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COMMENTARY

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LLOYD'S

EPIDEMICS OF EQUINE INFLUENZA IN JAPAN and Australia during the summer of 2007 have raised questions regarding the failure of quarantine and influenza vaccination to control the spread of disease.

Expanding and increasingly mobile equine populations have changed the dynamics of equine infectious disease. For example, an increased number of Thoroughbred stallions are shuttling between the Northern and Southern hemispheres. Horses were traditionally shipped by sea, allowing time to recover during transit from diseases such as equine influenza. With widespread acceptance of air travel, horses arrive in the importing country 48 hours after departure, within the incubation period for influenza. Consequently, horses may have clinical signs on arrival, and more importantly, are shedding vast quantities of virus, infecting every horse with which they come in contact.

As a result, epidemics of influenza occurred among equine populations of South Africa in 1986 and 2003, Hong Kong in 1992, and Japan and Australia in 2007. These epidemics occurred despite imported animals being subjected to officially approved quarantine and vaccination against influenza.

Investigation of the outbreaks in Hong Kong and South Africa exposed flaws in quarantine that permitted animals to be released into the general population while still infectious. Contaminated personnel and equipment also inadvertently carried the virus outside the quarantine facility.

The outbreak in Australia has been calamitous, given its equine population had not been exposed to influenza and therefore was susceptible to infection. The social and economic disruption to the equine industry in Australia is significant. Federal and state authorities in that country continue to struggle to control the disease and provide massive financial support to sustain the industry.

The impact has prompted a major inquiry into circumstances surrounding the outbreak, particularly failure of the quarantine facility in Sydney to contain the outbreak among imported stallions shuttled from several countries in the Northern Hemisphere. The findings will be important in developing guidelines for more effective approaches to equine quarantine in all importing countries.

A procedure that could be applied immediately is to test horses in quarantine for influenza using polymerase chain reaction (PCR) or enzyme-linked immunosorbant assay (ELISA) rapid test kits. In the hands of trained laboratory personnel, the test kits are simple to use and inexpensive. They also provide good sensitivity and specificity, the requisite hallmarks of a reliable testing system. Such testing has successfully been used by the Hong Kong Jockey Club since 1992 to screen all imported horses, including those from Europe and North America, which are areas known to be endemic for equine influenza.

Veterinary regulatory authorities would do a considerable service to the equine industry by incorporating these modern technologies in their import certification protocols. The practical application of such a proposal during pre-export and/or post arrival will require very careful consideration prior to implementation. An agreed plan of action must also be in place if a positive result is reported.

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INTERNATIONAL
Third Quarter 2007

THE INTERNATIONAL COLLATING CENTRE, Newmarket, England, and other sources reported the following disease outbreaks.

The United States Department of Agriculture (USDA), as of October 30, reported on the Equine Health Monitoring and Surveillance Web site www.aphis.usda.gov/vs/nahss/equine 130 cases of Eastern Equine Encephalitis (EEE) during 2007, the majority occurring in the southern states of Mississippi (30), Florida (17), Louisiana (38) and Texas (19).

A single case of West Nile Virus (WNV) infection was reported from the United Arab Emirates in a non-Thoroughbred horse that recovered. The USDA reported 407 equine cases of WNV during 2007 up to November 6, widely distributed throughout the USA. The highest numbers of WNV cases were in Texas (59), Montana (32), California (28), and Colorado (29). By comparison, 1,086 equine cases were reported in the USA in 2006. As of November 6, the Centers for Disease Control reported 3,265 human cases for 2007, with 92 fatalities.

Cases of equine herpes virus (EHV) respiratory disease were widely reported among a variety of breeds in France. In the United Kingdom, respiratory disease attributable to EHV-1 was diagnosed among a group of donkeys and to EHV-4 among a group of foals. Cases of EHV-1 abortion were diagnosed in June on three premises in the Western and Eastern Cape Provinces of South Africa. The neurological form of EHV-1 was confirmed among six horses on a single premise and coital exanthema (EHV-3) on two premises in the United Kingdom.

Equine Infectious Anemia (EIA) was identified during August in a horse imported from Romania to central Germany, and during September in a 12-year-old saddle mare that died in Ardeche, France.

Further details have been provided regarding the outbreak of Equine Viral Arteritis (EVA) in France. The outbreak, occurring between May and September, originated with a Percheron stallion shedding the virus in the semen and other stallions at the Haras du Pin National Stud. Cases were subsequently diagnosed on 28 premises in five different areas. Several hundred mares and foals, mainly draught and saddle horses, were found to be seropositive. Five foals died and several mares aborted. Thoroughbreds were not affected. Equine arteritis virus was transmitted following artificial insemination and natural mating. Symptoms in mares were mild or subclinical, with foals developing acute signs including fever, respiratory signs, and edema of the sheath and limbs.

The outbreak of equine influenza in Australia, which began in mid-August, remains confined to New South Wales and Queensland. It originated in the Sydney quarantine facility that housed imported shuttle stallions, with escape of virus resulting in widespread infection of the indigenous, naive population. As of November 26, 5,391 infected premises are in New South Wales and 2,166 in Queensland. In place are zoning, movement restrictions, biosecurity, and influenza vaccinations in buffer zones and of strategic horse populations. High value horses, generally Thoroughbred racehorses, are being vaccinated in New South Wales, Queensland, and Victoria. Vaccination has been initiated using a live recombinant vaccine produced by Merial.

The extensive outbreak of influenza in Japan first diagnosed at the Miho Training Center of the Japan Racing Association (JRA) on August 15 has continued, but with a decreased rate of occurrence. This is the first outbreak of influenza in Japan since 1972, and it has affected Thoroughbreds, non-Thoroughbreds, and pleasure horses throughout the country. Thoroughbred racehorses are routinely vaccinated twice a year, having received their last vaccination in May 2007 with a Japanese-manufactured killed vaccine. Following confirmation of the outbreak, movement restrictions were imposed. Six race meetings scheduled for August 18 and 19 at Sapporo, Niigata, and Kokura were cancelled. Racing resumed on August 25 under strict veterinary supervision. On September 4 the ban on the movement of horses between JRA and non-JRA facilities was partially lifted. Equine influenza was also confirmed on eight premises in Ireland.

Twenty-five cases of Potomac horse fever (PHF) were reported between June and August in Kentucky and Ohio by the University of Kentucky Livestock Disease Diagnostic Center, Lexington. The majority of cases were identified and treated by equine practitioners in the early stages of disease, resulting in low mortality.

Clinical cases of equine piroplasmosis were reported from South Africa, Switzerland, and Turkey. Turkey also reported a single case of

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equine rabies during July. Rotavirus infection was confirmed on five premises in Ireland.

Strangles was reported from Denmark, on two premises involving numerous horses; from Ireland, 14 premises with 19 cases; from South Africa, at least 50 horses on five premises in the Western Cape and Gauteng provinces; and Switzerland, involving animals on three premises.

On-Site Test for Equine Glanders

EDITORIAL NOTE: This paper is a follow-up to a report on glanders that appeared in the October 2003 Lloyds Equine Disease Quarterly *http://www.ca.uky.edu/gluck/Q_issues.asp.*

Diagnostic testing is evaluated according to how precise the test is in identifying a truly positive animal versus animals that are truly negative.

Sensitivity is the proportion of true positives (bacterial culture positive) that are identified by the test as being positive and is a measure of how accurately the test can identify animals with the disease.

Specificity is the proportion of true negatives (in this study, Pakistan Army horses) identified by the test as being negative. This is a measure of how accurately the test can identify animals without the disease.

Positive predictive values reflect the proportion of animals with a positive test result that are truly positive for the disease; negative predictive values reflect the proportion of animals with a negative test result that are truly negative for the disease.

A test with high sensitivity and specificity would be ideal; however, no test exists that is 100% sensitive and 100% specific. Higher sensitivity tests are important to identifying diseased animals. Higher specificity tests are useful for identifying disease-free animals.

Figure 1.

Results for Five Glanders Test Methods.

Test	*Sensitivity*	*Specificity*	*Positive Predictive Value*	*Negative Predictive Value*
IHA	97.1%	100%	100%	98.2%
CF	91.4%	100%	100%	96.7%
RBT	90.0%	100%	100%	94.0%
mCIE	81.4%	<100%	90.5%	88.6%
mallein	75.7%	100%	100%	86.6%

GLANDERS IS AN IMPORTANT INFECTIOUS equine disease of imposing antiquity. It is endemic in Brazil, Turkey, United Arab Emirates, Iran, Iraq, India, Pakistan, and some other countries. Equine glanders has been eradicated from North America and Europe, but its potential as a weapon of bio-sabotage still haunts professionals as well as many others.

Researchers in developing countries are still working on diagnosis, treatment, and vaccine development. Since a confirmatory laboratory diagnosis isn't available in many areas, diagnosis often has been made based on clinical signs and the mallein test. The mallein test is a cornerstone for the field diagnosis of glanders, but its limitation in terms of sensitivity, specificity, and accuracy are well recognized. These pitfalls of the mallein test prompted Russian scientists to develop a new on-site screening test for glanders, the Rose Bengal plate agglutination test (RBT). This test has not as yet been fully validated in terms of sensitivity, specificity, and accuracy under field conditions.

To this end, 70 positive (culture positive; gold standard) sera were collected from two separate outbreaks of glanders that occurred among horses of the Faisalabad Metropolis Mounted Police and Lahore Polo Club in Pakistan. Ninety-six sera from clinically normal but potentially exposed equines (in contact animals/cohort at the Lahore Polo Club) were also included. One-hundred and ten sera collected from the Pakistan Army establishment (Mona Depot) served as negative controls.

Antigen for RBT was prepared from three different strains of *Burkholderia mallei* as per the Office International des Epizooties (OIE) (2003) method. Indirect Haemagglutination (IHA), Complement Fixation (CF), and modified Counter immunoelectrophoresis (mCIE) tests were run in tandem. The results of these tests are summarized in Figure 1. Both mallein and mCIE tests were significantly ($P<0.05$) less sensitive (81.4 and 75.7%) than IHA, CF, and RBT tests. The low sensitivity of the mallein

test in this study concurs with that previously documented by this institution (Muhammad et al., 1998; J. Equine Sci. (Japan), 9(3):93–96). All tests but mCIE gave 100% specificity when testing sera from animals belonging to the glanders-free group (Pakistan Army horses).

In the present study the mallein test was least sensitive (75.5%) for clinical and bacteriologically positive equines. The RBT test in comparison with the mallein test was more sensitive and gave higher proportion of agreement to CF and IHA tests.

In the light of technically acceptable sensitivity, specificity, and negative and positive predictive values, the Rose Bengal test seems to be a good on-site field diagnostic tool for equine glanders. In comparison with other serological tests (IHA, CF, and mCIE) evaluated in this study, RBT is economical, rapid, and compatible with the technical capabilities of practicing veterinarians.

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GLANDERS FAST FACTS

ETIOLOGY: *Burkholderia mallei*, a gram-negative, aerobic bacteria.

SPECIES: Horses, donkeys, and mules primarily, although other mammalian species can occasionally be infected.

TRANSMISSION: Exposure of mucous membranes or breaks in the skin to infective exudates or respiratory discharges; for example, through use of common water and feed containers. The incubation time is variable and can be several months.

CLINICAL SIGNS: Cutaneous, nasal, and pulmonary forms of the disease can occur. Can include swelling of lymphatic vessels that eventually burst and drain on the skin, purulent nasal discharge, enlarged lymph nodes, septicemia, pneumonia, and high fever. Death can occur in days, or horses may have chronic clinical signs.

DIAGNOSIS: Several diagnostic tests are available. The mallein test involves an intradermal injection of antigen in the neck or eyelid or administration of eye drops and subsequent observation for a hypersensitivity reaction.

REPORTING: Glanders is an animal disease foreign to the United States. Positive cases must be reported to the United States Department of Agriculture (USDA) and the Office International des Epizooties (OIE) in Paris.

ENVIRONMENTAL ISSUES: Can remain viable outside the equid for variable times, depending upon environmental conditions. It is susceptible to many disinfectants.

PREVENTION: No vaccine exists. Strict quarantine and testing of imported animals and rapid containment of positive cases are critical.

PUBLIC HEALTH: Glanders is a zoonotic disease.

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Sellon DC and Long MT eds. Equine Infectious Diseases. Elsivier, St. Louis, MO (2007).
Center for Food Security and Public Health Fact Sheets: http://www.cfsph.iastate.edu/

NATIONAL

Investigating Foreign Animal Diseases

SURVEILLANCE FOR AND THE INVESTIGATION of suspected foreign animal diseases is a high priority for state and federal animal health regulatory officials, because the movement of animals and animal products, including semen and embryos, in international commerce could introduce disease and pests into our domestic animal populations. The introduction of a disease of foreign origin would threaten the U.S. share of international markets and would impose trade restrictions and costs for control and eradication. An economic study conducted in 2000 by the U.S. Department of Agriculture (USDA) estimated that the loss of equine exports due to the diagnosis of a communicable infectious disease of horses would have a total impact on the U.S. economy of 1.6 billion dollars[1].

The Animal Plant Health Inspection Service (APHIS) of the USDA regulates the importation of animals and animal products into the United States. Furthermore, animals imported from outside North America are required to enter through a few selected ports where they are examined for evidence of contagious, communicable diseases and samples are collected to test for specific disease conditions. (For more information, go to http://www.aphis.usda.gov/ and click on *Import and Export.*

Should a foreign animal disease (FAD) gain

entry into the United States, private veterinary practitioners who are accredited by USDA to participate in federally regulated activities (for example, issuing certificates of veterinary inspection) would provide the foundation for surveillance and early detection. APHIS, in cooperation with state departments of agriculture, would provide the administrative structure and regulatory oversight for reporting and investigating these disease events. In support of this effort, APHIS provides specialized hands-on training in the diagnosis of foreign animal diseases to state and federal veterinarians. These foreign animal disease diagnosticians (FADD) are located throughout each state and are available 24 hours a day to investigate suspected cases.

Since 2003, a total of 2,704 FAD investigations have been conducted nationwide. Of these, 1,170 (43%) were for disease conditions of horses, the majority of which were conducted due to vesicular conditions of the skin and muzzle and for animals showing central nervous system signs.

FAD investigations are generated primarily via a system that relies on producers and practitioners to report suspect cases. Investigations have, however, been initiated at the request of extension agents following reports of dead or dying animals or on the basis of a suspicious laboratory test result. (Figure 2). Depending on the type and quality of information received, the decision to conduct an FAD investigation is generally at the discretion of the state veterinarian and/or the federal area veterinarian in charge (AVIC), who then assigns an FAD diagnostician to the case.

Once a decision has been made to investigate, the diagnostician is required to initiate the farm visit within 24 hours of notification by the state or USDA area veterinarian in charge. In reality, most investigations are conducted within a few hours of receiving the report of a suspicious illness. The diagnostician will routinely visit the premises to make an assessment of the disease situation and to collect and submit samples. Depending on the species being investigated, the specimens will be shipped to either the National Veterinary Services Laboratory in Ames, Iowa, or to the Foreign Animal Disease Diagnostic Laboratory, Plum Island, New York.

There is never a fee for an FAD investigation, but laboratory testing is restricted to ruling out diseases of foreign origin. Preliminary results can be expected within 24 to 48 hours, depending on the priority of the investigation and the infectious agent under consideration.

Based on the presenting signs, relevant clinical history, or other pertinent epidemiology information, the FAD diagnostician, in consultation with the state veterinarian, will have some discretion as to further regulatory actions imposed on the suspect premises. At a minimum, a quarantine is issued and the owner/operator instructed that no animals or animal products such as semen are allowed to move off the premises until the receipt of laboratory findings and notification from the Office of the State Veterinarian.

On occasion, regulatory officials are asked to provide assistance with the investigations of diseases of unknown origin but which are not considered to be FADs. The investigation of Mare Reproductive Loss Syndrome is just such an example. Once an FAD had been ruled out, APHIS provided personnel and technical assistance with the collection and analysis of epidemiological data. (See http://www.ca.uky.edu/gluck/MRLSindex.asp for further information.)

A list of federally reportable equine diseases is found in Figure 3. Veterinarians, diagnostic laboratories, extension agents, animal owners, and farm managers are all part of the early detection system for foreign animal diseases. Contact information, such as that for Kentucky in Figure 4, should be readily available, especially for veterinarians.

Figure 4.

Kentucky Contact Information:

STATE VETERINARIAN
Dr. Robert Stout
Phone: 502-564-3956
Fax: 502-564-7852

USDA, APHIS, VS
Area Veterinarian in Charge (AVIC)
Phone: 502-227-9651
Fax: 502-223-7121

EMERGENCY OPERATIONS CENTER:
800-255-2587

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1. USDA (2000). Potential impact of the Contagious Equine Metritis-like organism on the equid industry in the U.S. USDA-APHIS-VS, CEAH, Center for Animal Disease Information and Analysis, Fort Collins, CO.

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KENTUCKY

Causes of Foal Mortality, a One-Year Snapshot

NEONATAL LOSSES ARE A SIGNIFICANT problem for the equine industry. Of the 1,294 fetal, neonatal, and juvenile equine cases presented to the University of Kentucky Livestock Disease Diagnostic Center from September 2004 through August 2005, 259 cases of neonatal mortality were evaluated for this study. Cases included only those that resulted from a live birth and those that had a specific age of less than 180 days, with cases in which the animals' age was listed as "juvenile" being excluded.

Deaths Up to 30 Days

The majority of all neonatal deaths occurred by 30 days of age (174/259, 67%). Of these, the top five causes of death include septicemia (44/174, 25%), musculoskeletal issues other than rib fractures (29/174, 17%), pneumonia (25/174, 14%), gastrointestinal problems (19/174, 11%), and rib fractures (18/174, 10%). All deaths from rib fractures occurred in this age bracket. (Rib fractures can result from multiple factors, including dystocia and trauma.)

All Neonatal Deaths *(Up to 180 Days)*

Of the 259 cases, septicemia was the predominant killer, causing 21% of all deaths (54/259). Of these, *Escherichia coli* (17/54, 31.5%) was the primary single organism grown from cultures obtained from septic animals; however, mixed cultures (17/54, 31.5%) were equally significant.

Deaths from musculoskeletal problems accounted for 51 (20%) of 259 cases, with 33 cases attributed to fractures. Trauma, other than those resulting in fractures, accounted for an additional nine cases of mortality.

When gastrointestinal problems (42/259, 16%) were identified as the cause of death, ulcers (9/42, 21.5%) and colitis (8/42, 19%) were the top two diagnoses.

Of the 15% of cases attributed to pneumonia (40 of 259), cultures failed to yield growth in 40% (16 of 40) of the cases. This could be attributed to treatment with antibiotics or to a viral or fungal etiology. *Rhodococcus equi* was cultured from 13 of the 40 (33%) pneumonia cases.

While the majority of the musculoskeletal causes cannot be prevented, improvements in orthopedic therapy allow for better outcomes. Prevention may be the best option for reducing deaths due to infectious etiologies, as foals are uniquely susceptible to infectious diseases. Therefore, it becomes imperative that veterinarians continue to educate horse owners and farm managers about vaccination, hygiene, and early recognition of clinical signs. More inclusive studies are currently being investigated on foal mortality.

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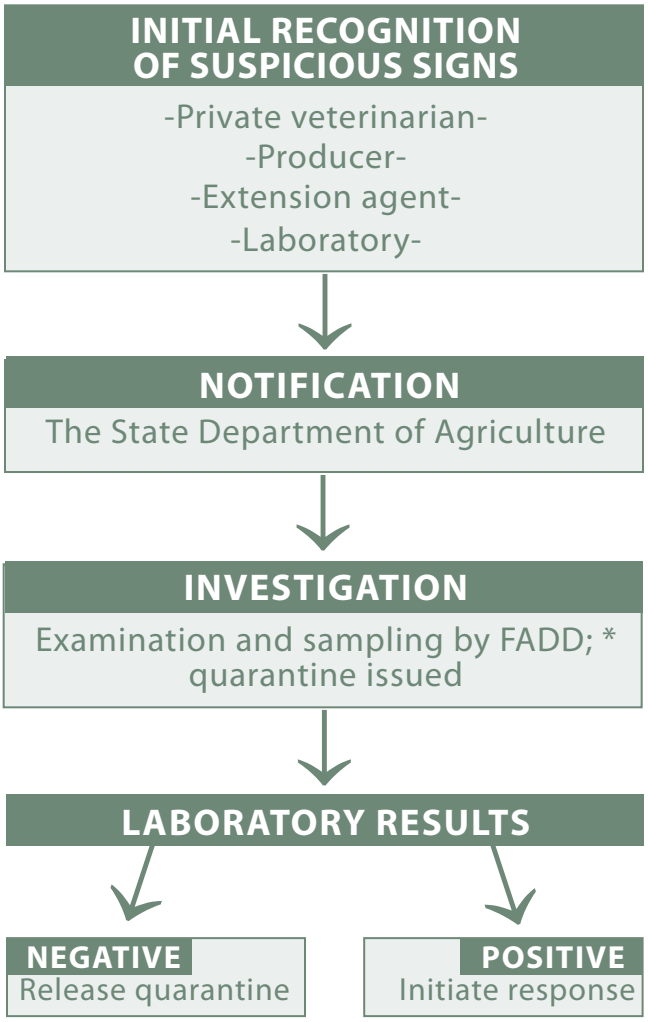
Figure 2.
Steps in Investigation of Suspected Foreign Animal Diseases.



** Foreign Animal Disease Diagnostician*

Figure 3.

Federally Reportable Diseases of Equine

- **African Horse Sickness** (virus)
- **Contagious Equine Metritis** *(Taylorella equigenitalis)*
- **Equine Piroplasmosis** (protozoal)
- **Dourine** (protozoal)
- **Glanders** *(Burkholderia mallei)*
- **Screwworm Myiasis** (parasite)
- **Vesicular Stomatitis** (virus)
- **Venezuelan Equine Encephalomyelitis** (virus)