCV were also detected within the cytoplasm of macrophages in areas of granulomatous inflammation. Nuclei of regenerating crypt epithelial cells were rarely positive. Kupffer cells in the liver were positive for both CPV-2 and DCV.

The presence of DCV viremia in surviving dogs was also evaluated, based upon its significance in *porcine circovirus* pathogenesis. Significantly, two surviving dogs, one from each outbreak, were viremic for DCV when tested 2 months to 1 year post-recovery.

Overall, the marked lymphoid depletion and the abundance of DCV in all lymphoid tissues suggest an immunosuppressive role of DCV, potentiating a concurrent CPV-2 infection. Alternatively, CPV-2 infection could induce the target cells for subsequent DCV replication.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2023183

TITLE: Point of Need Detection of Canine Respiratory Disease Pathogens on POCKIT, a Portable Molecular Detection System

ABSTRACT BODY:

Narrative: *Canine Distemper virus* (CDV), *Canine Herpes virus-1* (CHV-1), *Canine Parainfluenza virus* (CPIV), *Canine Respiratory Corona virus* (CRCoV), *Canine Adenovirus-2* (CAV-2), Canine Influenza virus (CIV), and *Bordetella bronchiseptica* are pathogens resulting in Canine Respiratory Disease (CRD). Proper diagnosis of CRD pathogens is paramount for patient care, population medicine, and biosecurity. Time to diagnosis is critical due to their highly infectious nature and ability to cause sometimes life threatening disease. When tested, samples are shipped to reference laboratories, delaying diagnosis and thus hindering infectious disease control. Here we evaluate pathogen specific insulated isothermal PCR (iiPCR) assays in the field deployable device, POCKIT, for the detection of important pathogens in the dog.

Published or de novo, real time PCR (qPCR) assays were validated as reference assays on the BioRad CFX96. Limits of detection (LOD) were determined via pathogen standards and were performed side by side for both platforms. Clinical samples (30 positive/30 negative) were tested side by side, in triplicate. When sufficient clinical samples were not available, various dilutions of the pathogen standard or vaccine were tested as surrogate positives.

Reference assay LOD for all canine pathogens fell one to 3 logs below one infectious unit except *B. bronchiseptica* with an LOD of one infectious unit. LOD for iiPCR assays on POCKIT are equivalent or within on log of the reference assays. Acceptable sensitivities for iiPCR assays on POCKIT are 98.5%, 100%, 93.3% and 96.7% for CIV, CDV, CAV-2 and CPIV assays. Specificity for all assays was 96-100%.For *B. bronchiseptica*, CHV, and CRCoV Sensitivity were 53.3%, 76.7% and 71%. These three reagent sets have been redesigned accordingly and evaluation of their sensitivity and specificity are underway.

POCKIT portable molecular detection system has exceptional performance in detection of several relevant pathogens such as Canine Distemper and Canine Influenza viruses and can have profound impact on infectious disease control in canine populations such as kennels, shelters and urban areas.

CURRENT CATEGORY/DISCIPLINE: Epidemiology | Virology

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AWARDS:

Trainee Letter: