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Evidence-Based Hand Hygiene in Equine Practice
Where “Clean Hands Save Lives” Becomes “Clean Hands Save Horses”

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Guidelines for hand asepsis have changed. Well-formulated alcoholic rubs have several advantages and are more efficient than traditional medicated soaps. Hand hygiene protocols are crucial in our fight against hospital acquired infections, and acquired resistance to chlorhexidine is an increasing problem. Authors’ addresses: Department of Large Animal Sciences, University of Copenhagen, Denmark (Verwilghen, van Galen); Department of Pathobiology, (Weese); and Department of Clinical Studies (Singh), Ontario Veterinary College, Guelph, ON, Canada N1G 2W1; e-mail: denis@equinespecialists.eu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Over the past few decades, the World Health Organisation (WHO) has put increased focus on hand hygiene campaigns under the slogan “Clean Hands–Save Lives” following the global recognition of hand hygiene being a key factor in reducing surgical site infections (SSI) and other hospital associated infections (HAIs). These campaigns have tackled both hand hygiene as part of routine patient care as well as improved pre-surgical hand preparation protocols.

As equine medicine progresses, we must adapt our decision-making in light of new scientific evidence. As much as possible, recommended protocols should now be evidence-based, recognizing that there are varying strengths of evidence and that equine-specific evidence may be limited. Regardless, increasing information from the human field and general understanding of infectious diseases indicate a need for changes in perceptions and practices in diagnostic methods and treatments in equine veterinary medicine, as has occurred (and continues to occur) in human medicine. Despite this paradigm shift, in the field of hand hygiene, things have slowly evolved. Surely, the field has advanced since the 1800s, when Semmelweis and Lister were making seminal (yet controversial) discoveries of the benefits of hand hygiene. They laid the foundations for the concepts of asepsis and vigorously fought the disbelief they were facing, yet while basic concepts of hand hygiene are now widely recognized, parallel actions in veterinary medicine are often lacking. Are we open-heartedly embracing the evidence today?

According to a survey performed in 2009 amongst ECVS and ACVS diplomates, only 6.7% of respon-
DETECTION, TREATMENT, AND BIOCONTAINMENT OF INFECTIOUS DISEASES

Students indicated that they were following WHO guidelines for pre-surgical hand asepsis. A subsequent survey in 2013 showed that, while practices had improved, 66% of the 218 respondents still did not follow the current WHO guidelines. So what do current guidelines and evidence say? Additionally, what is right: current guidelines or current practices?

2. Hand Preparation Today

The goal of surgical hand hygiene is not to sterilize the skin. Rather, it is to reduce or eliminate the burden of potentially pathogenic transient skin organisms and to reduce resident microbiota for the duration of a surgical procedure in order to reduce the risk of an SSI. These goals should be achieved while minimizing damage to the skin that might promote rebound bacterial overgrowth or compromise future hand asepsis attempts. The transient microbiota is acquired by contact with people, animals, and contaminated surfaces. It colonizes the superficial layers of the skin and is the most common cause of SSIs. The resident microbiota is a more established deeper component of the microbiota that is regarded as less pathogenic. Elimination of this component may be undesirable because its presence is a means of defense against the growth of opportunistic transient pathogens. Techniques involving aggressive cleaning of the skin with alkaline medicated soaps, like chlorhexidine gluconate (CHX) scrubs will have deleterious effects on the skin’s local defense mechanisms. Moreover, while brushing and scrubbing does not necessarily have any beneficial impact on bacterial reduction, they can cause small excoriations and, therefore, damage to the skin, further increasing the risk of skin colonization by pathogenic species. Despite the suboptimal features of the surgical scrub, 44% of the respondents to a survey of veterinary surgeons stated that they always use brushes. Current recommendations are to use soft sponges, if any, for hands and forearms, only if visibly soiled and brushes for fingertips.

Ideally, the chosen method must balance antimicrobial activity, skin compatibility and ease of use to be optimally effective and used. A variety of methods and products for surgical hand preparation are available. Aqueous solutions containing either povidone-iodine (PVP) or CHX have been widely used for many decades. Alcohol-based hand rubs (AHRs) have been described for surgical hand preparation for more than a century, although only recently have they become widely recommended for surgical hand preparation. Although no randomized controlled trials have been conducted showing any significant differences in SSI rates between the aforementioned methods, the AHR method is considered superior for a number of reasons. The antibacterial efficacy of products containing high concentrations of alcohol was shown to significantly surpass that of any medicated soap currently available. The initial reduction of the resident skin flora (microbiota) is so rapid and effective with AHRs that bacterial regrowth to baseline values on the gloved hand takes more than 6 hours. These observations were also confirmed in a veterinary trial that compared the activity of an AHR solution to CHX and PVP soap. In that study, a 1.5 min application of an AHR solution was performed with the 3 h residual effect on colony forming units (CFU) reduction of AHRs significantly better than for traditional hand scrubbing with PVP and CHX. Another potential benefit of AHRs is the lack of any known (or plausible) mechanism of alcohol resistance in bacteria. Therefore, there is no concern that repeated alcohol application could select for alcohol-resistance or co-select for resistance to antibiotics. It should be noted that when choosing an AHR solution, the product should meet the EN12791 or equivalent standard required for presurgical hand-rub formulations. Many available gel products will not meet these surgical standards and are therefore not recommended.

About 17% of respondents of one survey used a medicated scrub technique combined with an AHR, in clear contradiction to published evidence and recommendations that do not advocate the use of both methods together. Medicated soaps are either less effective or have a similar efficacy to the hand rub. An initial scrub with medicated soaps will not result in ultimately increased reduction of microbiota after AHR application. On the contrary, long-term use of medicated soaps increases the risk of dermatitis, making the skin more difficult to decontaminate and the combination of medicated soaps and AHR likely to increase the risk of skin damage, while providing no demonstrable positive effect. Furthermore, prior hand washing can alter the effectiveness of AHR solutions, particularly if hands are not completely dried before AHR application. Hand washing also increases preparation time, cost, carbon footprint, and water usage. It is estimated that 20 L of water is used per hand when preparing with medicated soaps. Faucets and basins can also be sources of Pseudomonas spp. and other Gram-negative bacteria, creating a potential for recontamination of the hands prior to gloving. Considering the aforementioned concerns, it has been suggested in human medicine there is no reason to include a hand wash before AHR solutions are applied and that hands should only be washed if they are visibly soiled. However, compared with human hospitals, the bacterial burden in veterinary settings may be higher, especially for equine surgeons. Until further objective data are available, it is reasonable to recommend a short hand-wash with a gentle soap (pH neutral) prior to the AHR application when the surgeon has handled animals or their environments. In this way, bacterial spores and organic material that might interfere with alcohol antisepsis can be eliminated.
3. Resistance
A new and more worrying aspect supporting the reduced use of medicated soaps is the emergence of increased acquired resistance towards antiseptics. In particular, the prevalence of Staphylococcus aureus carrying the chlorhexidine resistance gene gacA/B has been shown to increase in the hospital environment. This is a concern not just because of resistance to chlorhexidine, but also because chlorhexidine resistance can potentially co-select for resistance to clinically-relevant antibiotics. The overuse of CHX scrubbing soaps, which results in the spreading of large quantities of diluted active substance that is more likely to be bacteriostatic than bactericidal, could be a contributing factor in antibiotic resistance among pathogens on the hands or in the environment. Due to the fast killing activity of alcohol and the lack of any known (or plausible) genetic mechanisms that would allow for transmission of inherent alcohol resistance, acquired resistance to AHRs has not been shown to date, nor is it likely to be encountered, providing another major reason to move towards these products.

4. Outside Surgery
Outside the closed environment of the surgery theater, the hands of healthcare workers are thought to play a crucial role in the occurrence of SSI and other HAI. Hand hygiene is therefore regarded as one of the most effective measures to prevent HAI. Several reports have shown a temporal association between interventions to improve hand hygiene measures or compliance rates and reduced infection rates. A recent British report evaluating the “Clean your hands” campaign showed that in the 4-year study period, the AHR use per bed day raised “Clean your hands” campaign showed that in the 4-year study period, the AHR use per bed day raised 30% whereas the AHR solution use per bed day raised only 1.5%. This corroborates the results from the aforementioned survey that identified surgeons using antimicrobial soaps as pre-surgical hand preparation methods as having lower overall hand hygiene rates. New technologies are underway to electronically monitor the compliance in human healthcare, with financial incentives for health care staff. Ultimately, however, increased awareness and personal responsibility by all personnel is critical for improving hand hygiene rates.

5. Skin Health
It is clear that the veterinary occupation also has a real impact on the health of our skin. Not only are human medical staff known to acquire more a pathogenic resident microbiota over time, a high proportion of healthcare workers develop occupational dermatitis, mostly related to repeated washing with chlorhexidine soaps. However, one of the most important determinants of healthcare workers’ resistance to change to AHRs is the unfounded belief that AHRs are more harmful to their skin than soap and water. In reality, each hand wash detrimentally alters the lipid layer of the superficial skin, creating the loss of protective agents such as amino acids and natural antimicrobial factors. Prolonged and repeated washing leads to a damaged barrier function of the stratum corneum, resulting in the skin becoming more permeable for toxic agents and bacteria. On the contrary, AHR solutions have been shown to have less detrimental effects on skin than soaps in both human healthcare and veterinary settings. This corroborates the results from the aforementioned survey that identified surgeons using antimicrobial soaps as pre-surgical hand preparation methods as having lower overall hand hygiene rates compared to those using AHRs. This was particularly true for skin moisture content, with surgeons using antimicrobial soaps reporting significantly dryer hands than those using AHR solutions (Table 1). This is mainly due to the absence of the aforementioned deleterious effects of soap washing combined with the fact that AHR products contain humectants and need shorter application times. Furthermore, although lipid layers are also dissolved with AHRs, they are not washed away but rather reincorporated by the action of rubbing and emollients that are included in virtually all AHR products can facilitate restoration of a normal barrier. As mentioned before, unnecessary washing should be avoided, particularly with hot water.

6. Compliance Issues
In our latest survey, 89 respondents stated they believe AHRs are superior to traditional hand scrubbing techniques for obtaining hand asepsis. Nevertheless, over 42% of these respondents report the use of antimicrobial soaps in their protocol, clearly indicating a lack of compliance with their own beliefs. Reasons for this have not been specifically evaluated, but it may be rooted in the fact that many
surgeons consider the act of scrubbing as an “ancestral ritual” towards preparation to surgery, something that may prevent them from shifting to more efficient protocols.\textsuperscript{25} In addition, this may highlight the fact that involvement of the hospital management in setting up changes in hand hygiene policies is essential.\textsuperscript{33} The use of campaigns and internal training sessions has been shown to be of great benefit\textsuperscript{24,25} in increasing hand hygiene compliance amongst staff in human medicine. Similar initiatives should be made in veterinary medicine in order to obtain better compliance with the application of current knowledge on hand hygiene. Considering the application technique of AHRs is important and the use of hand scrubs is probably less prone to errors as all parts of the hands and arms get wet under the tap, good education is mandatory when introducing AHRs.

7. Conclusions

There have been significant changes made in the basic science of hand hygiene in the past decades, though the veterinary community does not yet seem to have embraced the changes.

In order to comply with current medical standards, the veterinary community should aim at introducing AHR solutions in both the pre-surgical and hygienic surgical hand disinfection protocols. The WHO statement “Clean Hands–Save Lives,” in an AAEP format: “Clean Hands–Save Horses!”

It is in our hands!

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

References


Table 1. Hand Health Scores From 2013 Surgical Preparation Survey\textsuperscript{4}

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Appearance</th>
<th>Intactness</th>
<th>Moisture Content</th>
<th>Sensation</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Antimicrobial scrub (n = 91)</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>6.25</td>
</tr>
<tr>
<td>B Antimicrobial scrub followed with AHR (n = 45)</td>
<td>7</td>
<td>6\textsuperscript{a}</td>
<td>5</td>
<td>7</td>
<td>5.75</td>
</tr>
<tr>
<td>C Neutral soap followed by AHR (n = 35)</td>
<td>7</td>
<td>6</td>
<td>6\textsuperscript{b}</td>
<td>7</td>
<td>6.75\textsuperscript{b}</td>
</tr>
<tr>
<td>D AHR only (n = 15)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6.75\textsuperscript{c}</td>
</tr>
<tr>
<td>E Alcohol chlorhexidine combination (n = 24)</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Based on “hand and skin self-assessment tool” WHO guidelines Appendix 3.\textsuperscript{8} Seven point based scale with 1 being abnormal and 7 normal. The overall score is based on the mean of the 4 individual assessments. Scores are reported as medians.

\textsuperscript{a}Intactness: significantly different from C and D.

\textsuperscript{b}Moisture content: C and D significantly different from A, B, and E but not from each other.

\textsuperscript{c}Overall scores: significantly different from A, B, and E.


How to Prepare a Biosecurity Program for an Equine Veterinary Hospital

Gillian A. Perkins, DVM, DACVIM*; and Jacqueline Bartol, DVM, DACVIM

1. Introduction
Biosecurity is essential for veterinary practices and maintaining trust and clientele. Whenever horses are transported and comingle with new animals, treated with antibiotics, eating an altered diet, or undergoing other stressors, there is a chance for a perfect storm where there is possible shedding and increased susceptibility to a contagious infectious agent. There is always a risk of acquiring an infectious disease in an equine hospital, whether it is Salmonella spp, an infection at the intravenous catheter site with a multidrug resistant (MDR) bacteria, or influenza. A biosecurity program needs to be in place to reduce these risks and is becoming a necessary part of standard-of-care expectations of our equine clients. The goals of a biosecurity program at a veterinary practice are to reduce the risk of nosocomial infections in hospitalized horses and to protect human health.1–4

2. Materials and Methods
The development of a biosecurity program begins with someone who is in charge and accountable. In most veterinary practices it would be best to have a veterinarian and a technician involved in the oversight of a biosecurity program. However, the entire practice needs to be engaged in proper biosecurity practices, as the program is only as good as the weakest link. If the veterinarians are practicing good biosecurity and lead by example, then the rest of the staff will follow suit. The training of personnel and keeping everyone informed of the protocols is a continuous activity. Preparing written biosecurity protocols using everyone’s input helps educate and inform the practice as a whole and is a very healthy process for the group. Once the documents are finalized, they should be easily accessible to everyone and referenced when needed. Having these documents in an easy-to-read format and providing it to visitors, such as visiting student externs before they arrive, sets the stage for a practice that cares about biosecurity and helps protect your clientele.

Formalizing a biosecurity program begins with putting protocols into place, then monitoring patients and assessing for problems, and training personnel. Continuous updating of the program is essential to its success.

Assessment and Critical Evaluation of Existing Protocols and Structure
If you are designing a new facility or wanting to formalize an infectious disease program at your
practice, these are the broad topics that you will want to investigate.

- Facility layout
- Traffic (people, horses, dogs, cleaning equipment, vehicles)
- Horse stabling
- Stall sanitation
- Manure, soiled bedding, and hay disposal
- Medical equipment sanitation
- Operating room sanitation
- Medical waste disposal
- Water source and disposal
- Feed and hay storage
- Vector and wildlife control (to include rodents, mosquitoes, ticks, and flies)
- Existing written infection control policies

An assessment of existing work-flow patterns and biosecurity protocols is a good place to start. Knowing your limitations will help you make critical decisions that will help protect your patients. For instance, deciding whether your clinic should admit a ‘fever of unknown origin’ patient. This initial assessment allows for a list of actionable items, interventions, and a long-term plan for improvements to your overall program.

This presentation will focus on general hygiene and effectively isolating patients to contain any infectious diseases.

General hygiene and cleaning and disinfection of the hospital and medical equipment is paramount to cleanliness and reducing pathogens in the environment. Some guidelines for cleaning the facilities and medical equipment are briefly summarized.

**Stalls, Treatment Rooms, Animal Facilities**

Remove all equipment, bedding, and organic material; then use a scrub brush to clean with soap and water getting into all corners and crevices, rinse with water, and then apply a disinfectant mix (Fig. 1). The disinfectant must be mixed as the label describes and appropriate protective clothing worn by the staff. The disinfectant should be allowed the amount of contact time specified on the label and then rinsed and the area allowed to dry. The disinfectant used in a hospital depends on safety, historical problems, cost, wear-and-tear on the facilities. For some pathogens, such as *Cryptosporidium sp.*, *Clostridium difficile*, and Rotavirus that are hardest to eliminate from the environment, a specific protocol may be set up for cleaning stalls and the surrounding area after the affected patient leaves. For example, 1:10 bleach solution is commonly used for eliminating *Clostridium difficile* spores. Rotavirus, a nonenveloped virus, requires an accelerated hydrogen peroxide, oxidizing agent, or phenolic compound.

**Medical Equipment Such as Ultrasound and Radiological Equipment**

This equipment should be spot cleaned after each use with a disinfectant wipe (Fig. 2). A regular schedule (every week, two-weeks, monthly) should be adhered to for a more aggressive cleaning of the entire piece of equipment and the cart upon which it is housed. In order to keep up with the regularly scheduled full-cleaning, have a sign-up sheet on the equipment with the date and signature of the person performing the cleaning.

**Other Equipment**

There are many items such as twitches, nasogastric tubes, brushes, and much more that come into contact with individual animals and should be cleaned between animals. If possible these items could be assigned for use in one particular patient and then cleaned after the patient is discharged.

**Hand Hygiene**

Hand hygiene is a very important part of a biosecurity program and cannot be stressed enough. Hands should be washed before and after handling each patient. Similar to human healthcare workers, one should wash hands when entering an examination room in front of clients. This sets a good example and shows that your practice cares about hygiene. Hands should be rinsed with water, soap applied, and then washed for about 20 s (sing “Happy Birthday”). All hand surfaces should be washed covering the top, bottom, between each fin-
ger, and sides. Following which, hands should be completely dried. Washing hands has the advantage of removing dirt and debris, as well as “rinsing pathogens” down the sink. Hand sanitizers are a good alternative in areas where a sink is not available, but do not replace the physical washing of the hands. In addition, most hand sanitizers consist of alcohol, which is not effective against Cryptosporidium spp. or Clostridial spores. Wearing examination gloves for certain situations such as changing bandages, assessing incisions and wounds, evaluating catheters (IV, urinary, etc.), treating eyes, when handling horses with infectious diseases, and when handling critical and immune suppressed patients can prevent personnel from infecting patients and vice versa. The examination gloves should be worn when handling the patient or the affected site and then gloves removed and hands washed and/or sanitized. Sometimes people become nonchalant when wearing examination gloves and contact other surfaces, for example, they evaluate the abdominal incision of a post-operative colic and then pick up their cell phone wearing the same gloves. One should always be cognizant of their actions and potential for contamination of other equipment surfaces and then indirect transmission to other patients. In order for staff to maintain good hand hygiene, sinks and hand sanitizers need to be easily accessible, stocked with soap and hand towels, and clean (Fig. 3).

Cleaning Personal Items
Personal items such as cell phones, stethoscopes, and pen-lights is dependent on the individual and is often overlooked. In surveys of hospitals for various pathogenic bacteria, cell phones have been positive in many instances.\(^5\) Disinfectant wipes are convenient and can be used to clean cell phones and other items. This should be done on a daily basis. The cell phone, in particular, goes from patient to patient and from hand to mouth and is extremely important to clean. Reminding staff to do so, or having daily sessions where everyone cleans together, can improve hygiene.

Eating and Drinking in the Hospital and Stable Areas
The fast pace of veterinary practice leads to eating lunch on the run. In general, a veterinarian and their staff should not be eating or drinking while handling a patient, especially in front of clients. This portrays a lack of professionalism, cleanliness, and disregard for personal health and the health of the animal. Eating and drinking should be limited to the office areas and a designated break room. In addition, hands should be washed before and
after eating. For compliance, a convenient location for eating and drinking needs to be provided to the employees.

Grouping of Patients Based on Risk

The housing and management of patients should be from low risk of shedding a contagious disease to high risk, with the least traffic taking place through the high risk patient areas. The order from least to highest risk is outpatients < elective surgical patients < colics and foals < infectious disease patients and should be housed separately (Fig. 4). For example, outpatient lameness and reproductive evaluations should be evaluated and treated away from the inpatient colics and neonatal foals. The elective surgical patients should also be housed separately—these patients are generally healthy but when undergoing surgery are often treated with perioperative antimicrobials, have undergone anaesthesia, have been held off feed, etc., putting them at risk. The colics should be kept separate from other populations of animals because they are at highest risk of shedding *Salmonella spp.* A separate area for foals is ideal to protect them because they are highly susceptible and also because they often present with diarrhea and are an infectious disease risk. It is ideal to also group personnel to work in each separate area on a given day. Ideally, there is a separate isolation facility for managing infectious or suspect infectious disease cases. Alternatively, a semi-isolation situation can be implemented in the hospital.

Preparation for and Handling the Contagious Infectious Disease Patients

Monitoring Patients for Infectious Diseases on Admission

In some instances, where a particular infectious disease is high in prevalence in the area, a veterinary hospital may decide to monitor for that infectious disease in all horses on arrival. For instance, the University of Guelph cultured nasal swabs of horses on arrival and throughout hospitalization for methicillin-resistant *Staphylococcus aureus* (MRSA). This allowed them to take action and isolate any positive patients and, hopefully, reduce in-hospital transmission of MRSA. Many veterinary teaching hospitals perform fecal *Salmonella spp.* cultures when admitting patients.4,7 The cost-benefit of such an approach needs to be considered and the time it takes for the diagnostic test results to be reported often limits the usefulness of this type of screening.

Contagious Infectious Diseases of Concern to Equine Veterinary Hospitals

Determining what infectious agents are likely to represent a risk to your hospital is important. Table 1 includes a list of agents that are communicable from horse to horse and are likely to result in nosocomial infections and impact the hospital’s ability to

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Table 1. Infectious Diseases of Most Concern for Horse-to-Horse Transmission in a Veterinary Hospital

<table>
<thead>
<tr>
<th>Respiratory</th>
<th>Gastrointestinal</th>
<th>Neurological</th>
<th>Surgical site, wound and catheter infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>(aerosol, direct</td>
<td>(fecal-oral, indirect</td>
<td>EHV-1 (EHM)</td>
<td></td>
</tr>
<tr>
<td>contact, indirect contact)</td>
<td>contact)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza</td>
<td><em>Salmonella spp.</em></td>
<td>EHV-1 (EHM)</td>
<td></td>
</tr>
<tr>
<td>EHV-1 and EHV-4&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>Clostridium difficile</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinitis A&amp;B virus&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Coronavirus</td>
<td>MRSA&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Strangles</td>
<td>Rotavirus</td>
<td><em>Resistant Enterococcus spp.</em></td>
<td></td>
</tr>
<tr>
<td>EVA&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>Cryptosporidium sp.</em></td>
<td>Other MDR bacteria</td>
<td></td>
</tr>
</tbody>
</table>

Note: EHV-1 = equine herpes virus-1; EVA = equine viral arteritis; EHM = equine herpes myeloencephalopathy; MRSA = methicillin resistant *Staphylococcus aureus*; MDR = multidrug resistant.<br>
<sup>a</sup>EHV-1 and EVA transmission is through respiratory secretions and direct nose-to-nose contact or exposure to an aborted fetus and placenta.<br>
<sup>b</sup>Horses can be nasally colonized with MRSA and their stall can be transiently infected.<br>
<sup>c</sup>Rhinitis virus has been found in horse urine 37 days post-infection.
admit patients. These agents have been listed by body system, which also represents their major mode of transmission, with most hospitals being concerned with fecal-oral, aerosolization, and direct and indirect contact transmission through fomites. Interfering in critical steps in the transmission cycle will help reduce disease in other hospitalized horses. For example, foot baths could be helpful in preventing spread of fecal pathogens but is limited in efficacy against respiratory pathogens.

**Identifying Patients With Infectious Diseases Prior to Admission**

Once your hospital has identified pathogenic agents of risk, the next step is identifying clinical signs that would alert you and your front office staff to take special precautions to protect the health of others in the hospital. The front office staff, technicians, and interns should be coached to have the clients or referring DVMs speak directly with the Biosecurity Officer prior to shipment of any horse with a possible contagious disease. Fever, diarrhea, nasal discharge, and multiple animals affected on a farm are syndromic signs that alert the clinicians of a possible infectious disease. Time and time again, some key element is lost in the history (no matter how thorough) prior to admission that later reveals itself during the initial diagnostic evaluation; therefore, err on the side of caution and isolate the patient until more information is gathered. Sometimes an initial assessment can be made in the horse trailer—taking the temperature, palpating mandibular lymph nodes, etc. and determining if the patient needs to be isolated or not. In addition, the horse could be worked up in a more separate area of the hospital and then it can be decided whether it requires isolation or not. This happens at Cornell University, not uncommonly with horses with colic that end up having a fever and leukopenia, where other reasons for colic are excluded and colitis becomes the primary differential. The horse then is admitted to the isolation unit and the colic evaluation area is closed and rigorously cleaned and disinfected.

**Criteria for Placing a Horse in Isolation**

Criteria should be set for placing a horse in isolation. Fever of unknown origin, leukopenia, diarrhea, respiratory signs, lymphadenopathy, multiple animals on a farm affected, and any acute onset ataxia with difficulty urinating and defecating could be clinical signs that warrant placement of a horse in isolation. The hospital should also have a protocol to handle patients with nonhealing wounds and possible MDR infections. Methicillin-resistant *Staphylococcus aureus* patients should also have special consideration because nasal colonization can allow for dispersal of the pathogen and lead to a heavily contaminated stall and surrounding area during hospitalization. Therefore, when entering the stall and handling the horse the veterinarian’s and staff’s clothing may become contaminated and allow for fomite transmission. Fortunately, MRSA does not persist in the environment and is not a long-term inhabitant of hospital surfaces, while other MDR pathogens such as *Enterococcus spp.* can survive for long periods in a hospital environment and cause nosocomial infections. Often, providing barrier precautions and semi-isolation within the hospital for these patients and bandaging the wounds can be sufficient to prevent high levels of environmental contamination. However, there are always scenarios that seem somewhat blurred and the person in charge of biosecurity should be contacted at any time of day to discuss the patient history, the current status of the hospital, and make a decision on where that particular horse should be housed. The convenience factor often comes into play and, generally, people regard an isolation situation as more work. Therefore, the biosecurity person in charge needs to hold their ground and handle push-back from colleagues in order to keep the hospital safe. Placing an animal in isolation versus doing clean-up of the entire hospital once an animal is diagnosed with something infectious is much easier for everyone in the long run. The team becomes much more vigilant when they have gone through an infectious disease scare together.

**Setting Up an Isolation Facility: Separate Facility or Semi-Isolation Within the Hospital**

**Separate Isolation Facility**

Having an isolation facility as a stand-alone building from the rest of the hospital has many advantages; however, this is somewhat costly. In general, the building should be separate but still easily accessible. The isolation area should have designated equipment and supplies. If planning to handle diseases spread by respiratory transmission, separate ventilation for each stall should be provided. An anteroom with space that provides the ability to dress in appropriate personal protective equipment (PPE) and prepare the necessary medications and supplies before entering the stall is a key element to an isolation unit. Since a separate isolation facility may not be cost effective for smaller haul-in equine facilities, this presentation will focus more on setting up perimeters to do semi-isolation within the hospital.

**How to Set Up Semi-Isolation Within a Hospital**

When a separate isolation facility is not available, animals can be housed in a hospital using a perimeter and barrier precautions upon entry into the area. This is not advised for respiratory viruses that can be aerosolized throughout a barn such as influenza and equine herpesvirus. In fact, nosocomial infections of equine herpesvirus-1 have been reported on numerous occasions when in-hospital semi-isolation attempts were made. Often the shared airspace is blamed, however, breaches in biosecurity protocols should not be overlooked and when the isolation is being done in the hospital it is...
more likely that these breaches impact other hospitalized patients.

The stall utilized for hospital semi-isolation would be best if it were at the end of a barn or away from major human and animal traffic in order to minimize exposure and perhaps a stall next-to and/or across from the proposed semi-isolation stall can remain vacant to allow for a “buffer zone.” The further away the suspect infectious case is away from the rest of your hospitalized patients, the better. A perimeter can be set up around the stall using ropes, caution tape, or small construction fencing. In order to enter the area one needs to don personal protective equipment (PPE) and step through a footbath or mat at the entry, a cart that holds PPE (in this instance, full body covers, plastic disposable boots, and examination gloves), and biohazard waste disposal. When exiting the stall, the PPE should be removed and placed in the medical waste container and hand sanitizer applied.

Fig. 5. A, Semi-isolation within the hospital involves an obvious perimeter (yellow caution tape) with a footbath or mat at the entry, a cart that holds PPE (in this instance, full body covers, plastic disposable boots, and examination gloves), and biohazard waste disposal. B, When exiting the stall, the PPE should be removed and placed in the medical waste container and hand sanitizer applied.

In general, the PPE should be one-time use items that are disposable. There is no way to get into a coverall that has been worn into a stall and is contaminated on the outside without contaminating oneself. Gowns where a person’s limbs are not covered are not as ideal as a full-body suit, especially for management of neonates where you are often straddling the foal to restrain or perform a procedure. A full-body suit is advised and one that is the least permeable to water. Cost can be a big consideration when deciding what type of PPE since these suits can be costly and the number of suits used per day can be very high in critical care patients. In general, it is best to pick one protocol for the PPE and use that in all infectious disease patients—otherwise, the staff becomes confused as to what to use and when.

The perimeter line should be crossed only once the PPE is on, similar to entering and exiting a surgical suite. A footbath may also be placed at this site. All personnel entering the site should follow these guidelines from clinicians to clients to stable hands. Upon exiting the site, the PPE is removed and placed in the medical waste basket that is also conveniently located. Hand sanitizers should be used and then the personnel should immediately go and wash their hands (Fig. 5B). The staff should be organized with their physical examinations and treatments, making every attempt to limit the number of times they enter the stall. The medical record should remain outside of the stall. The stall should have a designated stethoscope and thermometer in addition to cleaning equipment. Any items necessary for treatments should be taken into the stall each time. Any hospital equipment, such as the ultrasound, must be cleaned after use in the isolated area, taking care to disinfect the wheels (even roll through the footmat, if possible, or spray the wheels with a disinfectant). In addition, the patient must remain in the stall at all times and not be walked throughout the hospital. Once an animal is discharged, the area should remain isolated until all of the items are picked up and the stall is cleaned and disinfected. If the patient is positive for an infectious disease, for example, Salmonella spp., environmental cultures of the stall, surrounding area, and areas of high human traffic after the cleaning and disinfection could be performed prior to opening this area to new patients.

Perform Diagnostic Testing on Infectious Disease Patients

A definitive diagnosis is imperative when dealing with infectious diseases and essential to a successful biosecurity program. It is always best to be “in the know” when it comes to managing infectious diseases in an equine hospital, so that you can disrupt the transmission cycle. Developing a good rapport with your veterinary diagnostic laboratory in order to expedite samples in urgent situations is also of
major importance. While many diseases are handled with the same good biosecurity practices, there are specifics of each infectious disease and durations of quarantine, etc. that can help in the overall management.

Active Surveillance and Monitoring
The biosecurity officer should be proactively monitoring for adverse events such as surgical site infections or diarrhea in post-operative patients and decide when a problem is occurring. As a problem arises, the biosecurity officer and staff may reinvestigate existing protocols, evaluate how patients are prepared for surgery, antimicrobial drug use, etc. Once a limitation of the program is determined, new strategies that could break the transmission cycle and limit disease in future equine patients could be implemented.

3. Results and Conclusions
Biosecurity and infection control are essential for equine hospitals where horses with enhanced susceptibility to infection are concentrated in a small area, thus increasing the risk of transmission of pathogens from one horse to another. Nosocomial infections are an inherent risk of hospitalization and attempts should be made to limit their occurrence through a well formulated biosecurity program. Prevention is far better than costly treatments, and sometimes, poor outcomes, when it comes to nosocomial infections.

Acknowledgments

Conflict of Interest
The Authors declare no conflicts of interest.

References and Footnote

Frequency of *Corynebacterium pseudotuberculosis* Infection in Horses Across the United States During a 10-Year Period

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*Corynebacterium pseudotuberculosis* infection in horses is increasing and occurs throughout the United States. Further studies to determine changes in annual incidence and to identify potential changing climatic conditions or vector populations associated with transmission of infection are warranted. Authors' addresses: The Department of Medicine and Epidemiology (Spier), the William R. Pritchard Veterinary Medical Teaching Hospital (Kilcoyne), University of California, Davis, CA 95616; the Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY 40511 (Carter, Smith); and the Department of Veterinary Pathobiology (Swinford) and the Equine Infectious Disease Laboratory, Department of Large Animal Clinical Sciences (Cohen), College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843; e-mail: sjspier@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

*Corynebacterium pseudotuberculosis* causes various clinical forms of infection, including external abscesses in the pectoral or ventral abdominal area, internal abscessation, or infection of the limbs. The purpose of this study was to examine the occurrence of *C. pseudotuberculosis* infection throughout the United States over a 10-year period.

2. Materials and Methods

State veterinary diagnostic laboratories in the United States were solicited to participate in a cross-sectional study. Data from 2237 horses culture-positive for *C. pseudotuberculosis* identified by 15 veterinary diagnostic laboratories were collected. Data requested included number of cases of *C. pseudotuberculosis* infection in horses seen per year, geographical regions from which the sample was obtained, month and year of sample submission, breed and age of horses, and category of clinical presentation (i.e., internal infection, external infection, or ulcerative lymphangitis).

3. Results and Discussion

Fifteen laboratories provided data from 23 states. The proportion of cases during 2011 and 2012 (54%; 1213/2237) was significantly (P < 0.0001) greater than that for period from 2003 to 2010...
C. pseudotuberculosis was recovered from horses in states where disease has not been previously recognized as endemic. Horses were identified year-round. No significant association between the clinical form of disease and age or breed was observed.

Acknowledgments
The authors would like to acknowledge the state diagnostic laboratories for collecting and contributing data to the study.

Conflict of Interest
The Authors would like to thank Zoetis for funding and declare no conflicts of interest.
Minimum Inhibitory Concentration Determination of Equine *Corynebacterium Pseudotuberculosis* Isolates from 1996 to 2012

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Equine *Corynebacterium pseudotuberculosis* has not developed appreciable changes to minimum inhibitory concentrations (MIC) patterns over time. Authors’ addresses: William R. Pritchard Veterinary Medical Teaching Hospital, (Rhodes); Department of Medicine and Epidemiology, (Magdesian, Spier, Edman); Department of Clinical Pathology, Microbiology and Immunology, (Byrne); and Department of Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, One Shields Ave., Davis, CA 95616; e-mail: sjspxier@ucdavis.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction

Few reports exist regarding the antimicrobial susceptibility patterns of *C. pseudotuberculosis*. The purpose of this study was to evaluate for temporal trends in the in vitro antimicrobial activities of 23 antimicrobials against equine *C. pseudotuberculosis* isolates. A secondary aim was to determine if a relationship exists between MIC and abscess location.

2. Materials and Methods

The study was retrospective and cross-sectional. Medical records were reviewed to obtain clinical and MIC data. Two hundred and seven *C. pseudotuberculosis* isolates from 196 horses were included. Minimum inhibitory concentrations were determined by the microdilution technique for isolates with missing MIC data. MIC values over three periods were compared (1996–2001, 2002–2006, 2007–2012).

3. Results

There were 140 horses with external abscesses, 20 with internal abscesses, 23 horses with both external and internal abscesses, and 4 with ulcerative lymphangitis. Commonly used antimicrobials with MIC$_{50}$ and MIC$_{90}$ values below the susceptibility break points used in this study included the aminoglycosides, doxycycline, erythromycin, azithromycin, chloramphenicol, enrofloxacin, rifampin, tetracycline, and trimethoprim-sulfamethoxazole. Ceftiofur, clarithromycin, ampicillin, and penicillin have variable break points depending on the micro-
organism used, complicating interpretation. There were no significant changes in MIC values over the study period, and there was no relationship between MIC patterns and abscess location.

4. Discussion

*C. pseudotuberculosis* shows susceptibility to many antimicrobials and has not developed resistance over time; however, it is important to consider pharmacokinetics and pharmacodynamic properties of antimicrobials when formulating a treatment plan.

**Acknowledgments**

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**Conflict of Interest**

The Authors declare no conflicts of interest.
Voluntary Surveillance Program for Equine Influenza
Virus in the United States From 2010 to 2013

Nicola Pusterla, DVM, PhD, DACVIM*; Philip Kass, DVM, MPVM, MS, PhD; Samantha Mapes, MS; Cara Wademann; Nina Akana; Craig Barnett, DVM; Cynthia MacKenzie, DVM; and Wendy Vaala, DVM, DACVIM

This study provides valuable and contemporary information on the frequency of equine influenza virus (EIV) detected by real-time polymerase chain reaction (qPCR) in the U.S. The results also point to the fact that older and previously vaccinated horses were susceptible to EIV. Author’s addresses: Department of Medicine and Epidemiology (Pusterla, Mapes, Wademan, Akana) and Department of Population Health and Reproduction (Kass), School of Veterinary Medicine, University of California, Davis, CA 95616; and Merck Animal Health (Barnett, MacKenzie, Vaala), Summit, NJ 07901; email: npusterla@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
Recent surveillance studies for equine respiratory viruses have shown that EIV continues to be a prevalent viral respiratory pathogen of horses throughout the U.S. and Europe. Due to the highly contagious nature and rapid spread of EIV, this virus has severe financial implications for the horse industry. The objective of this study was to gain a better understanding of the prevalence and epidemiology of EIV shed by horses presented to veterinarians with an upper respiratory tract infection from March 2010 to November 2013.

2. Materials and Methods
Nasal secretions from 2375 equids with acute onset of respiratory disease were tested by qPCR for EIV. Categorical analyses were performed to determine the association between observations and EIV. Furthermore, observations from EIV positive study horses were compared to previous data from March 2008 to February 2010. Sequencing analysis of the HA1 gene of EIV was performed on 29 EIV isolates.

3. Results and Discussion
A total of 230 (9.7%) equids tested qPCR positive for EIV. The EIV positive equids in the 1 to 5 and 6 to 10 year age group, as well as Quarter Horses, were over-represented. Fever, nasal discharge, and coughing were more commonly observed in EIV positive horses than the entire study population. The EIV qPCR positive study cases were significantly older and more often vaccinated against EIV compared to EIV qPCR positive animals from the 2008 to 2010 study period. All of the sequenced EIV isolates belonged to the clade 1 Florida sublineage.

Acknowledgments
Conflict of Interest
This study was supported by Merck Animal Health.
Detection of Modified-Live Equine Intranasal Vaccine Pathogens in Adult Horses Using Quantitative Polymerase Chain Reaction

Corey Harms*; Samantha Mapes, MS; Nina Akana; Daniel Coatti Rocha, DVM; Nicola Pusterla, DVM, PhD, DACVIM

The duration of detection of modified-live intranasal vaccine pathogens in nasal secretions by qPCR appears to be dependent on vaccine schedule and ambient temperature. Authors’ address: Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616; e-mail: charms@ucdavis.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
Horses displaying signs of respiratory disease are routinely tested using real-time polymerase chain reaction (qPCR) analysis of nasal secretions. However, qPCR testing from a nasal swab does not distinguish between modified-live vaccine and wild-type pathogens. Thus, if a horse recently vaccinated intranasally subsequently develops signs of respiratory disease it would be unclear whether the qPCR detection of that specific pathogen is from the recently administered vaccine or represents true infection. Therefore, the objective of this study was to provide a timeline for how long after intranasal vaccination a horse will continue to shed modified-live S. equi subsp. equi (S. equi) and equine influenza virus (EIV).

2. Materials and Methods
Twenty-three adult horses were randomly assigned to one of two vaccine groups (S. equi = 12 horses; EIV = 11 horses), with two of the horses in each group to remain unvaccinated. All horses to be vaccinated received the vaccine in the left nostril at different periods according to the manufacturers’ recommendations. After each vaccination, both nostrils of every horse were swabbed daily for 5 days. The swabs were processed and analyzed via qPCR for the presence of S. equi and EIV.

3. Results and Discussion
For both vaccine pathogens, qPCR detection was limited to 2 days following first vaccination (S. equi and EIV) and 1 day at second vaccination 3 weeks later (S. equi). Following a boost vaccination at 6 months, qPCR detection lasted up to 5 days for both vaccine pathogens. The authors hypothesize that lower environmental temperatures during the 6 month revaccination period caused prolonged pathogen shedding times, possibly due to enhanced replication within the upper respiratory airways and impaired mucociliary clearance.

Acknowledgments
Conflict of Interest
This study was supported by Merial and a grant from The Center for Equine Health, University of California, School of Veterinary Medicine at Davis. The vaccines were kindly provided by Zoetis and Merck Animal Health.
How to Use and Interpret Quantitative Polymerase Chain Reaction for the Laboratory Diagnosis of Equine Herpesvirus-1 Infection

Nicola Pusterla, DVM, PhD, DACVIM*; Christian M. Leutenegger, Dr. Med. Vet., PhD, FHV

1. Introduction

Rapid laboratory tests for detection of equine herpesvirus-1 (EHV-1) are most useful in potential epidemic situations because immediate identification of the causative agent is often critical for guiding management strategies. Real-time polymerase chain reaction (qPCR) has become the diagnostic test of choice due to its quick turn-around-time and high analytical sensitivity and specificity. The qPCR is an enzymatic exponential DNA amplification technique that, under optimal conditions, is capable of detecting a small number of target viral genes. The PCR detection of EHV-1 is routinely performed in respiratory secretions from a nasal or nasopharyngeal swab and uncoagulated blood samples collected into Ethylenediaminetetraacetic acid (EDTA) tubes. Many conventional PCR protocols (single or nested PCR) targeting specific genes of EHV-1 have been published for the molecular detection of EHV-1.1–6

The increasing application of PCR for the molecular detection of EHV-1 in practice settings has presented new dilemmas with regard to how test results are interpreted and used by both equine practitioners and regulatory veterinarians since routine PCR assays are unable to differentiate between replicating (lytic), nonreplicating, or latent virus. Advances in technology and the use of novel PCR platforms, such as qPCR, allow calculation of viral loads for equine herpesviruses.7–13 Although considerable progress has been made in developing PCR assays, the lack of protocol standardization between laboratories and the variability in the use of contamination and quality assurance controls remain an ongoing challenge.

Viral load testing, although not routinely offered by many veterinary laboratories, represents a major improvement in the interpretation of PCR results in the EHV-1 field, allowing better characterization of disease stage, better assessment of risk of exposure to other horses, and better monitoring of response to treatment. The objective of this paper is to discuss the use and interpretation of quantitative PCR for the laboratory diagnosis of EHV-1 infection and to illustrate viral kinetics using field cases.
2. Materials and Methods

Quantitative PCR for EHV-1

Biological samples (whole blood and nasal secretions) were collected from symptomatic and asymptomatic horses naturally infected with EHV-1. The horses either presented to the William R. Pritchard Veterinary Medical Teaching Hospital, University of California, School of Veterinary Medicine at Davis or were part of recent outbreaks of EHV-1. Blood samples and nasal swabs were processed for nucleic acid purification using an automated nucleic acid extraction system, according to the manufacturer’s recommendations. All samples were assayed for the presence of the equine glycer-aldehyde-3-phosphate dehydrogenase (eGAPDH) and the glycoprotein B (gB) gene of EHV-1 using previously reported TaqMan PCR assays. Absolute quantitation of EHV-1 target molecules was performed using standard curves for EHV-1 and eGAPDH and expressed as EHV-1 gene copies per million cells.

Molecular Surrogate for Lytic EHV-1

The lytic cycle of infection for EHV-1 results in the release of new virus particles from the infected cells. During lytic infection, viral gene transcription and regulation is sequentially regulated into three distinct phases: immediate early, early, and late phase. The detection of transcripts for late genes has been successfully used as a surrogate for lytic infection of equine gammaherpes viruses. One hundred and thirty dual blood and nasal secretion samples from naturally infected horses were used to correlate the presence or absence of late gene transcripts, by measure of messenger RNA (mRNA) of the gB gene of EHV-1, with absolute quantitation of EHV-1 target genes. Total RNA purification and transcription of RNA to complementary DNA (cDNA) was performed according to previously reported protocols.

Characterization of Disease Stage

To determine viral load signature profiles of horses in different stages of EHV-1 infection, blood and nasal secretions were processed and quantitation was performed as mentioned earlier. The study population was comprised of 27 horses with neurological signs (EHM group), 28 horses with fever and no neurological signs (EHV-1 group), and 52 exposed but asymptomatic horses (asymptomatic group). The statistical difference \( (P < 0.05) \) in EHV-1 viral loads between the groups was determined using the Mann-Whitney Test.

Monitoring of Response to Treatment

Twelve horses presenting to the William R. Pritchard Veterinary Medical Teaching Hospital at Davis from 2006 to 2013 with acute onset of neurological deficits and laboratory confirmation of EHV-1 infection were sampled daily during their hospitalization period to determine the viral kinetics of EHV-1 in blood and nasal secretions. All horses received similar medical treatment, which included the administration of dexamethasone (0.05 mg/kg, 0.1 mg/kg IV q 24 h for 2–3 days), valacyclovir (30 mg/kg PO q 8 h for up to a total of 10 days), flunixin meglumine (0.5–1.1 mg/kg IV q 12 h), \( \alpha \)-tocopherol (10,000 IU PO q 24 h), di-methyl sulfoxide (1 g/kg IV diluted in fluids q 24 h for 3 days) and pentoxifylline (10 mg/kg PO q 8 h). Duration of EHV-1 detection in blood and nasal secretions was determined for each horse.

Indicator for Survival

Absolute quantitation for EHV-1 was performed on admission for 22 EHM horses presenting to the William R. Pritchard Veterinary Medical Teaching Hospital, University of California at Davis. The horses were grouped into survivors and non-survivors. Non-survivors were horses euthanized because of progression of disease despite intensive treatment and did not include horses euthanized because of financial reasons. Statistical difference \( (P < 0.05) \) in EHV-1 viral loads between the two groups was determined using the Mann-Whitney Test.

3. Results

The presence or absence of mRNA transcripts for the gB gene directly related to the absolute quantitation of this gene at the genomic DNA level (Fig. 1). Nasal secretions with the presence of lytic EHV-1 showed an average of 170 times higher viral load than nasal secretions with no lytic EHV-1 \( (P = 0.0001) \). Differences also occurred at the blood
level, with a 7-fold difference in viral load between lytic and nonlytic EHV-1 ($P = 0.0001$).

Only 17 of the 27 horses with EHM tested PCR positive in blood samples, whereas EHV-1 was detected in the nasal secretions of all horses with EHM (Fig. 2). When comparing only the samples from the 17 neurological horses with dual positive results, viral loads in nasal secretions (median $= 5.16 \times 10^5$ gB gene copies/million cells) were significantly higher ($P = 0.0001$) than in blood samples ($250$ gB gene copies/million cells). Equine herpesvirus-1 was detected in samples of both blood and nasal secretions from all 28 EHV-1 group horses. Viral loads were similar ($P = 0.76$) between blood samples ($1.6 \times 10^4$ gB gene copies/million cells) and nasal secretions ($6.0 \times 10^3$ gB gene copies/million cells). Only 13 asymptomatically infected horses had positive PCR results in blood ($459$ gB gene copies/million cells), while 51 of the 52 asymptomatic horses tested PCR positive in nasal secretions ($3.7 \times 10^3$ gB gene copies/million cells). When viral loads in blood were compared amongst the three infected groups, the EHV-1 group horses had significantly higher viral loads ($P = 0.0001$) than EHM and asymptomatic horses. Significant differences were also found for viral loads in nasal secretions between the EHM horses and EHV-1 horses ($P = 0.003$) and between the EHM horses and asymptomatic horses ($P = 0.0001$). No significant difference was found in the nasal viral loads between EHV-1 horses and asymptomatic horses ($P = 0.26$).

All 12 horses treated at the William R. Pritchard Veterinary Medical Teaching Hospital at Davis for EHM tested PCR positive for EHV-1 in blood and nasal secretions on admission. The detection period of EHV-1 in nasal secretions ranged from 3 to 9 days (median 6.5 days) with steadily decreasing viral loads (Fig. 3). The EHV-1 in blood was detected for 2 to 5 days (3 days) with rapidly declining viral loads (Fig. 4).

Viral loads from EHM horses on admission were a reliable indicator of outcome. The viral loads in nasal secretions and blood from non-survivors were an average of 300 and 10 times higher, respectively, when compared to the viral loads in survivors (Fig. 5; $P = 0.0025$).

4. Discussion

In recent years, qPCR has supplanted conventional cell culture for the detection of EHV-1 based on its quick turn-around-time, high sensitivity/specificity, and cost-effectiveness. Furthermore, quantitative PCR represents a major improvement in the detec-
tion of infectious pathogens by allowing characterization of disease stage, assessment of risk of exposure to other animals, and monitoring of response to therapy. This approach has long been used in the human medical field, where the molecular quantification of specific pathogens, such as HIV or hepatitis C virus, is considered standard of care.15–19 In recent years, novel molecular platforms, such as qPCR, have enabled the study of viral kinetics of EHV-1 in respiratory secretions, blood samples, and tissues after experimental or natural infection.7–12 However, research and diagnostic laboratories should use quantitative methods with the least evidence of variability between samples and extraction protocols. In order to improve standardization of quantitative PCR, a recent study compared viral load in nasal secretions using 4 different absolute quantitation methods.20 Protocols normalizing results against a preselected volume (i.e., eluted DNA or volume of nasal secretions) were more prone to interlaboratory variations than protocols standardizing viral load to the entire swab, a house-keeping gene, or an arbitrarily chosen amount of extracted DNA. It is important that diagnostic laboratories use an internal quality control system to increase the reliability of results and minimize the risk of reporting false-negative results.

The use of qPCR for the detection of the gB gene of EHV-1 and its transcripts allowed the characterization of the viral state in 130 blood samples and nasal secretions. The molecular approach used in this study represents a crude measure of very complex biological processes that are still not fully understood. Furthermore, one must bear in mind that during an ongoing EHV-1 infection, the viral state rarely fulfills the “all or none” rule, but rather progressively transitions from one state to the next. The lytic or replicating state is molecularly easy to assess based on the detection of transcripts from essential genes, such as the gB gene, that are absolutely required for virus replication. Detection of virus in the lytic state is a strong indicator of active infection and potential for viral transmission and environmental contamination. Lytic infection in nasal secretions was characterized by the presence of transcriptional activity of the gB gene and, as expected, was associated with high viral loads at the DNA level (range 2 × 10^3–1.7 × 10^9 gB gene copies/million cells), while nonreplicating virus was routinely seen with low viral loads (62–1.0 × 10^5 gB gene copies/million cells). Because of the greater difference between lytic and non-lytic EHV-1 in nasal secretions, a threshold of 10^5 gB gene copies/million nasal cells was able to differentiate between lytic and nonlytic virus in all samples. The difference between lytic (200–3.3 × 10^6 gB gene copies/million cells) and nonlytic (18–2.0 × 10^4 gB gene copies/million cells) EHV-1 in blood samples was narrower, making a specific threshold more difficult to establish. However, one must bear in mind that the detection of EHV-1 gB gene DNA with no detection of transcriptional activity of the same gene may have other possible biological (i.e., abortive EHV-1 infection without the expression of late genes) and technical (i.e., differences in analytical sensitivity of the assays, sample processing) explanations. From an infectious disease control standpoint, one can assume nasal secretions to contain infectious EHV-1 based on a high viral load (≥1 × 10^5 gB gene copies/million cells) and/or evidence of transcripts for the gB gene.

The study showed differences in viral loads determined by qPCR between disease stages in adult horses as well as between clinically and subclinically infected horses. Neurological deficits associated with EHV-1 infection appear during the viremic phase of infection and the interval between infection and subsequent onset of neurological disease is usually between 6 and 10 days.21 The 27 neurological horses showed low viral loads in blood and up to one million-fold higher viral loads in nasal secretions. Anecdotally, many veterinarians mistakenly believed that horses with neurological manifestations of EHV-1 infection were no longer shedding virus. The results of this study clearly demonstrate that horses with EHM shed high loads of replicating EHV-1 in nasal secretions and, therefore, pose a substantial risk to other horses. From a biosecurity standpoint, horses affected by EHM should be isolated and confined away from other horses in order to decrease the risk of exposure to susceptible individuals. Although the risk of transmission of EHV-1 from neurologically affected horses has not
been thoroughly evaluated, indirect spread by fomites and personnel is the more likely reason for the rapid spread of infection during outbreaks of EHM. Recent outbreaks at racetracks, riding schools, and veterinary hospitals provide ample documentation of the contagiousness of neurologically infected horses.\textsuperscript{22–25} Viral loads in blood and nasal secretions of horses with EHM were different from the viral loads encountered in febrile and subclinical horses. All 28 horses presenting for fever without neurological deficits had detectable EHV-1 in both blood samples and nasal secretions with similar viral loads between the two sample types. Our results are in agreement with experimental studies showing that viremia commonly coincides with the pyrexia peak in EHV-1 infection.\textsuperscript{10} While the minority of the subclinically infected horses had detectable EHV-1 in blood, almost all had detectable EHV-1 in nasal secretions, with 21% of them displaying viral loads $\geq 1 \times 10^5$ gB gene copies/million cells. These results highlight the infectious nature of exposed but asymptomatic horses and the need to test both symptomatic and asymptomatic horses during an outbreak in order to help guide infectious disease control measures.

Equine herpesvirus type-1 in 12 horses with EHM remained detectable in blood and nasal secretions up to day 5 and day 9 of hospitalization, respectively. Viral loads slowly decreased in the blood and nasal secretions of all horses over the hospitalization period. The results are in agreement with a recent study determining nasal shedding in 16 horses with EHM.\textsuperscript{24} In that study, the last day of EHV-1 shedding in horses with EHM was day 9. The effect of specific treatment could not be determined since all diseased horses received a similar treatment.

The evaluation of viral loads in blood and nasal secretions in horses with EHM on admission showed remarkable differences between survivors and non-survivors. However, based on the small number of horses per group, one must interpret the results carefully and always use clinical findings and response to treatment to determine prognosis, rather than single laboratory values. Non-survival in 4 horses with EHM was associated with the development of clinical signs of encephalopathy. The biological basis for the variable virus burdens in the different equine patients remains elusive. One recent study on severe acute respiratory syndrome-associated coronavirus in human beings determined that the viral load was a determinant of clinical outcome and was associated with polymorphism of genes involved in innate immunity.\textsuperscript{26} It remains to be determined if a similar mechanism applies to horses with EHM.

In conclusion, the present study showed that the EHV-1 viral load and viral state can be determined using real-time PCR on field samples collected during various EHV-1 outbreaks. This molecular approach can help assess the risk of exposure to other horses and permits determination of viral kinetics in infected animals. The results of this study represent the first attempt in the development of guidelines for the use and interpretation of qPCR results from EHV-1 cases. This information is important to determining the risk of exposure to other horses and to helping practitioners and regulatory officials make appropriate decisions regarding the management of horses that test positive for EHV-1 by PCR.

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Conflict of Interest

The Authors declare no conflicts of interest.

**References and Footnote**


*CAS-1820 X-tractor Gene, Corbett Life Science, Sydney, Australia.*