

Glanders Testing Is A Slowly Evolving Process

After an international dressage horse turns up with a false positive, this contagious disease, the USDA testing protocol comes under fire

BY LINDSAY BERRETH



After returning from Europe, Chase Hickok's Sagacious HF tested positive for glanders and spent more than two weeks in a quarantine facility and would have had to return overseas if his connections hadn't been able to prove it was a false result. JENNIFER KEELER PHOTO



Chase Hickok wasn't anticipating anything unusual as she drove to the U.S. Department of Agriculture quarantine center at the Miami International Airport on Aug. 8 to pick up Grand Prix horse Sagacious HF after a successful European tour.

But when she got a phone call from the flight broker telling her the 18-year-old Dutch Warmblood gelding (Welt Hit II—Judith, Cocktail) had tested positive for the highly contagious disease glanders, a routine pick-up turned into something much more stressful.

Sagacious, owned by Hyperion Farm, has traveled the world with Hickok, 27, as well as his former riders Caroline Roffman and Lauren Sammis. Hickok had just returned to Wellington, Fla., from a trip representing the United States on Nations Cup teams at Hickstead (England) and Falsterbo (Sweden).

When horses come back to the United States from Europe they undergo a quarantine period and get tested for glanders along with dourine, equine piroplasmiasis and equine infectious anemia.

Glanders, which has been eradicated from the United States, is caused by the bacterium *Burkholderia mallei*. It's transmitted horse to horse through infected nasal discharge, muzzle to muzzle contact, or shared water and food troughs. Objects that have been in contact with infected horses, such as brushes, halters and even human clothing can also spread the disease.

It's characterized by ulcerating nodules, usually in the upper respiratory tract, lungs and skin, as well as a high fever and thick nasal discharge.

However, while a horse may become acutely infected and show clinical signs, that's not always the case.

"Horses tend to be chronically

infected by and large," said equine infectious disease expert Peter J. Timoney, Ph.D., FRCVS, and a professor at the University of Kentucky's Gluck Equine Research Center. "To the naked eye they are very often clinically normal."

There is no vaccine for glanders, and affected horses are most often euthanized because even if they survive, they may then become carriers. *Burkholderia mallei* is a zoonotic agent, which means humans can contract the disease from infected horses.

Different tests exist to identify glanders, and the two most common are the complement fixation test and the Western blot test (see "Getting Scientific" sidebar).

The CFT is the standard test at USDA quarantine facilities, and Sagacious tested positive twice, but he showed no signs of infection.

"I flew with him on the plane and had another horse on the plane as well that was in the same box as he was for the entire duration of the flight," said Hickok. "That horse, from Day 1, has tested negative."

Sagacious hadn't traveled to any area where he would be at risk of exposure to glanders either, but after the second test came back positive, Hickok and owner Al Guden were told to send the gelding back to Europe or euthanize him.

An Established System

Donna Karlsons, public affairs specialist at the USDA/Animal And Plant Health Inspection Service, said APHIS uses CFT because it's an established, accurate test that's been used for many years.

"It is described in the test manual of the World Animal Health Organization (OIE) and is used by other importing countries (in Europe and South America)," she explained via email.

"For well over 100 years, the classical traditional test, that serological test that we've used to detect antibodies for this bacterium, just the same as it was for many other bacterial diseases of horses, was the complement fixation test," said Timoney. "It's a very old test. It's a very good test when used in the



Glanders has been eradicated in the United States. Horses suffering from glanders have a thick nasal discharge and ulcerating nodules, usually in the upper respiratory tract, lungs and on the skin. PHOTO COURTESY OF THE BROOKE

appropriate context. But like everything else, with the passage of time we've come to know a great deal more about the advantages and, needless to say, the shortcomings of the test."

The Western blot or immunoblot test uses a different procedure to identify the disease (see "Getting Scientific" sidebar), but it's not USDA policy to offer this option if a horse comes up positive via CFT.

"Laboratories around the world are practiced in and rely on the CFT," said Karlsons. "This extensive experience with a specific test generally allows better interpretation and understanding of test results, with better knowledge of how animals in different stages of an infection react to the test."

Karlsons added that the Western blot is not as widely used by interna-

tional laboratories, and therefore they have less experience interpreting and understanding the test results.

But the fact that CFT has existed for so long and in so many places does have some drawbacks.

Timoney pointed to research coming out of the OIE World Reference Center in Riems, Germany, that showed complement fixation tests for glanders are not the same all over the world.

"There are three known sources of antigen that labs have used in their tests to detect antibodies to [glanders]," said Timoney. "Depending on which antigen-based assay you're using, you'll get a variation in both sensitivity and specificity. By specificity I mean that the result, if you get a positive result, it is a bonified positive result and not a spurious positive result."

Of the three antigen-based assays, the one the USDA uses was 100 percent sensitive but did not have as good specificity as the other two, according to Timoney.

He also pointed to cross reactions, as there are bacteria that are similar to glanders. "Glanders is unique, but it does have relatives, and those relatives can contribute to the problems we find occurring with respect to the false positives in the CF test," said Timoney.

Another issue that may arise with the CFT is the very length of time the test has existed. "The antigen is prepared from a strain that it's been prepared from for decades and decades and decades," said Timoney. "It means that when that strain was initially isolated and used to prepare the antigen, used in the test, it probably had a higher level of sensitivity and specificity. Over time that antigen is not necessarily the most reflective of strains of glanders bacillus that are in current circulation."

But trying to identify the current strain of glanders bacillus most likely to menace the United States is tough because we don't have the disease in this country.

A study led by Mandy C. Elschner and published in 2011 demonstrated the Western blot "showed a markedly

► Getting Scientific: Western Blot Vs. Complement Fixation Tests

The Western blot and complement fixation tests used to detect glanders in horses are serological assays, meaning they detect antibodies specific for the disease organism, *Burkholderia mallei*, in the serum or blood fraction of infected horses, according to Donna Karlsons, public affairs specialist at the USDA/Animal And Plant Health Inspection Service.

"They do not detect the infecting organisms themselves, rather the antibodies that the animal produces shortly after being infected with the bacteria," she said. "These antibodies remain in the animal well after an infection occurs."

"In the CFT the antibodies react with a certain segment, the lipopolysaccharide fraction, of a reference strain of the infecting bacteria," Karlsons continued. "This reaction 'fixes' and depletes an immune molecule called complement. The complement is then unavailable to react with an indicator antigen-antibody reaction, creating a visual change that is read by trained/experienced personnel."

"In the Western blot, certain segments, or antigens, of three strains of the bacteria are separated by size using electrophoresis, then transferred to a membrane," she added. "The serum is put in contact with this membrane containing the antigens, and any antibodies present in the serum bind to these antigens. The bound antibodies are then detected with chemiluminescent reagents. The assay is read by a machine, looking for distinctive banding patterns on the membrane, focusing solely on one of the antigens—the lipopolysaccharide antigen, the same antigen used in the CFT."

"Subject to the test being run appropriately, the antigen being prepared from an appropriate source correctly, then [Western blot] does represent the most sensitive test," said Peter J. Timoney, Ph.D., FRCVS. "What you're doing is you're taking a complex mixture of antigens—in this case of this particular bacterium—and you electrophorese, or you pass a current through them in a gel, and that separates out those antigens into a series of bands, so each individual antigen has its own band that migrates at a different rate in the gel."

"Then you attempt to identify whether the bands that you get following testing a particular horse's serum correspond to the bands that you know are proven to be specific for *Burkholderia mallei*," he added.

Timoney explained that with Western blot, it's not a positive or negative reaction. "You are actually identifying the specific individual antigens that in fact are immunoreactive in the test," he said. "It's both a highly specific test and a highly sensitive test. It's not as a quick, it's not as rapid, it's more labor intensive, but it's still a very definitive test."

higher diagnostic specificity when compared to the prescribed CFT and therefore can be used as a confirmatory test."

However, Elschner noted that the CFT "remains the test of choice for routine testing of glanders due to its high sensitivity, its feasibility using standard laboratory equipment and its worldwide distribution in diagnostic laboratories."

Elschner's study pointed to the fact that CFT has a specificity of only 94.5 percent, and "consequently, a considerable number of CFT false positive tested animals must be expected when horses from glanders free regions are tested by the current OIE standards."

Another Chance

Hickok and Guden had no interest in euthanizing Sagacious or sending him back to Europe, and once they realized false positives do occur, they were determined to come up with another solution.

"As we were starting to fact find, our friend in California, Christine Traurig, sent an article about a race horse in a similar situation," said Hickok. "Her owners had moved heaven and earth to get her released and enlisted the help of an attorney. Immediately I got on the phone and started calling these race horse owners and left messages and sent emails, not really expecting them to get back to me, but thinking, 'What the heck? It can't hurt at this point.' Sure enough, 30 minutes later I got an email from this attorney, Chapman Hopkins."

Hopkins had worked on the 2016 California case where a Thoroughbred mare named Tooreen Dancer had a false positive CFT after she was imported from Ireland.

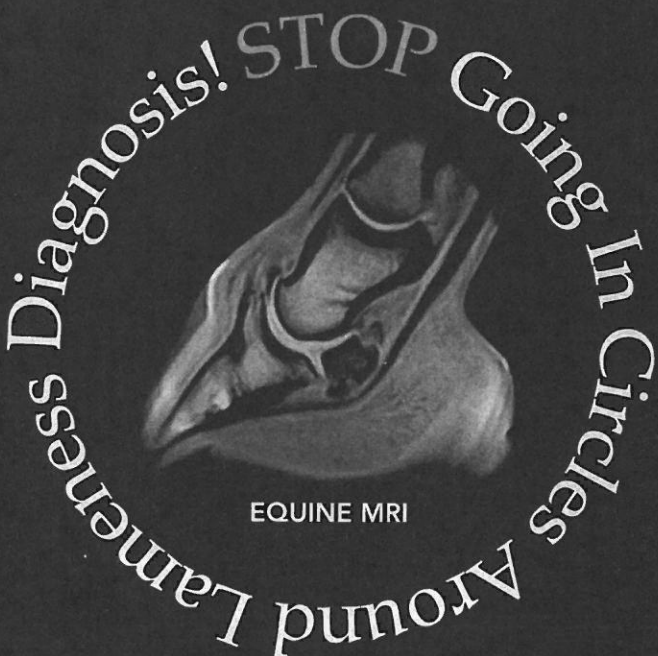
The Lexington, Ky.-based Hopkins said that before Tooreen Dancer, nobody had seen a glanders testing issue to that extent, and nobody had ever pushed the USDA. He reached out to industry groups, including the National Thoroughbred Racing Association, which was instrumental in getting through to U.S. Senator Mitch McConnell (R-KY).

"It was all hands on deck—it happened right before Christmas—pulling at every resource and connection I had in the equine industry and in the U.S. government," said Hopkins.

With pressure from McConnell's office, the NTRA and Timoney's expert testimony, the USDA changed their protocol for Tooreen Dancer, ultimately allowing for repeated CF

tests. She eventually tested negative and was able to stay in the country after spending 30 days in quarantine.

"Part of this was showing the USDA why their CFT test protocols were deficient, why they led to false positives," said Hopkins. "Dr. Timoney was very good about putting the deficiencies in layman's terms and helping us convey those to the USDA."




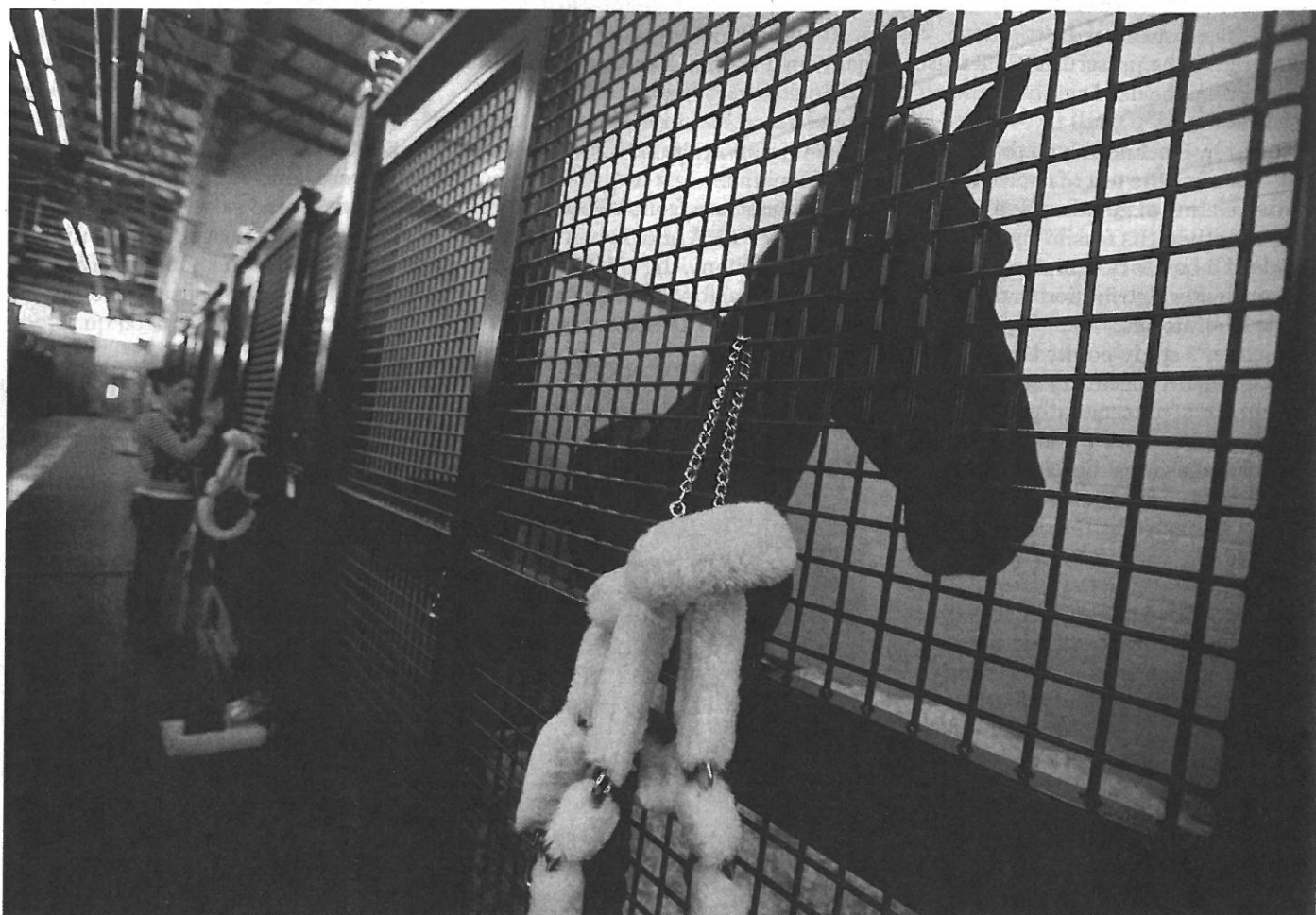
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"To that point in time there had never been a horse that had tested positive twice under the complement fixation test and then been permitted to stay in the country and do an alternative protocol," Hopkins added. "There had been instances where they'd tested positive once and then they shipped them back out of the country [see 'An Expensive Journey' sidebar]."

Hopkins advised Hickok and Guden not to ship Sagacious out of the country, and he agreed to help them petition the USDA for another test. And 24 hours after Hopkins contacted the USDA, officials agreed to test Sagacious using the Western blot test, which is more commonly used in Europe. That test, conducted on Aug. 11, came back negative.

"That was a huge sigh of relief for us," said Hickok. "The USDA has been nothing but understanding of our situation and said, 'This CFT test we're using is maybe not the most

reliable or accurate. Let's see what we can do and figure out a way to make this work for everybody.'"

The gelding had to stay in quarantine until Aug. 24 for another round of testing using both tests, and when those came back negative he was released.

"APHIS recognizes that additional pieces of information—including the history of the animal and tests on additional samples—can help clarify the horse's health status," said Karlsons.

She pointed to the USDA policy of conducting follow-up tests if the CFT comes back "non-negative."

"In rare instances, APHIS has allowed a horse to remain in quarantine and have further testing done," Karlsons said. "In these very few instances, there was not a set number of retests to be completed. The horses were sampled until the test was negative."

U.S. Department of Agriculture quarantine centers, like those at The Ark at JFK Airport in New York, are the first step to preventing the spread of disease to animals in the United States. THE ARK/CORNELL UNIVERSITY PHOTO

The Wheels Of Change Turn Slowly

According to Karlsons, APHIS officials routinely evaluate all the test methods available and incorporate the ones that are the most appropriate.

"We are currently evaluating testing methods for equine diseases assessed in our quarantine requirements, and the policy may change based on this evaluation," she said.

After the Tooreen Dancer case, Hopkins has continued to work with the NTRA to encourage the USDA to re-examine testing protocol. He said that because the USDA is a government agency, change takes time. They aren't allowed to deviate from

► An Expensive Journey

When Terry Trimble Catlett, DVM, saw the story of Sagacious HF's false positive glanders test, she knew all too well the struggle that Chase Hickok and Al Guden were going through.

In October 2015, Catlett, a Dartmoor Pony breeder based in Boyce, Va., went to England with a group of Dartmoor owners.

The group brought back two stallions and four weanling fillies, including one that Catlett purchased, Springwater Alicia (Moortown Honeyman—Springwater Anna, Springwater Intermezzo).

The ponies shipped from Amsterdam's Schiphol Airport to JFK Airport in New York. Springwater Alicia had a fever when she got to Amsterdam. She received Banamine, and the fever subsided by the time she got to New York.

She tested positive on her complement fixation test at JFK. Twenty-four hours later she was retested and labeled a suspect. All of the ponies were held.

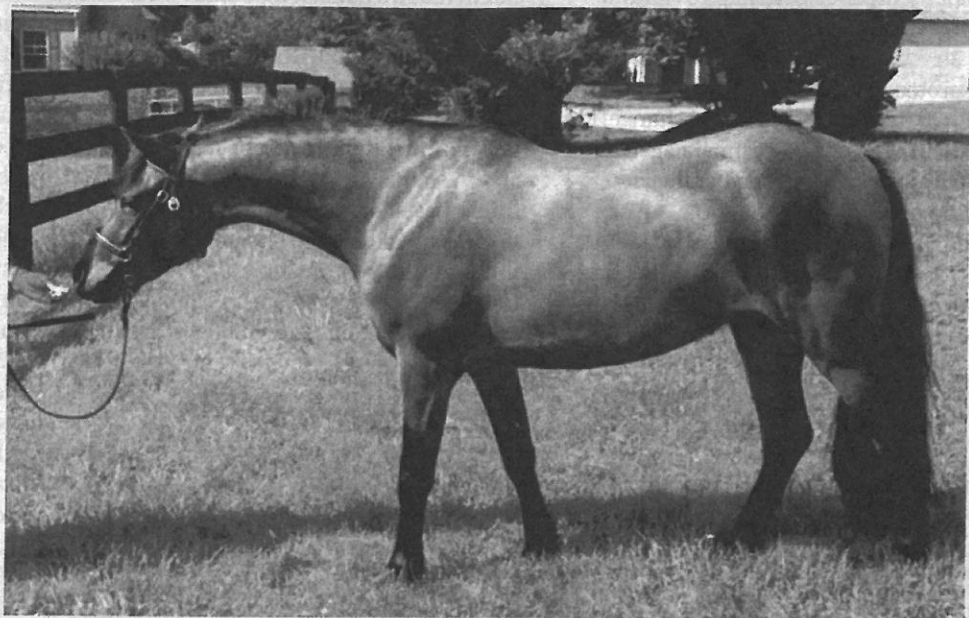
Catlett was given the option to send the filly back or euthanize her. "It doesn't make sense—how can you ship something that you think has a bioterrorism threat back to your ally?" said Catlett. "But that's what they're telling you to do. England's quarantine system is very different than ours. It's not government run. They have private contractors. Who wants to accept a pony that's labeled as a suspect?"

Because Catlett had bought the pony from her breeder, they were able to connect her to a quarantine barn.

She was retested in England via CFT, and that came back negative, so Springwater Alicia was released from the Department of Environmental Foods And Rural Affairs certified quarantine facility back to her breeder on Dec. 9.

Catlett then had another veterinarian draw blood for three more CFT tests before deciding to try importing the pony to the United States once again.

"As far as the Western blot, we requested it but do not have documentation that they ran it," she said. "DEFRA would not communicate with the local vet, which he found unusual and disconcerting. We also



had communicated with a [World Animal Health Organization] reference lab while she was in quarantine in the U.S. They would have run the Western blot on Springwater Alicia but only if requested by the USDA. The USDA refused."

When the filly returned to the airport in Amsterdam, there was another problem.

"All the horses that were due to fly with her on that flight wouldn't fly with her because of the fear of getting held up for two weeks," Catlett said. "At this point she has more negative bloodwork than anybody on the airplane."

So Springwater Alicia shipped back to the United States by herself. Finally on Feb. 14, 2016, she was released to Catlett after testing negative for glanders. Catlett said she spent more than \$100,000 on the process.

"When we went through this the USDA was not particularly helpful," she said. "We contacted our representatives, and we got a conference call where they said, 'We just have to stay to our protocol. This doesn't happen very often, and we're safeguarding the herd.' All reasonable things. Glanders would be a much bigger problem if we didn't test for it. It's not that we shouldn't test for it, it's that you have to work through those false positives. Perhaps all of the horsemen coming together will get them to change it."

Terri Trimble Catlett went through a nightmare importing her Dartmoor Pony Springwater Alicia due to a false positive test for glanders. PHOTO COURTESY OF TERRI TRIMBLE CATLETT

► FALL HORSE CARE ISSUE

protocol unless the legislature enacts new regulations.

"That's why it was crucial to get to a senior member of Congress to communicate with the USDA that these changes are necessary, that the deviation from the protocol was appropriate," he said. "I think to the extent they didn't change, it's just a product of a government agency strictly complying with what their protocol calls for."

Hopkins saw the fact that the USDA had already changed their protocol, first allowing Tooreen Dancer to stay and undergo more testing, and then just eight

months later re-testing Sagacious using Western blot, as progress.

"My understanding is that to the extent that it's taken longer than we'd hoped for to get a revised protocol in place, it's not coming from a place of reluctance to change the protocol," he said. "They want to make sure that when they do amend the protocol, it's accurate and the best setup they can achieve. I think they should be applauded in that respect—that they are changing, and they are trying. It's just taking a long time to get there."

In his arguments for why the USDA

needs to change, Hopkins has focused on explaining why a black and white policy doesn't work for glanders testing.

"You're telling us that your complement fixation test is accurate, and if your accurate test is resulting in the horse testing positive for glanders, how from a liability perspective can you possibly expect me to advise my clients to stick a positive horse on a plane and send it back to a country of origin?" he said. "Because God forbid the horse actually is positive. Then we've knowingly spread glanders to a European country. I'm not going to advise my client to do that."

"You're really putting us in a box where the only thing we can do is euthanize the horse, and we're going to be doing that when everybody knows the horse is probably negative for glanders," he continued. "At that point it becomes an animal welfare issue."

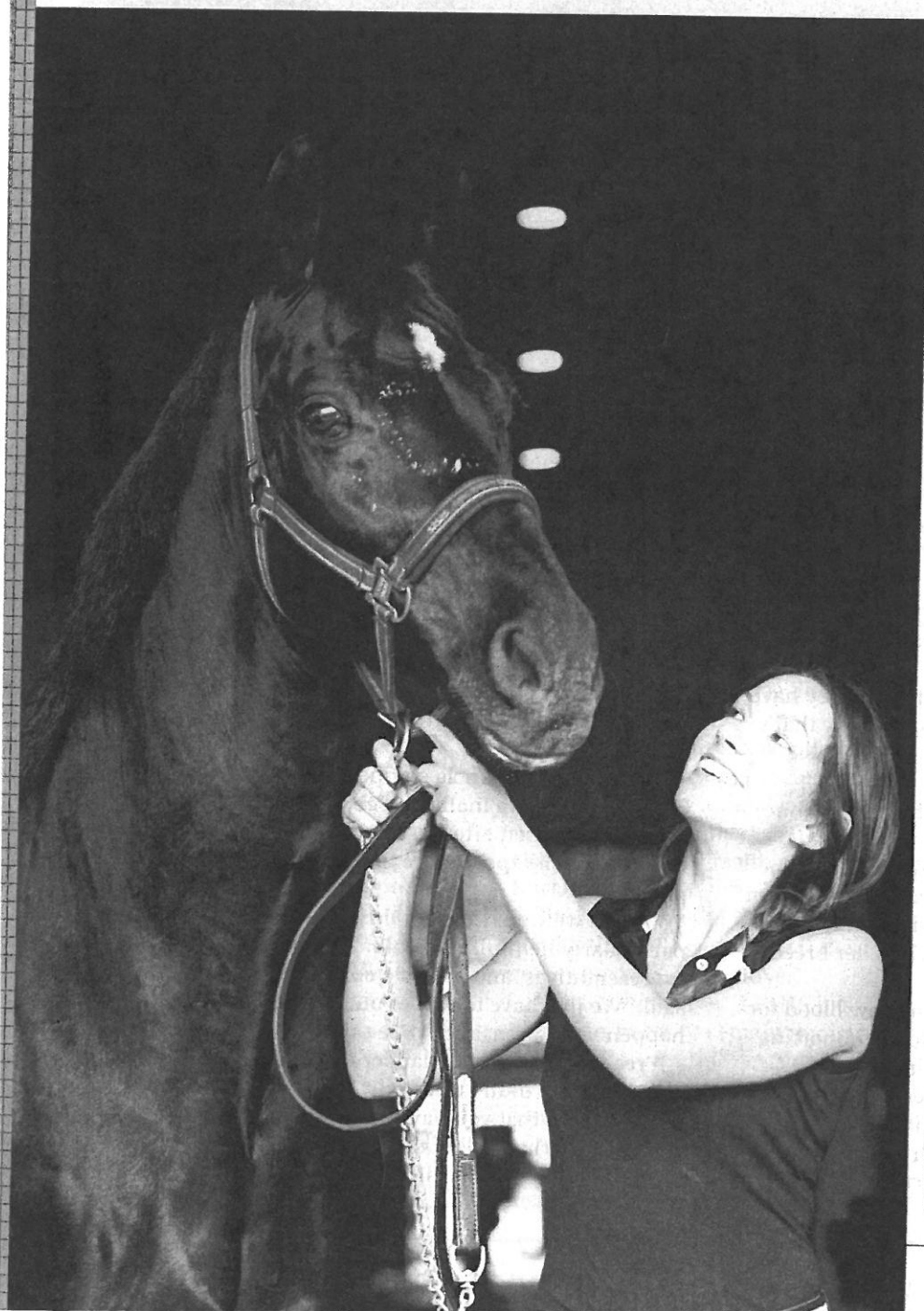
Cliff Williamson, director of health and regulatory affairs for the American Horse Council, said the AHC sometimes gets calls about issues with different health tests for imported horses. He's heard of three cases of false positive glanders tests in the past 1½ years, including Sagacious.

"Preventing a new disease from coming into the country is of paramount importance, and the Horse Council takes that very seriously," he said. "Everybody has to think about the entirety of the horse population over the convenience of one animal. But that said, we also petition the USDA annually to continue to investigate and research new, potentially better testing alternatives. We have reached out at the behest of both horse owners and horse organizations to pressure the USDA to do follow-up testing and things like that."

He added that the AHC has confidence that the USDA protocols work,

"I really hope we can effect some change to this and help to streamline the procedures and make it not such a confusing and scary and devastating process for so many people," said Chase Hickok after a routine test turned up a false positive for glanders for her Grand Prix mount Sagacious HF during his re-entry to the United States after a European tour.

JENNIFER KEELER PHOTO



► How Do You Eradicate A Disease?

Glanders used to be prevalent at the turn of the century in the United Kingdom and most of Western Europe when working horses spread the disease via common drinking troughs in the town squares. But when the complement fixation test was developed, it provided a means to get rid of glanders.

"They used complement fixation test, and they slaughtered out," said Peter J. Timoney, Ph.D., FRCVS, and a professor at the University of Kentucky's Gluck Equine Research Center. "The CF test is a great herd test. It will separate out the negative animals, and over time hopefully you'll be able to detect the positive ones as well. It doesn't work as effectively if you use it purely on an individual animal basis. People don't grasp the difference."

Which is to say that when you aren't dealing with horses that have great sentimental or financial value or both, they don't get a second chance. If they tested positive, they were euthanized. Some healthy horses most likely died, but they were sacrificed for the greater good.

Today, that argument doesn't work, as the owners of horses flying back and forth across the ocean for international competition are far more attached to the individual animals.

"Horses are different from any other livestock species or domestic animal species," said Timoney. "No. 1: They live the longest. And that presents problems for them and for those of us who take care of them. They have the potential to be exposed multiple times to various infections that other species are not. No. 2: They travel more than other species except humans. That wasn't the case years ago, but it is the case today. No. 3: The individual unit invariably has a name, not a number, and it's of inestimable value."

Timoney said the Western blot test is in the process of being validated by the World Animal Health Organization, and researchers expect it will become the most definitive test for glanders, as it has both the sensitivity and specificity necessary for testing on an individual animal basis.

But that doesn't mean it will become the routine test, as CFT still works well for the majority of horses and is already established all over the world. Western blot could just become the routine backup procedure.

"What you're trying to do is speed up the process and yet at the same time take full advantage of what we know scientifically in terms of diagnosis of these respective infections," Timoney said.

but they support alternative testing options for these instances.

Williamson also noted that should the USDA decide to adopt the Western blot test as part of their protocol, things take time in Washington, D.C.

"The Western blot test has been on the radar for several years, but the notion that the United States needed to change is a relatively new conversation," he said. "It is to be expected that there will be some delays in getting everything adopted, but they are aware of the test and do acknowledge some of the research that's been done."

Limited resources and financial

considerations also play a role in the speed at which new testing methods are implemented.

"It is frustrating, and I certainly sympathize with any horse owner who's been put into this kind of situation, but we all have to take a step back and recognize that the most important thing for the country is to protect the national herd health," Williamson said.

Timoney also referred to the national herd health, pointing out that horses travel more than ever now.

"With the increased frequency of movement, you can no longer accept in all instances that there



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isn't a potential risk of introduction of a disease through movement, either legal or illegal," he said. "And don't forget if we're dealing with animals coming in from [the European Union], there is absolutely zero testing required when a horse leaves from the easternmost EU member state to the westernmost member state within the EU.

"We cannot presume anymore under different trade agreements that we enjoy the same rigid control that we used to many years ago," he said. "That's just part of doing trade. What you're trying to do is arrive at a compromise, not totally restrict movement to make it impossible to trade, but put enough safeguards in place such that there is a very minimal risk."

But despite the increased risk of diseases moving around, or perhaps because of it, Timoney said it's more important than ever to have a testing

"I've been contacted by so many people who have had similar stories."

—CHASE HICKOK

protocol that takes into account the fact that false positives can occur.

"It should not have required external intervention or pressure, especially political pressure, to have given rise to a response," he said. "To have a test at the side that they weren't using routinely to screen an animal, but given a circumstance where a particular serum was reacting in the CF test, to subsequently subject it to the immunoblot test. That should have been automatic. It shouldn't have required some external agency or

individual to have prompted that type of response.

"I'm not blaming USDA," Timoney continued. "I'm just saying there needed to be a greater consciousness, awareness. You've got to be aware of not just the individual affluent owner in this industry but the rank and file horse owner who may not have any means of interceding with the agency with respect to getting their animal retested using different tests."

Hickok is relieved that Sagacious was able to come home, and she hopes her story will shine a light on false positive glanders tests.

"I've been contacted by so many people who have had similar stories, some with the same outcome, some with not such a good outcome," she said. "We've learned [false positive glanders tests are] way more prevalent and way more common than we ever thought.

We're hopeful that between all of us who have gone through this experience we can try to make some changes and reexamine the protocol.

"It's the emotional burden, the financial burden," she continued. "It's such a stressful experience, and I really hope we can effect some change to this and help to streamline the procedures and make it not such a confusing and scary and devastating process for so many people."

"It's an issue that's out there, and something that needs to be fixed sooner rather than later," Hopkins added. "It's just going to take a group effort from industry participants, owners, attorneys, trainers and press. It's going to take that to finally get it pushed through." ☉

After a lengthy import ordeal thanks to a false positive test for glanders, Chase Hickok's Grand Prix partner Sagacious HF was able to safely return home.

KIMBERLY LOUSHIN PHOTO

