NEWSLETTER

Winter – 2021

Iowa State University
College of Veterinary Medicine

Iowa State University
Veterinary Diagnostic Laboratory
1850 Christensen Drive
Ames, IA 50011-1134

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1850 Christensen Drive
Ames, IA 50011-1134

Phone: 515-294-1950
Email: isuvdl@iastate.edu

Equine Herpesvirus-1 (EHV-1) Infection in Horses

Several equine herpes virus (EHV) infections affect horses – those belonging to the alpha herpesivirus family (EHV-1, EHV-3 and EHV-4) and those belonging to the gamma herpesvirus family (EHV-2 and EHV-5). EHV-1 most commonly causes uncomplicated upper respiratory tract infection, but in some cases, secondary viremia can result in equine herpes myeloencephalopathy (EHM), abortion and neonatal illness.

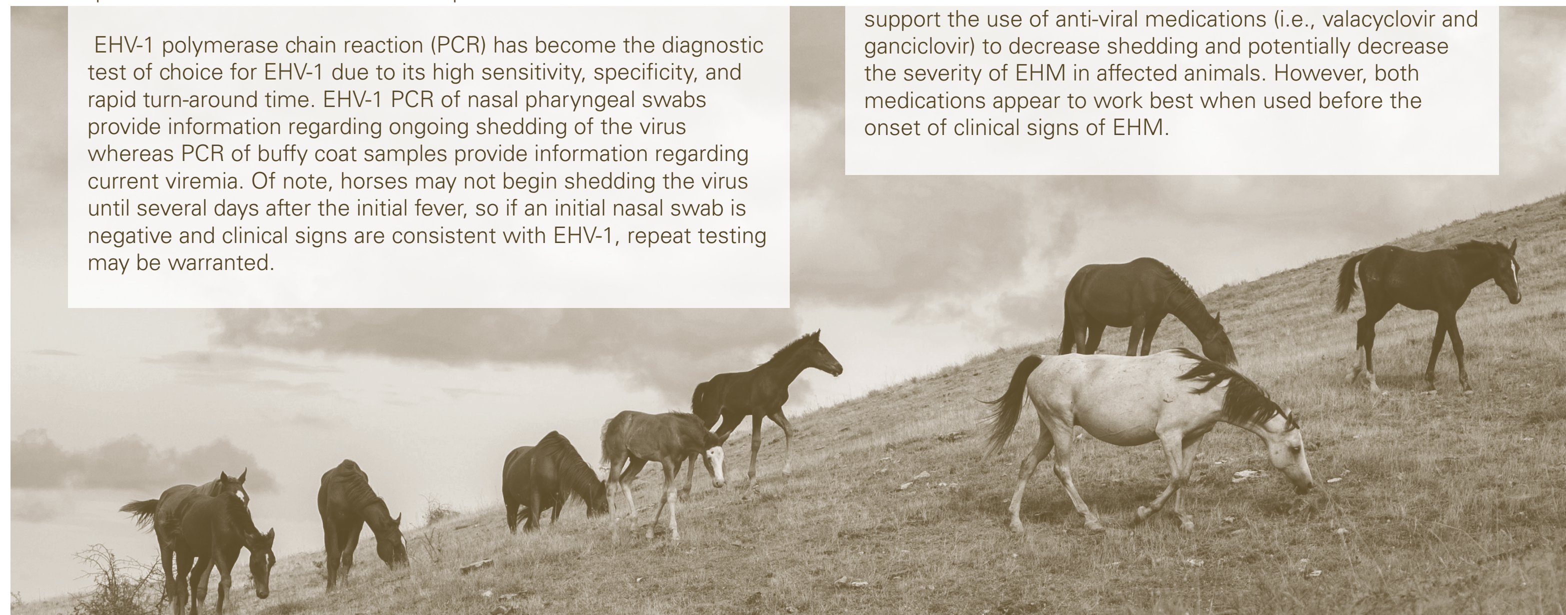
80-90% of horses have been infected with EHV-1 by the time they are two years of age. Once infected, most horses are latently infected for life and shedding of the virus may recrudesce under times of immunosuppression.

Initial clinical signs of EHV-1 infection can include pyrexia, serous nasal discharge and occasionally a cough. In pregnant mares, abortion commonly occurs 2-12 weeks after the initial infection. After the initial upper respiratory clinical signs, some horses develop a cell-associated viremia where infected leukocytes disseminate the virus throughout the horse which can result in vasculitis of the central nervous system resulting in neurological disease (EHM). Although there is a genetic mutation (D752) that results in higher order levels of viremia, thus making neurologic sequela more likely or prevalent during an outbreak, any strain of EHV-1 can result in EHM. Classic clinical signs associated with EHM include hindlimb weakness, ataxia and urine dribbling due to inability to effectively empty the bladder. Horses can also experience cranial nerve defects and changes in mentation. In horses with EHM, there is an approximately 50% case fatality rate. If you have an EHM suspect, please contact the state veterinarian for further advice regarding quarantine measures as EHM is a reportable disease.

EHV-1 polymerase chain reaction (PCR) has become the diagnostic test of choice for EHV-1 due to its high sensitivity, specificity, and rapid turn-around time. EHV-1 PCR of nasal pharyngeal swabs provide information regarding ongoing shedding of the virus whereas PCR of buffy coat samples provide information regarding current viremia. Of note, horses may not begin shedding the virus until several days after the initial fever, so if an initial nasal swab is negative and clinical signs are consistent with EHV-1, repeat testing may be warranted.

Unfortunately, EHV-1 is highly contagious via respiratory secretions and fomite transmission. EHV-1 positive horses should be quarantined from the rest of the herd to limit transmission and routine biosecurity measures should be implemented by their caretakers to limit fomite transmission. Vaccination for EHV-1 is effective at decreasing nasal shedding and the incidence of abortion and respiratory disease but unfortunately has not been efficacious in preventing EHM. Thus, early identification of infected animals and early implementation of biosecurity measures remain paramount in decreasing the risk of horses developing EHM.

Treatment for EHV-1 respiratory disease is largely supportive in nature. Some research studies have found evidence to support the use of anti-viral medications (i.e., valacyclovir and ganciclovir) to decrease shedding and potentially decrease the severity of EHM in affected animals. However, both medications appear to work best when used before the onset of clinical signs of EHM.



Jenny Jones
Client Services

Jenny spent 14 years working in the billing department on the human medicine side before starting at Iowa State in 2007 where she was a receptionist at the Lloyd Small Animal Hospital and Large Animal Hospital. In 2012, she made the jump to the ISU VDL.

Jenny enjoys building relationships with submitting clinicians and their office staff and helping assist them with their VDL needs. Jenny wears many hats. Most of the time, you will find her on the phone assisting clients, but she also processes billing for clients, books travel for employees, and coordinates external testing. The ISU VDL has changed immensely since Jenny arrived in 2012, but she notes, technology has helped submitters more easily send in samples which keeps things running quickly at the VDL all the while the ISU VDL continues to see an increase in its caseload every year. She is excited to continue her service to our clients in the new VDL soon. Unlike many of her co-workers, Jenny grew up with a Shih-tzu and a random hamster and had no farm experience prior to her time at the VDL. Because of this, each day she learns more about hows and whys of farm life.

Jenny lives in Story City with her husband, Steve, of 25 years. She's 15 minutes away from her childhood home where her two siblings and parents still reside today. Jenny and Steve have a son, Bryan, who is an active biker. In their free time, they enjoy attending Bryan's cycle cross racing and wonder where he got his drive and athleticism from. Additionally, they enjoy a warm day on their Harley and camping with family and friends. A great story around a campfire is a great way to end the day.

STAFF HIGHLIGHT

Performance of a Mycoplasma hyopneumoniae (MHP) serum ELISA for antibody detection in processing fluids

Ronaldo Magtoto BS PhD, Betsy Armenta-Leyva DVM, Precy Dizon-Magtoto DVM MS, Ting-Yu Cheng DVM PhD, Maria J. Clavijo DVM PhD, Clayton Johnson DVM, Will Lopez DVM PhD, David Baum DVM PhD, Jeffrey Zimmerman DVM PhD, Luis G. Giménez-Lirola BS MS PhD

This article has been edited for length. The full article has been accepted for publication into the Journal for Swine Health and Production.

BACKGROUND

Mycoplasma hyopneumoniae (*MHP*), the etiological agent of enzootic pneumonia (EP) and a major player in the "porcine respiratory disease complex" (PRDC), is one of the most economically important pathogens of swine, costing the U.S. swine industry approximately $400 million annually. Sow herd stability is key to the reduction of *MHP* losses in growing pigs because piglets, *MHP*-free at birth, become infected by contact with sows shedding the microorganism. For this reason, control programs typically focus either on enhancement of sow herd immunity (vaccination or intentional exposure of gilts) or complete elimination of *MHP*. Regardless of the approach, testing for *MHP*-specific DNA or antibody is needed to establish the status of the breeding herd population. Since each diagnostic approach has its advantages and disadvantages, the choice is determined by which best fits the farm's *MHP* control strategy and yet is practical in terms of sampling and testing.

Processing fluid (PF), the serosanguineous fluid recovered from testicles and tails obtained at the time of piglet processing, is an easily collected specimen with high diagnostic utility. Sow herd surveillance based on PF was first reported in 2010 and has been widely adopted by the industry. Therefore, the purpose of this study was to evaluate the diagnostic performance of PF samples for the detection of *MHP* antibodies using a commercial ELISA.

METHODS

DESIGN

Processing fluid samples (n = 494) from commercial farms (n = 3) were tested for the presence of *MHP* antibodies using a commercial *MHP* indirect serum antibody ELISA at a 1:10 dilution. Based on intervention program and/or historical monitoring, one farm was considered *MHP*-positive (246 PF samples) and two farms were considered *MHP*-negative (248 samples). Receiver operating characteristic (ROC) curve analysis was used to analyze diagnostic performance using farm *MHP* status as a proxy of sample status. From this analysis, diagnostic sensitivity and specificity and 95% confidence intervals were estimated over a range of cutoffs.

PROCESSING FLUID (PF) SAMPLES

Samples were collected from three commercial swine farms between 2018 through 2020 for the purpose of monitoring porcine circovirus type 2 (PCV2) and/or porcine reproductive and respiratory syndrome virus (PRRSV). The criteria to establish *MHP* status corresponded to the *MHP* status of gilts used for the original stocking at each farm. The status of the *MHP*-negative farms was established based on their stocking history, syndromic and routine surveillance. The latter comprised of monthly serum collection tested by *MHP* ELISA. Both *MHP*-negative farms did not implement *MHP* vaccine for piglets, gilts or sows. The *MHP*-positive herd was stocked with *MHP*-positive gilts and received commercial *MHP* vaccine as weaned gilts and again at pre-breeding. There was no mass vaccination of the sow herd and the piglets did not receive any *MHP* vaccine prior to weaning. Clinical signs of *MHP* in that herd were only identified sporadically in the gilt development unit in gilts aged 15-20 weeks, including mild coughing for 2-3 weeks with no noticeable performance impact.

PF samples were collected at the time of piglet processing. Each PF sample represented between 14 to 56 litters of 3- to 5-day-old piglets. At the end of processing, the liquid was transferred to a tube and sent to the Iowa State University Veterinary Diagnostic Laboratory.

MHP INDIRECT ANTIBODY ELISA

The *MHP* ELISA (*M. hyo* Ab test, Idexx Laboratories Inc) was used in the study. Samples were tested for the presence of *MHP* antibodies following the instructions provided by the manufacturer with the exception that samples were tested at a 1:10 dilution. Plates were read on an ELISA reader (EMax® Plus Microplate Reader, Molecular Devices) using SoftMax pro 7.0 (Software, Molecular Devices) and optical density (OD) results converted to sample-to-positive (S/P) ratios:

$$MHP\ \text{ELISA S/P} = \frac{(\text{Sample OD} - \text{Negative control mean OD})}{(\text{Positive control mean OD} - \text{Negative control mean OD})}$$

STATISTICAL ANALYSIS

Diagnostic sensitivities and specificities for specific ELISA S/P cutoffs were estimated by ROC analysis (R version 4.0). To perform the analysis, *MHP* ELISA S/P results with negative values were truncated to zero and sample status (positive, negative) was assumed to match farm status (*MHP*-positive or *MHP*-negative). Estimation of 95% confidence intervals for diagnostic sensitivity and specificity for every ELISA S/P cutoff was performed.

RESULTS

A frequency distribution of *MHP* ELISA S/P responses by farm status is given in Figure 1. Among all samples from the two *MHP*-negative farms (n = 248), 246 (99.2%) had S/P values < 0.20 and all 248 (100%) had S/P values < 0.40. Among samples from the *MHP*-positive farm (n = 246), 240 (97.6%) had S/P values ≥ 0.40. A cutoff of S/P ≥ 0.4 provided 98.8% (97.2, 100) and 100% (100, 100) diagnostic sensitivity and diagnostic specificity, respectively.

DISCUSSION

Routine surveillance based on DNA and antibody detection is crucial for tracking *MHP* in commercial herds. In sow herds, serum antibody testing is a common approach, but serum-based *MHP* surveillance is constrained both by the labor required for collecting blood samples and the number of samples required for statistically valid surveillance. However, other specimens have been described to contain detectable levels of *MHP* antibody and could potentially be used for surveillance, e.g., colostrum, milk, meat juice, and processing fluids. In this regard, processing fluids are of particular interest because they are easily collected and achieve better detection at a lower cost at the population level than individual pig sampling.

The use of processing fluid antibody testing for sow herd surveillance was first reported in 2010 and has since been described for the nucleic acid- or antibody-based surveillance of a variety of pathogen.

Antibody in processing fluids from piglets primarily represents circulating maternal antibody (primarily IgG). That is, colostral IgG is transported from the piglet's intestinal tract and into the lamina propria by non-selective endocytosis, then enters the intestinal lymphatic system and, finally, the circulatory system. Therefore, antibody detection in processing fluid samples provides the means to surveil sow herd *MHP* antibody status - not the piglet humoral immune response against *MHP* infection.

The present study determined that the manufacturer´s recommended cutoff (S/P ≥ 0.40) provided 97.6% (95% CI: 95.5, 99.2) and 100.0 (95% CI: 100, 100) diagnostic sensitivity and diagnostic specificity, respectively. However, since near-perfect diagnostic specificity to minimize false positives results is mandatory for surveillance, users may elect to use a higher cutoff using the cutoffs and associated diagnostic sensitivities and specificities provided in Table 2. (see full paper)

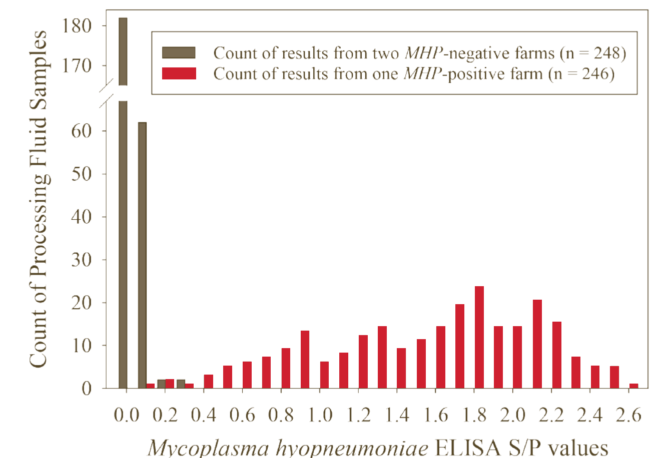
One limitation of the study was the fact that sample classification was based on farm status rather than individual sow status. Notably, four samples from the *MHP*-positive herd had S/P values < 0.4. The overall impact of this small number of misclassified samples on the analysis would be to slightly underestimate the diagnostic sensitivity of the ELISA, but this will have little impact on the utility of this population-based surveillance tool. Still, the

Performance of a Mycoplasma hyopneumoniae (MHP) serum ELISA for antibody detection in processing fluids (cont.)

lack of detection in the *MHP*-negative dataset suggests a high level of specificity of this sample type and test. It is also important to note that the *MHP* ELISA cannot differentiate between vaccine or acquired antibodies. Thus, positive processing fluid samples used from this study may have resulted from the use of vaccine in the breeding herd and not maternal antibodies derived from natural infection. This is an important point that will need to be considered for routine surveillance of vaccinated but antigen-free herds.

Overall, this study demonstrated that processing fluids could be used for detection of *MHP*-specific antibodies. The convenience and low cost nature afforded by processing fluids, combined with its potentially high herd sensitivity, make it highly promising for monitoring naïve herds. Future investigation would need to determine the sensitivity of this sample type compared to serum or deep tracheal swabs for timely detection of *MHP* antibodies in *MHP*-naïve herds.

Figure 1.



ANNOUNCEMENTS:

Upcoming University Holidays:

New Year's Eve — Friday, December 31st
MLK Jr. Day — Monday, January 17th

HATS will be closed on New Year's Eve but will be receiving drop-offs until 3pm on Saturday, January 1st for PRRS and PEDV/PDCoV/TGEV testing.

HATS will be receiving drop-offs as normal, Monday, January 17th.

Questions?

Please contact ISU VDL Client Services
515-294-1950 — isuvdl@iastate.edu