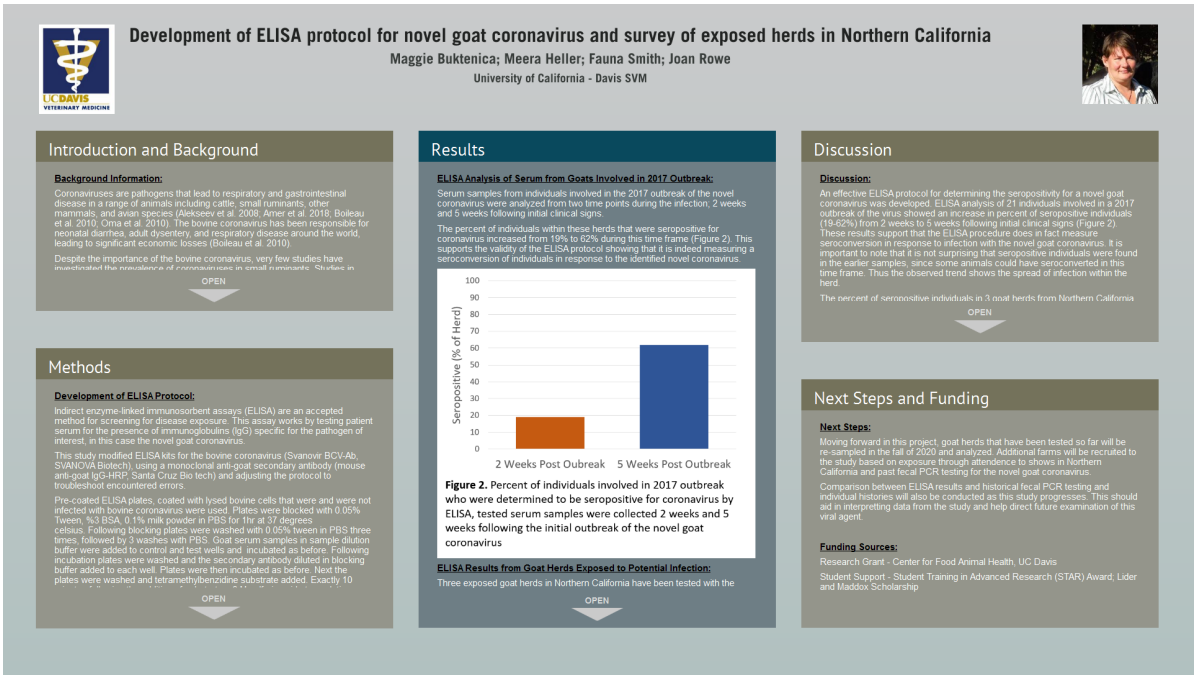
Development of ELISA protocol for novel goat coronavirus and survey of exposed herds in Northern California



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PRESENTED AT:

AAVMC Association of American Veterinary Medical Colleges

VSSS Veterinary Summer Scholars Symposium

INTRODUCTION AND BACKGROUND

Background Information:

Coronaviruses are pathogens that lead to respiratory and gastrointestinal disease in a range of animals including cattle, small ruminants, other mammals, and avian species (Alekseev et al. 2008; Amer et al. 2018; Boileau et al. 2010; Oma et al. 2010). The bovine coronavirus has been responsible for neonatal diarrhea, adult dysentery, and respiratory disease around the world, leading to significant economic losses (Boileau et al. 2010).

Despite the importance of the bovine coronavirus, very few studies have investigated the prevalence of coronaviruses in small ruminants. Studies in Sweden, Spain, Turkey, South Korea, Idaho, and Montana have found 0-19.3% and 1-3.3% of sheep and goats, respectively, to be seropositive for a bovine like Coronavirus (Amer et al. 2018, Traven et al. 1999).

In 2017, outbreaks of enteric and respiratory disease occurred at two goat shows in Northern California. The pathogen was identified by fecal PCR, and sequencing revealed it to be a novel coronavirus related to the bovine coronavirus. The disease was characterized by a change in fecal consistency, lethargy, decreased milk production, anorexia, and fever, and was highly contagious within affected herds.

Similar cases of diarrhea and respiratory disease were reported in 2018 and 2019 show seasons, however an etiologic agent was not identified.

Key Aims:

1. Develop an ELISA protocol to screen for antibodies for the novel goat coronavirus

2. Characterize seropositivity in exposed herds and exposed individuals over time.

METHODS

Development of ELISA Protocol:

Indirect enzyme-linked immunosorbent assays (ELISA) are an accepted method for screening for disease exposure. This assay works by testing patient serum for the presence of immunoglobulins (IgG) specific for the pathogen of interest, in this case the novel goat coronavirus.

This study modified ELISA kits for the bovine coronavirus (Svanovir BCV-Ab, SVANOVA Biotech), using a monoclonal anti-goat secondary antibody (mouse anti-goat IgG-HRP, Santa Cruz Bio tech) and adjusting the protocol to troubleshoot encountered errors.

Pre-coated ELISA plates, coated with lysed bovine cells that were and were not infected with bovine coronavirus were used. Plates were blocked with 0.05% Tween, %3 BSA, 0.1% milk powder in PBS for 1hr at 37 degrees Celsius. Following blocking plates were washed with 0.05% tween in PBS three times, followed by 3 washes with PBS. Goat serum samples in sample dilution buffer were added to control and test wells and incubated as before. Following incubation plates were washed and the secondary antibody diluted in blocking buffer added to each well. Plates were then incubated as before. Next the plates were washed and tetramethylbenzidine substrate added. Exactly 10 minutes following the addition of substrate a 2 M sulfuric acid stop solution was added to each well. Within 15 minutes of adding stop solution the absorbance of each well was read at 450 nm to determine the optical density (OD) of each sample.

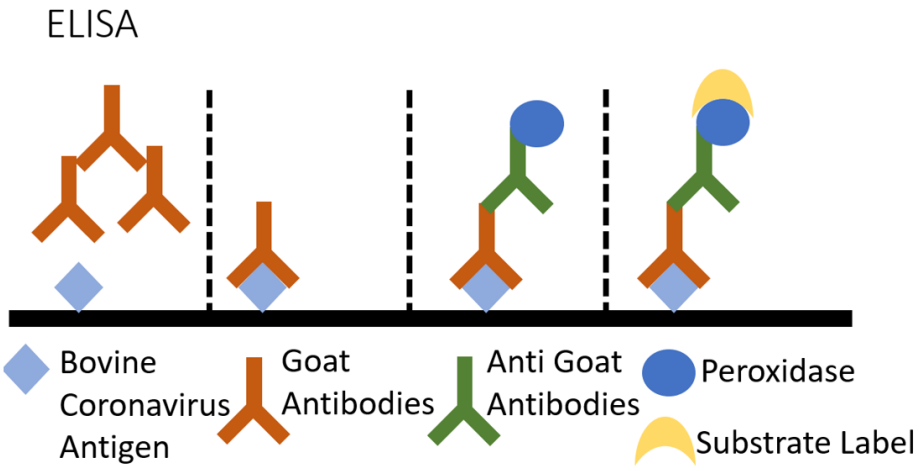

Figure 1. Depiction of ELISA assay.

Determination of positive cutoff value:

Serum samples from PCR positive individuals in 2017 were considered positive controls (n=14). Serum samples from an isolated goat herd were considered negative controls (n=33).

Cut-Off Absorbance was determined as:

Mean of negative controls + 3 x Standard Deviation (of the negative controls)

Sample Population:

Samples were taken from 3 exposed goat herds (including the herds with PCR positive animals) All individuals over 1 year of age were sampled in each herd. Historical samples from 2017, 2018, 2019 were available from some or all of the herds. All herds were sampled in 2020. A total of 141 serum samples, from 102 Individuals were assayed.

RESULTS

ELISA Analysis of Serum from Goats Involved in 2017 Outbreak:

Serum samples from individuals involved in the 2017 outbreak of the novel coronavirus were analyzed from two time points during the infection; 2 weeks and 5 weeks following initial clinical signs.

The percent of individuals within these herds that were seropositive for coronavirus increased from 19% to 62% during this time frame (Figure 2). This supports the validity of the ELISA protocol showing that it is indeed measuring a seroconversion of individuals in response to the identified novel coronavirus.

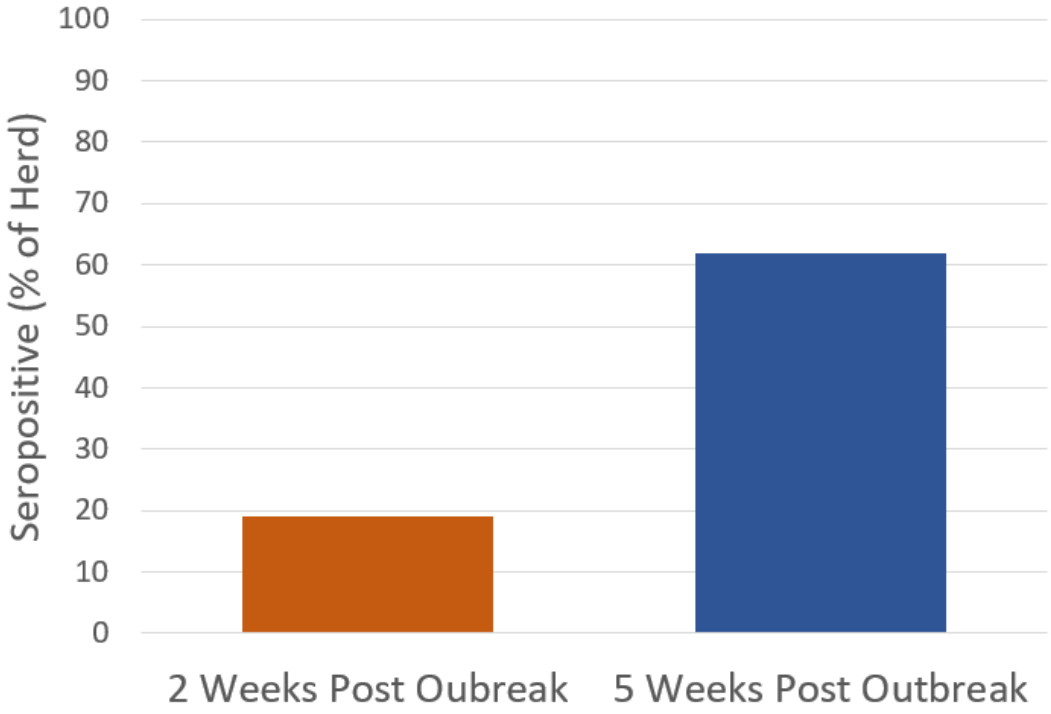

Figure 2. Percent of individuals involved in 2017 outbreak who were determined to be seropositive for coronavirus by ELISA, tested serum samples were collected 2 weeks and 5 weeks following the initial outbreak of the novel goat coronavirus

ELISA Results from Goat Herds Exposed to Potential Infection:

Three exposed goat herds in Northern California have been tested with the developed ELISA protocol. The results from these analyses have shown 62-100% of individuals within these herds to be seropositive against coronavirus (Figure 3). Furthermore, one of these herds was tested in 2017, 2019 and 2020 and found an increase in the percent of seropositive animals (62-100%).

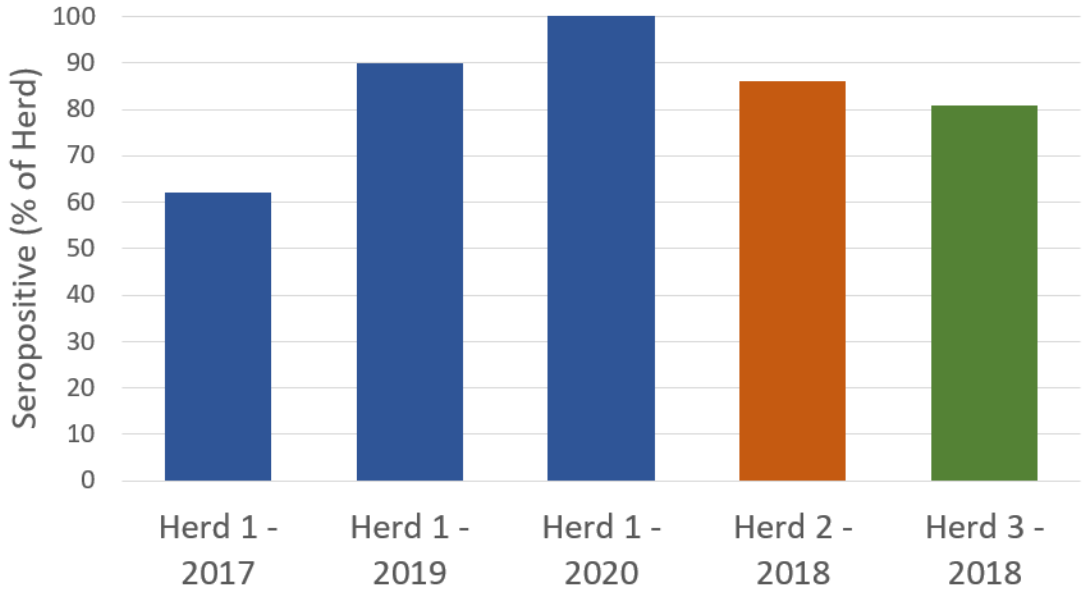

Figure 3. Percent of individuals in 3 goat herds from Northern California who were determined to be seropositive for coronavirus by ELISA, where the Herd 1 – 2017 samples were those collected 5 weeks following the initial 2017 outbreak of the novel goat coronavirus and the other samples were collected during the years indicated

ELISA Results from Individuals Tested at Multiple Time Points:

Eight individuals with samples from 2017, 2019 and 2020 all showed an increase in titer over the years, as reflected by an increase OD reading (Figure 4). Thus these individuals either maintained antibodies against this novel coronavirus for years, or that the virus continued to circulate within the herd.

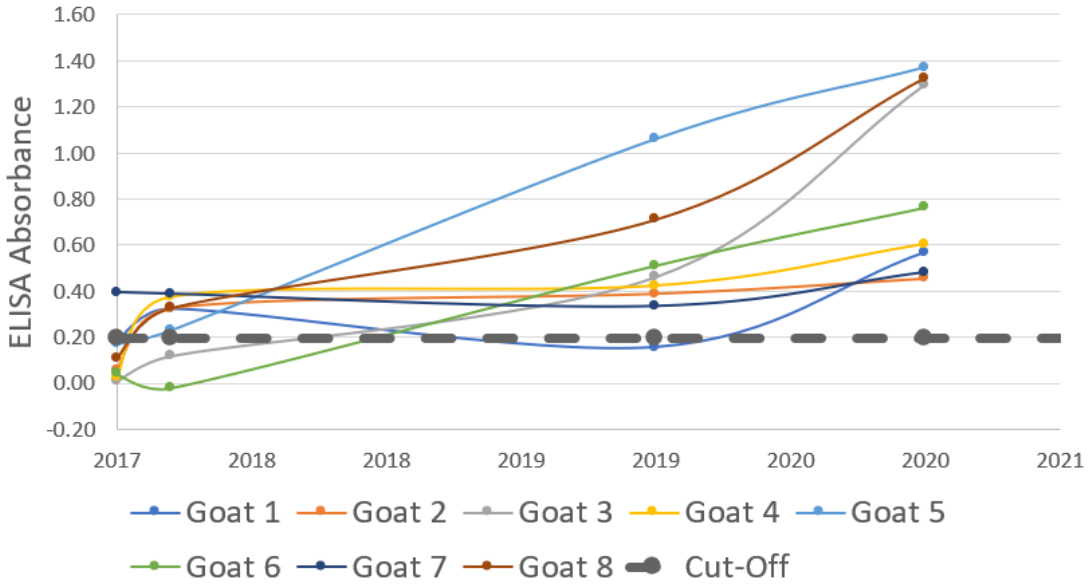

Figure 4. Change in final ELISA absorbance of eight goats from 2017 to 2020, where the line labeled cut-off marks the separation between seropositive and negative individuals

DISCUSSION

Discussion:

An effective ELISA protocol for determining the seropositivity for a novel goat coronavirus was developed. ELISA analysis of 21 individuals involved in a 2017 outbreak of the virus showed an increase in percent of seropositive individuals (19-62%) from 2 weeks to 5 weeks following initial clinical signs (Figure 2). These results support that the ELISA procedure does in fact measure seroconversion in response to infection with the novel goat coronavirus. It is important to note that it is not surprising that seropositive individuals were found in the earlier samples, since some animals could have seroconverted in this time frame. Thus the observed trend shows the spread of infection within the herd.

The percent of seropositive individuals in 3 goat herds from Northern California were determined using serum samples from 2017, 2018, 2019, and 2020 (Figure 3). The measured percentages of seropositive individuals (62-100%) were higher than had been previously measured in small ruminants (Amer et al. 2018, Ozmen et al. 2006; Traven et al. 1999; Yang et al., 2008), and indicate that this coronavirus is a significant pathogen within the Northern California show goat population.

The individual OD values of eight individuals were tracked from 2017 to 2020, and were all found to increase within the time frame (Figure 4).These results indicate that individuals may be able to maintain antibodies against this novel coronavirus for years, or that the virus continues to circulate within the herd. Further investigation into the clinical histories of these individuals and additional tracking of individual titers are required to make conclusions from this data.

NEXT STEPS AND FUNDING

Next Steps:

Moving forward in this project, goat herds that have been tested so far will be re-sampled in the fall of 2020 and analyzed. Additional farms will be recruited to the study based on exposure through attendance to shows in Northern California and past fecal PCR testing for the novel goat coronavirus.

Comparison between ELISA results and historical fecal PCR testing and individual histories will also be conducted as this study progresses. This should aid in interpreting data from the study and help direct future examination of this viral agent.

Funding Sources:

Research Grant - Center for Food Animal Health, UC Davis

Student Support - Student Training in Advanced Research (STAR) Award; Lider and Maddox Scholarship

DISCLOSURES

The authors have no conflicts of interest related to this research meterial.

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ABSTRACT

Coronaviruses are pathogens that lead to respiratory and gastrointestinal disease in a range of animals including humans, cattle, small ruminants, other mammals, turkey, and other avian species. A few studies have investigated the seroprevalence of coronaviruses in small ruminants, such as sheep and goats, and have found only very low levels. The first recorded instance of coronavirus infection in goats within the United States, was in 2017. During two separate goat shows in Northern California, outbreaks of enteric and respiratory disease were observed. The virus was identified by fecal PCR, and sequencing revealed a novel coronavirus closely related to bovine coronavirus. The purpose of this study was to determine the within-herd prevalence of this novel coronavirus in show goats in Northern California. Specifically, this study used goat serum samples collected in 2017, 2018 and 2020 from herds with known exposure and lack of exposure, to validate an ELISA protocol for screening goats' for immunity to this novel virus. This was done using an ELISA kit commonly used for bovine coronavirus modified for use with goat serum. Following validation of this protocol, ELISA analysis was conducted on goat herds with risk of exposure to the novel coronavirus. The results from these analyses indicate that goats in Northern California do have significant exposure to this virus. The next steps of this project are to continue to determine within herd immunity to the coronavirus in order to determine to prevalence duration of seropositivity.

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