President’s Message

Calving season is my favorite part of the beef cattle calendar. Most of the beef calves in this part of the country are born in late winter and early spring. Even when I was a real vet, after assisting a calving at three in the morning, watching a newborn struggle to its feet looking for a meal is a sight I hope I never tire of. My seventeen-year-old son gives me a hard time about making a living with my arm in a cow, but when one of his calves is born, he appreciates what you and I do. I hope you and your clients are having a successful calving season.

Our winter Board meeting was held February 5th at the FASS office in Savoy, IL. All in attendance commented that it was a very productive meeting. The GMO Resolution Committee reported that we are making progress. There have been settlement negotiations between the AETA and the defendants and the banks. As before, we will keep you notified.

Audit Committee members, Dr. Richard Whitaker and Dr. Dan Hornickel, met at the FASS headquarters on February 4th to review the 2004 AETA financial records. They reported that they were satisfied with all of the records and documentation. Dan and Whit also met with the accounting firm of Martin, Hood, Freise, and Associates to discuss the future need for an audit or accounting services.

The Certification Committee made a few recommendations that the Board adopted. The Committee recommended that we continue to certify the company and not the individual. The committee would have preferred to certify the individual if the EEC list of approved collection teams did not exist. Although USDA–APHIS personnel have indicated that they did not foresee any problems, the Committee was not certain the EEC officials would be as cooperative. There was also concern about how USDA–APHIS would handle inspection fees for multi-person practices if individuals were certified. The Committee also made changes in the requirements for application for certification. Please see the Certification Committee article.

The Government Liaison committee has been very busy the last few months. An update by Dr. Whitaker is included in this Closer Look.

The Board of Directors gave its unanimous support to the Cooperative Committee and our association with USLGE. A meeting between USLGE CEO, Mike Phillips, and FASS-AETA personnel, Keely Roy and Jennifer Gavel, is scheduled for March 29th in Savoy. The purpose of this meeting is to help the staff understand the Foreign Agriculture Service programs and the paperwork that is involved. The Cooperative Committee would welcome any suggestions from the membership for additional countries for potential embryo marketing projects.

The Board created a new AETA student membership program that is open to any full-time student at recognized colleges and universities. Check the AETA website for details. Please make an effort to contact your local university or your alma mater and inform them of this new membership.

The drug compounding issue that we addressed in Tampa continues to be a controversial issue. After a lengthy discussion, the Board adopted this position, “The AETA supports the FDA guidelines as stated in the Animal Medicinal Drug Use Clarification Act of 1994 [AMDUCA].” More information about this topic can be found at [http://www.avma.org/scienact/amduca/amducal.asp](http://www.avma.org/scienact/amduca/amducal.asp).

The plans for our annual convention this year in Minneapolis are well under way. We are meeting again with CETA September 8–10 at the Marriott City Center. Dr. Pat Richards and Dr. David Duxbury have a great list of speakers and topics with emphasis on the practical aspects of embryo transfer.

Our good friend and colleague, John Hasler, was admitted to a Ft. Collins hospital on March 13 suffering from congestive heart failure. John contracted a bacterial infection that created a vegetative endocarditis on the mitral valve. After six days in the hospital, he is at home on long-term antibiotic therapy and rest. There is a great likelihood that he will have to have a mitral valve replacement. Our thoughts and prayers go out to John and Marilyn.
On Monday, March 21st, the Board was notified by FASS that Chuck Sapp, the Executive Vice President, had tendered his resignation. In a letter to the Board, FASS President James Lauderdale commented “Be assured that FASS is dedicated to providing the utmost level of consumer service to our client members. All FASS operations will continue as they have, via the abilities of the professional, excellently cross-trained, and dedicated personnel.” The Board discussed this new development via a conference call that evening. Dr. Jerry Baker, the Executive Director for the American Society of Animal Science, will serve as the interim Business Manager for FASS. Well, the saga continues. Someone once told me that the only constant in life is change. The AETA has experienced a great deal of change the last two years. Let’s wish Chuck well.

**Government Liaison Committee Update**

We’ve made some administrative changes in our Committee this winter that should spread the workload among the Committee members. We each took a region of the world as follows:

- Chuck Gue: South and Central America, Australia, New Zealand
- Dave Duxbury: Asia, Middle East, and Africa
- Richard Whitaker: EU and Canada

You may continue to contact Richard Whitaker, Chair, with your concerns, or you may contact the appropriate committee member directly. The final (interim) directive has been published for the EU with bilingual certificates posted on IREGS. This directive is due to expire January 1, 2006. This tug of war could resume then…maybe not.

The Committee is arranging to meet with the Import/Export staff in Riverdale, Maryland this spring. We are hoping to introduce some standards to the inspection process we all endure for USDA ET accreditation. This may entail some training process for APHIS staff. Other issues include notification procedures for area offices of the ever-changing regulations.

### Future Meeting Dates

<table>
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<tr>
<th>Event</th>
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<tr>
<td><strong>AETA</strong></td>
<td>2005</td>
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<td>Oct. 5 – 7</td>
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<td><strong>IETS</strong></td>
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<td><strong>AABP</strong></td>
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<td>2007</td>
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<td><strong>SFT/ACT</strong></td>
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### NOTICE TO READERS

Articles published in *A Closer Look* are not peer-reviewed or refereed. All statements, opinions, and conclusions contained in the articles in *A Closer Look* are those of the author(s) and are not necessarily those of the American Embryo Transfer Association unless specifically approved by the AETA Board of Directors.
II. REQUIREMENTS FOR APPLICATION FOR CERTIFICATION

A. To be eligible for certification, an ETB must employ at each business site location, at least one practitioner who holds either (1) a Doctor of Veterinary Medicine (DVM) or other equivalent veterinary degree with a license to practice within the United States of America, or (2) a Ph.D. with emphasis in reproductive physiology, where the applicant’s college transcript and dissertation have been reviewed and determined to be acceptable by the Board of Directors of the AETA.

B. An ETB must be in business for a minimum of twelve months from the first documented embryo transfer to make application for certification.

B. The qualified individual(s) taking the examination must have performed a minimum of 50 embryo recoveries of superovulated females and handled 250 embryos of which at least 100 were transferred to recipients females and at least 100 were frozen within the last 12 months.

C. The application must be postmarked no later than 21 days prior to the Professional Competence Examination.

D. The ETB must have necessary equipment available and the knowledge to freeze and thaw embryos.

E. The ETB must pay an application fee, as outlined on the application for each separate business site where the embryo transfer company operates.

F. A qualified individual may apply for and take the certification exam without being presently associated with a certified ETB. An individual, not presently associated with a certified ETB, may maintain his or her status as a fully qualified individual by continuing to meet the testing or CE requirements and by paying an annual fee equal to that paid by a certified ETB. This individual could subsequently act as a qualified individual for a certified ETB upon their association with an ETB, which has a current Operating Agreement with the AETA.

Please Note: Because of the timeliness of critical announcements between the Government Liaison Committee and certified ETBs, all newly certified ETBs will be required to supply electronic contact information (e-mail) prior to certification authorization. For additional questions, or to submit your electronic contact information, please contact AETA at (217) 398-2217 or aeta@assochq.org.
 Plans for the 2005 Annual Meeting in Minneapolis have gotten underway, and it is shaping up to be an exciting meeting.

The Headquarters hotel is the Marriott City Center, 30 South 7th Street Minneapolis, Minnesota, Phone: 1-612-349-4000. We have reserved a block of rooms at a rate of $119 per night; reservations will open up as we get closer to the meeting.

Here is a list of speakers who have committed at this time:

**Rueben Mapletoft, Saskatoon, SK, Canada:** Bovine ET 101 Wet Lab

**Gabriel Bo, Cordoba, Argentina:** a) Efficiency of programs that control follicular development and ovulation for the donor superovulation without estrus detection and b) Application of fixed-time AI and embryo transfer programs in beef cattle operations.

**Vic Cortese:** a) BVD impact on pregnancy and control in ET herds and b) *Leptospira hardjo* bovis and perinatal immunology impacts to reproduction in cattle operations

**Cliff Lamb, Grand Rapids, MN:** a) Management considerations for donor cows and b) Factors affecting IVF pregnancy rates in recipients.

The following additional topics/events will also be included in the program:

- Equine ET 101 Wet Lab
- Practitioner’s Forum
- Certification Update
- Exhibits
- Banquet
- Golf Tournament
- Companion Tours

As more information becomes available, we will post it on the website (www.aeta.org/mtg.asp) and include it in subsequent newsletters.

*We look forward to seeing you in Minneapolis!!*
Your Embryo Transfer Professionals. Embryo Transfer is our only business. Customer service is our priority. Customer satisfaction is our only goal.

We now have a FLAWLESS Y Foley Connector which is SOLVENT FREE that will fit and HOLD in ANY CATHETER, including silicone catheters.

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- Complete Flush Media W/ BSA
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NEW

AETA STUDENT
MEMBERSHIP

Who is eligible? Full-time students at recognized colleges and universities

Cost: $25 per year

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Benefits include:
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- Access to online newsletter, A Closer Look (quarterly)
- Access to online, searchable membership directory
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- $25 annual conference registration fee for the scientific sessions only
- Free conference (redeemable at one meeting attended during the first three years after graduation)

Apply now at www.aeta.org
Q Fever

Etiology
Q fever results from infection by *Coxiella burnetii*. This organism is an obligate intracellular pathogen and has been traditionally placed in the family Rickettsiaceae; however, recent phylogenetic studies have demonstrated that *C. burnetii* is more closely related to Legionella, Francisella and Rickettsiella in the gamma subdivision of Proteobacteria. *C. burnetii* forms unusual spore–like structures that are highly resistant to environmental conditions. The organism also has two distinct antigenic phases. Phase I is pathogenic and is found in infected animals or in nature; phase II is less pathogenic and is recovered after bacteria are passaged repeatedly in cell cultures or eggs.

Geographic Distribution
Q fever has been found worldwide, except in New Zealand.

Transmission
*C. burnetii* can be transmitted by aerosols or direct contact; it is also spread by ingestion of an infected placenta, other reproductive discharges or milk. Organisms localize in the mammary glands, supramammary lymph nodes, uterus and placenta in domestic ruminants and other susceptible species; bacteria can be shed in milk, the placenta and reproductive discharges during subsequent pregnancies and lactations. *C. burnetii* can also be found in the feces and urine. Ticks seem to spread infections among ruminants and sometimes people. Transmission has occurred in blood transfusions and by sexual contact in humans. Organisms have also been found in the semen of bulls. Vertical transmission is possible but rare.

*C. burnetii* is highly resistant to environmental conditions and is easily spread by aerosols; infectious airborne particles can travel a half–mile or more. Viable organisms can be found for up to 30 days in dried sputum, 120 days in dust, 49 days in dried urine from infected guinea pigs, and for at least 19 months in tick feces. At 4–6°C, organisms can survive for 42 months in milk and 12 to 16 months in wool.

Disinfection
*C. burnetii* is highly resistant to physical and chemical agents. Variable susceptibility has been reported for hypochlorite, formalin and phenolic disinfectants; a 0.05% hypochlorite, 5% peroxide or 1:100 solution of Lysol® may be effective. *C. burnetii* is also susceptible to glutaraldehyde, ethanol, gaseous formaldehyde, gamma irradiation or temperatures of 130°C for 60 min. High temperature pasteurization destroys the organism.

Infections in Humans
Incubation Period
In humans, the incubation period varies from 2 to 40 days; the typical incubation period is approximately 2 to 5 weeks.

Clinical Signs
The symptoms of Q fever appear acutely and can include fever, chills, a severe headache, fatigue, malaise, myalgia and chest pains. The illness generally lasts from a week to more than 3 weeks. A nonproductive cough, with pneumonitis on X–ray, sometimes develops during the second week. In severe cases, lobar consolidation and pneumonia may occur; severe infections are particularly common in elderly or debilitated patients. Hepatitis is seen in approximately one third of patients with prolonged disease; the clinical signs may include fever, malaise, right upper abdominal pain, hepatomegaly and sometimes jaundice. In pregnant women, infections can result in premature delivery, abortion and placentitis. Complications are not common but may include chronic hepatitis, aseptic meningitis, encephalitis, osteomyelitis, vasculitis and endocarditis. Endocarditis usually occurs in people who have pre–existing damage to the heart valves. The symptoms are similar to subacute bacterial endocarditis.

Communicability
Person to person spread is very rare but has been seen in people with pneumonia.

Diagnostic Tests
In humans, Q fever is usually diagnosed by serology. Serologic tests can be done as early as the second week of ill-
ness; they may include immunofluorescence, ELISA, agglutination or complement fixation. Antibodies to the protein antigens found in phase II organisms appear in acute Q fever; antibodies to the lipopolysaccharide of phase I organisms indicate chronic Q fever. Organisms are occasionally found in stained tissue samples but this test is not routinely used in humans.

Isolation of C. burnetii is dangerous to laboratory personnel and is rarely done. Organisms can be recovered from blood samples; bacteria are isolated in cell cultures, embryonated chicken eggs or laboratory animals including mice, hamsters and guinea pigs. Blood cultures from patients with endocarditis are usually negative.

**Treatment and Vaccination**

Antibiotics can shorten the course of acute illness and reduce the risk of complications. Treatment of chronic cases is more difficult and may require long–term antibiotic therapy. Surgical replacement is sometimes necessary for damaged valves.

Effective vaccines may be available for people who are occupationally exposed. A licensed vaccine is available in Australia. In the United States, an investigational vaccine can be obtained from special laboratories such as the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

**Morbidity and Mortality**

Most cases of Q fever occur in people occupationally exposed to farm animals or their products: farmers, abattoir workers, researchers, laboratory personnel, dairy workers and woolsorters have an increased risk of infection. Approximately 60% of cases are thought to be asymptomatic. An additional 38% of infected people experience mild illness, while 2% develop severe disease and require hospitalization.

Q fever is usually a self–limiting illness; most cases resolve spontaneously within 2 days to 2 weeks. The mortality rate is 1% in untreated cases and lower in those who are treated. A biological attack with aerosolized organisms is expected to be similar to a natural outbreak.

**Infections in Animals**

**Species Affected**

Sheep, goats and cattle are the most common domestic animal reservoirs. Dogs, cats, rabbits, horses, pigs, camels, buffalo, rodents, pigeons, geese and other fowl may carry C. burnetii. Antibodies to C. burnetii have been found in badgers, coyotes, raccoons, opossums, badgers, jackrabbits, feral pigs, black bears and musk ox. Ticks and wild birds can also harbor this organism.

**Incubation Period**

The incubation period is variable; reproductive failure is usually the only symptom in animals. Abortions generally occur late in pregnancy.

**Clinical Signs**

Abortion, stillbirth, retained placenta, endometritis, infertility and small or weak offspring can be seen in ruminants, cats, dogs, rabbits and other species. Most abortions occur near term. Several abortions may be followed by uncomplicated recovery, particularly in small ruminants; in other cases, the disease may recur yearly.

With the exception of reproductive disease, animals are usually asymptomatic. Goats sometimes have a poor appetite and are depressed for 1 to 2 days before an abortion. Clinical signs including fever, anorexia, mild coughing, rhinitis and increased respiratory rates occur in experimentally infected sheep but have not been reported in natural infections. Experimentally infected cats develop fever and lethargy.

**Communicability**

Yes. Large numbers of organisms are found in the placenta, fetal fluids, aborted fetus, milk, urine and feces. Serologically negative animals may shed organisms.

**Diagnostic Tests**

C. burnetii can be detected in vaginal discharges, the placenta, placental fluids and aborted fetuses, as well as milk, urine and feces. Organisms are not shed continuously in milk and colostrum. In the placenta, organisms can be identified in exudates or areas of inflammation with a modified Ziehl–Neelsen or Gimenez stain; C. burnetii is an acid–fast, pleomorphic, small coccoid or filamentous organism. This organism is not usually detected by Gram stains. Bacterial identity can be confirmed by immunohistochemistry. Polymerase chain reaction techniques are also available in some laboratories. Fresh, frozen or paraffin–embedded samples of serum,uffy coat, milk, feces, vaginal exudates, cerebrospinal fluid, bone marrow, placenta, liver, cardiac valve, fetal tissue and other tissues can be tested by PCR.

A number of serologic tests are available; the most commonly used tests include indirect immunofluorescence, enzyme–linked immunosorbent assay (ELISA) and complement fixation. Cross–reactions have been seen between some strains of C. burnetii and Chlamydia in ELISA and immunoblot assays.

C. burnetii can be isolated in cell cultures, embryonated chicken eggs or laboratory animals including mice, hamsters and guinea pigs; however, isolation is dangerous to laboratory personnel and is rarely used for diagnosis.
Treatment and Vaccination

Little is known about the efficacy of antibiotic treatment in ruminants or other domestic animals. Treatment is sometimes recommended to reduce the risk of abortion. Antibiotics may in some cases suppress rather than eliminate infections. Isolating infected pregnant animals and burning or burying the reproductive membranes and placenta can decrease transmission.

Vaccines are not available for domestic ruminants in the United States but are used in other countries. Vaccines may prevent infections in calves, decrease shedding of organisms and improve fertility in infected animals. They do not eliminate shedding of the organism.

Morbidity and Mortality

Information on the prevalence of infection is limited. In an endemic region in California, 18 to 55% of sheep had antibodies to C. burnetii; the number of seropositive sheep varied seasonally and was highest soon after lambing. In other surveys, 82% of cows in some California dairies were seropositive, as well as 78% of coyotes, 55% of foxes, 53% of brush rabbits and 22% of deer in Northern California. In Ontario, Canada, infections were found in 33 to 82% of cattle herds and 0 to 35% of sheep flocks. Close contact with sheep appears to increase the risk of infection in dogs.

Significant morbidity can be seen in some species. In sheep, abortions can affect 5 to 50% of the flock. In one California study, Q fever may have been responsible for 9% of all abortions in goats. Deaths are rare in natural infections.

Post–Mortem Lesions

Placentitis is the most characteristic sign in ruminants. The placenta is typically leathery and thickened and may contain large quantities of white–yellow, creamy exudate at the edges of the cotyledons and in the intercotyledonal areas. In some cases, the exudate may be reddish–brown and fluid. Severe vasculitis is uncommon, but thrombi and some degree of vascular inflammation may be noted. Fetal pneumonia has been seen in goats and cattle and may occur in sheep; however, the lesions in aborted fetuses are usually non–specific.

Internet Resources

Animal Health Australia. The National Animal Health Information System (NAHIS)  
Material Safety Data Sheets –Canadian Laboratory Center for Disease Control  
Medical Microbiology  
http://www.gsbs.utmb.edu/microbook

Office International des Epizooties (OIE)  
Manual of Standards for Diagnostic Tests and Vaccines  
http://www.oie.int/eng/normes/mmanual/a_summary.htm  
Q Fever: An Overview  
United States Animal Health Association  
http://www.usaha.org/speeches/speech01/s01conch.html  
The Merck Manual  
http://www.merck.com/pubs/mmanual/  
The Merck Veterinary Manual  
http://www.merckvetmanual.com/mvm/index.jsp  
USAMRIID’s Medical Management of Biological Casualties Handbook  
http://www.vnh.org/BIOCASU/toc.html

References


2 Correlation between oxygen respiration rates and morphology, sex, diameter and developmental stage of single bovine IVP-embryos.


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dMTT Agrifood Research Finland, 31600 Jokioinen, Finland.

A simple, non-invasive, rapid and sensitive oxygen microsensor system was developed to investigate correlations between oxygen respiration rates of individual bovine embryos and their morphology, sex, diameter and developmental stage. Bovine IVP-embryos (n = 78; Holm et al. Theriogenology 52; 683-700) were analysed around the 8-cell stage (Day 3; n = 18) and at various blastocyst stages (Day 7; n = 60). Each embryo was morphologically evaluated, its outer diameter measured and was then loaded into a glass tube (i.d. 0.68 mm, length 3 mm). After 1 h, oxygen concentration gradients generated by the embryo’s respiration were measured over approx. 8 min with an oxygen microelectrode (www.unisense.com). Five embryos were measured in one round together with an empty tube as control. The procedure was repeated twice for each embryo with approx. 1 h interval. Individual respiration rates in nL O2/embryo/h (nL/h) were calculated from these gradients. The measurements were performed at 38.5°C under constant flow of humidified 5% CO2 in air (approx. 19% O2). After this, 64 embryos (14 Day 3; 50 Day 7) were lysed for sex diagnosis by PCR. Values are given as mean ± SD. The sensitivity of the oxygen measurement system was high (controls: 0.034 ± 0.035 nL/h, n = 15) and its repeatability from 1st to 2nd measurement was 99.7 ± 9.8% (n = 71). The average embryo respiration rate was 0.39 ± 0.05 nL/h on Day 3 (n = 18) and 1.31 ± 0.52 nL/h on Day 7 (n = 60). For Day 7 embryos, the respiration rates varied according to their morphological quality, being 1.87 ± 0.46a (n = 18), 1.17 ± 0.32b (n = 23), 0.95 ± 0.27bc (n = 14) and 0.72 ± 0.24c (n = 4) nL/h for quality 1, 2, 3, and 4 embryos, respectively (Proc Mixed,abc: P<0.05; values with different superscripts differ significantly). The sex ratio (male:female) was 9:5 (Day 3) and 32:18 (Day 7), and on Day 7 this ratio varied between qualities: 11:2, 12:8, 8:4, and 1:3 for quality 1, 2, 3, and 4, respectively. The average respiration rate on day 3 was the same for males and females, as it was on day 7 (1.22 ± 0.43 nL/h (females) and 1.31 ± 0.58 nL/h (males), P>0.05). There was a correlation between embryo diameter and respiration rate (r² = 0.65, n = 74), which was even stronger for Day 7 male embryos (r² = 0.72, n = 32). In conclusion, a highly reliable, repeatable and sensitive system was established for measuring respiration rates in single bovine embryos, even at early developmental stages. The respiration rate was lower on day 3 compared to Day 7 embryos, and it was correlated with the morphological embryo quality on Day 7. Oxygen consumption could be a valuable supplementary indicator of embryo viability, especially in difficult evaluations (e.g. quality 2 and 3 after IVP). It remains to be demonstrated if such measurements can also reveal quality differences already at Day 3, which would be of interest in, e.g., the human field.

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28 Cloned Embryos Can Be Produced Using Donor Cells Obtained from a 72-Hour Cooled Carcass.

S. Arat¹, H. Bagis¹, H. Odaman Mercan¹, A. Dinnyes²

¹Research Institute for Genetic Engineering and Biotechnology, TUBITAK, Kocaeli Turkey;

There are few reports on the use of cells from a dead mammal for nuclear transfer (NT). So far, most calves have been cloned from live adult cows or fresh fetal samples. The ability to produce cloned animals using postmortem tissue can provide an additional application to the field of NT. This study was conducted to investigate whether viable cells could be obtained from tissues chilled for 72 h and whether these cells could be used for NT. Bovine oocytes isolated from slaughterhouse ovaries were matured in TCM199 supplemented with 10% fetal calf serum (FBS), 50 µg/mL sodium pyruvate, 1% v:v penicillin-streptomycin (10,000 U/mL penicillin G, 10,000 µg/mL streptomycin), 10 ng/mL EGF, 0.5 µg/mL FSH, and 5 µg/mL LH. A cell line (MC) was established from leg muscle of a cow carcass stored at 0°C for 72 h. Tissues from muscle were cut into small pieces. Tissue explants were cultured in DMEM-F12 supplemented with 10% FBS at 37°C in 5% CO₂ in air. Bovine granulosa cells (GC) were isolated from ovarian follicles and used for NT as control cells. Prior to NT, all somatic cells were allowed to grow to confluency (G1/G0) in DMEM-F12 medium supplemented with 10% FBS. Cumulus cells were removed by vortexing with hyaluronidase at 18 h after the start of maturation. Matured oocytes labeled with DNA fluorochrome Hoechst 33342 were enucleated under UV to ensure full removal of the chromatin. A single cell was inserted into the perivitelline space of the enucleated oocyte. Oocyte-cell couples were fused by a DC pulse of 133V/500 µm for 25 µs. After fusion, NT units were activated using a combination of calcium ionophore (5 µM), cytochalasin D (2.5 µg/mL) and cycloheximide (10 µg/ml) and cultured for 7 days in BARC or G1.3-G2.3 medium. Differences (developmental potential and cell numbers) among groups were analyzed by one-way ANOVA after arcsin square transformation. The results are summarized in Table 1. The results suggest that viable cells can be obtained from muscle of a cow carcass stored at cold temperature for 72 h and that these cells have ability to generate NT blastocysts at rates similar to those obtained with fresh GCs. In addition, G1.3 and G2.3 culture medium supported embryo development better than BARC medium.

This study was supported by a grant from TUBITAK, Turkey (VHAG-1908 and Turkey-Hungary bilateral project VHAG-2022).

Table 1. In vitro development of NT embryos

<table>
<thead>
<tr>
<th>Donor cell</th>
<th>Medium</th>
<th>NT units</th>
<th>Cleaved (%)</th>
<th>Blastocyst (%)</th>
<th>No. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>G1.3-G2.3</td>
<td>78</td>
<td>46 (58.9)²</td>
<td>18 (23.0)²</td>
<td>96.0 ± 11.5</td>
</tr>
<tr>
<td>MC</td>
<td>BARC</td>
<td>66</td>
<td>24 (36.3)²</td>
<td>6 (9.0)²</td>
<td>100.6 ± 15.0</td>
</tr>
<tr>
<td>GC</td>
<td>BARC</td>
<td>43</td>
<td>22 (51.2)²</td>
<td>5 (11.6)²</td>
<td>93.7 ± 14.6</td>
</tr>
</tbody>
</table>

Values within each column with different superscripts are significantly different (P<0.05).
A Preliminary Study of the In Vitro Development of Asian Elephant Cloned Embryos Reconstructed Using a Rabbit Recipient Oocyte.

P. Numchaisrika†, R. Rungsiwivut†, A. Thongpakdee†, M. Techakumphu†

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Interspecies nuclear transfer is an important tool for studying the interaction between the cytoplasm of one cell and the donor nucleus of another (Chen et al. 2002 Biol. Reprod. 67, 637-642). The aim of this experiment was to investigate the possibility of developing in vitro an asian elephant cloned embryo using a rabbit recipient oocyte. The elephant donor cells were obtained from the ear skin of a stillborn Asian elephant (Elephus maximus) and the in vivo-matured recipient oocytes were obtained from FSH-stimulated New Zealand White doe rabbits. Enucleation was accomplished by aspiration of the first polar body and the metaphase II plate together with a small amount of cytoplasm. Successful enucleation was confirmed by UV examination after staining with 5 µg mL-1 Hoechst 33342. The donor cells were introduced into the perivitelline space of the enucleated oocytes immediately after enucleation. The elephant-rabbit reconstructed embryos were fused in 0.3 M manitol with 0.1 mM Ca2+ and Mg2+ using two types of electrical pulses: E1 (n = 61): 3.2 kV/cm, 3 pulses, 20 µs (Chesne. et al. 2002 Nat. Biotechnol. 20, 366-369); E2 (n = 69): 2.0 kV/cm, 2 pulses, 20 µs (Chen et al. 2002 Biol. Reprod. 67, 637-642). The fused embryos were activated 1 h after fusion by electrical pulses to those used for fusion and then incubated in 5 µg mL-1 cyclohexamide and 2 mM 6-DMAP for 1 h. Subsequently, the activated embryos were cultured in B2 medium containing 2.5% fetal calf serum. The developmental rate was observed every 24 h for 7 days and the differences in the percentages of embryos developing to a particular stage were determined by chi-square analysis. The results showed that the fusion and cleavage rates of elephant-rabbit cloned embryos fused and activated by E1 were significantly higher than for E2 (P<0.05; see Table 1). Compared with rabbit-rabbit cloned embryos using adult skin fibroblast as a donor cell and E1 for both fusion and electrical activation, we found that the cleavage and blastocyst rates of elephant-rabbit cloned embryos was higher than for the rabbit-rabbit ones (65% (28/43) versus 58% (28/48) and 7% (3/43) versus 4% (2/48) respectively). Results from this study showed that either of the electrical pulses, 3.2 kV/cm, 3 pulses, 20 µs or 2.0 kV/cm, 2 pulses, 20 µs, can be used to fuse elephant somatic cells to rabbit ooplasm and the rabbit oocytes can be served as recipient oocytes to support the development of elephant cloned embryos up to the blastocyst stage.

This work was supported by Rajadapisek Sompoj Fund, Chulalongkorn University.

Table 1. Developmental rate of elephant-rabbit cloned embryos after being fused by different electrical pulses

<table>
<thead>
<tr>
<th>Electrical Reconstructed n (%)</th>
<th>Fused/Culture n (%)</th>
<th>Cleavage n (%)</th>
<th>Blastocyst pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1 61</td>
<td>43 (70.5a)</td>
<td>28 (65a)</td>
<td>3 (7a)</td>
</tr>
<tr>
<td>E2 69</td>
<td>36 (52.2b)</td>
<td>17 (47b)</td>
<td>1 (3b)</td>
</tr>
</tbody>
</table>

Values with different superscripts within the same column differ significantly (P<0.05).
68 Reproductive performance of cloned bulls.

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The generation of cloned animals by somatic cell nuclear transfer has been reported in a number of countries worldwide. However, progress has been impeded by the extremely low efficiency of cloning and health of some of the cloned animals. Surprisingly, little is known of the reproductive performance of viable clones when compared to the original cell donor, given that a major motivation of cloning is dissemination of superior genotypes. The aim of this study was to compare semen collected from three cloned bulls (Clones A1, B1, and B2) to that of the original donor bulls (Donors A and B). Parameters examined included ejaculate volume, sperm concentration and the motility characteristics of frozen/thawed semen using computed-assisted semen analysis (Hamilton Thorne Biosciences, Inc., Beverly, MA, USA). The fertilization ability of each semen sample was examined using in vitro matured oocytes derived from abattoir-source ovaries. Frozen/thawed semen samples from donor and cloned bulls were prepared on a Percoll gradient and diluted with fertilization medium to a concentration of 1 million sperm/mL prior to fertilization (IVF). The number of blastocysts and total cell counts were analyzed on Day 7 of culture. Finally, in vitro-fertilized blastocysts (Day 7) were transferred to synchronized recipients (n = 49) to examine in vivo development. Proportional data for the in vitro development of embryos and subsequent pregnancy rates were analyzed by chi-square test, and embryo cell numbers were analyzed using Student’s t-test. Progressive motility percentage between donor and cloned bull did not differ: Donor A (62.25 ± 3.89, n = 12); Donor B (66.69 ± 4.47, n = 13); Clone A1 (71.37 ± 8.57, n = 8); Clone B1 (73.75 ± 2.42, n = 12); Clone B2 (72.41 ± 3.26, n = 12). No obvious differences in kinetic motility parameters were evident between cloned and non-cloned donor animals. However, blastocyst rates were significantly higher in cloned bulls (Clone A1: 30.9%, 81/262; Clone B1: 34.4%, 98/285; and Clone B2: 42.9%, 120/280) compared to donor bulls (Donor A: 20.7%, 54/261; Donor B: 20.9%, 76/364). Total embryo cell numbers did not differ significantly between donor bulls (Donor A: 138.3 ± 5.3, n = 39; Donor B: 133.2 ± 5.2, n = 47) and cloned bulls (Clone A1: 126.3 ± 4.4, n = 45; Clone B1: 134.4 ± 7.1, n = 26; and Cloned B2: 140.1 ± 3.9, n = 46). Initial pregnancy rates on Day 30 were also not different between Donor A (42.3%, 11/26) and Clone A1 (47.8%, 11/23). Preliminary observations from the small data set on postpubertal cloned bulls indicate that semen production, semen morphology, and reproductive performance (in vitro and in vivo) were similar in terms of semen characteristics and reproductive performances when compared to their original donor bulls.
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It is said that work, well done, is art. At Diamondback Drugs, we continually strive to perfect the art and science of veterinary pharmaceuticals.
Methicillin-resistant Staphylococcus aureus infection was identified in 2 horses treated at a veterinary hospital in 2000, prompting a study of colonization rates of horses and associated persons. Seventy-nine horses and 27 persons colonized or infected with MRSA were identified from October 2000 to November 2002; most isolations occurred in a 3-month period in 2002. Twenty-seven (34%) of the equine isolates were from the veterinary hospital, while 41 (51%) were from a thoroughbred farm in Ontario. Seventeen (63%) of 27 human isolates were from the veterinary hospital, and 8 (30%) were from the thoroughbred farm. Thirteen (16%) horses and 1 (4%) person were clinically infected. Ninety-six percent of equine and 93% of human isolates were subtypes of Canadian epidemic MRSA-5, spa type 7 and possessed SCCmec IV. All tested isolates from clinical infections were negative for the Panton-Valentine leukocidin genes. Equine MRSA infection may be an important emerging zoonotic and veterinary disease.

Methicillin was first introduced in human medicine in the 1950s for the treatment of penicillin-resistant staphylococci, and within a few years, methicillin-resistant isolates of Staphylococcus aureus (MRSA) were identified (1). Since then, MRSA has emerged as an important problem in human medicine internationally, especially in the hospital setting (2–6). Methicillin resistance is mediated by production of an altered penicillin-binding protein (PBP2a), which confers resistance to all ß-lactam antimicrobial agents. The gene that encodes this altered PBP, mecA, resides on a large, mobile genetic element called the staphylococcal chromosomal cassette mec (SCCmec). Three types of SCC (types I, II, and III) were originally described in hospital-acquired MRSA strains, most of them isolated before 1990. A fourth type (type IV) was recently described, initially in community-acquired MRSA isolates (2–4). Although MRSA have been typically hospital acquired (5,6), reports of community-acquired MRSA in persons have increased (7–9). In Canada, 6 epidemic clones, designated CMRSA (Canadian epidemic MRSA) 1 through 6 based on pulsed-field gel electrophoresis (PFGE), are recognized (10,11). In addition to PFGE, SCCmec typing and DNA sequencing of the X region of the protein A gene (spa typing) can be used to further differentiate strains for epidemiologic analysis (12).

The role of MRSA in veterinary medicine has not been well characterized. Isolation of MRSA has been reported in horses, cattle, and dogs (13–20). In addition, MRSA infection in 2 horses treated at the Ontario Veterinary College Veterinary Teaching Hospital (OVC-VTH) was first documented in 2000. To evaluate the apparent emergence of MRSA infection and colonization in horses, nasal swabs from horses and persons at OVC-VTH and a select group of farms from southern Ontario were cultured.

Materials and Methods

Veterinary Hospital Facility

The Large Animal Clinic at OVC-VTH is a tertiary-care referral center with a caseload of ~2,000 horses per year. Hospital personnel that are in contact with horses include senior clinicians (internists, surgeons, other specialists), residents, interns, veterinary technicians, agricultural assistants, and veterinary students.
Sampling Procedures

A limited organized MRSA screening of horses at OVC-VTH was performed from October 1 to October 5, 2000, after the first 2 clinical cases were recognized. A more thorough screening program was performed from May 17 to November 16, 2002, after further cases were recognized. With this program, nasal swabs were collected from all horses at the time of admission, weekly during hospitalization, and at the time of discharge. Intermittent screening of horses on 1 Ontario breeding farm (farm A) was performed from May 11 to September 15, 2002, after infected horses were identified. Nasal swabs were collected from horses at 9 other Ontario farms after MRSA infection in a resident horse was identified at OVC-VTH. Nasal swabs were collected from all horses that were present on these farms on the day of sampling.

Voluntary screening of OVC-VTH Large Animal Clinic personnel was performed on 2 occasions in 2002 in response to identification of clusters of nosocomial MRSA colonization in horses. Periodic nasal cultures of horses and personnel were initiated at farm A from May 11 to September 15, 2002.

For persons, cotton-tipped swabs were used to sample both anterior nares. For horses, the swab was inserted ~10 cm into 1 anterior nare and rubbed against the mucosa as the swab was removed. Swabs were placed in liquid Stuart’s or Amies medium and stored at 4°C until processing. Colonization or infection identified within 72 h of admission was classified as community acquired.

MRSA Identification, Characterization, and Typing

Nasal swab samples were injected onto mannitol-salt agar with 2 µg/mL oxacillin and incubated aerobically at 35°C for 48 h. Colonies were identified as *S. aureus* based on colony morphology, Gram stain appearance, ability to ferment maltose, and positive tube coagulase test or latex agglutination test (Pastorex Staph Plus, Bio-Rad Laboratories Ltd., Mississauga, Canada).

Screening for methicillin resistance in all *S. aureus* was by growth on Mueller-Hinton agar with 4% NaCl and 6 µg/mL oxacillin. Confirmation of methicillin resistance was by detection of PBP 2a by using the MRSA SCREEN antibody kit (Denka Seiken Co. Ltd, Tokyo, Japan) (21).

Antimicrobial susceptibility testing was performed by broth microdilution as per NCCLS guidelines (22). Detection of inducible *erm* gene-mediated clindamycin-resistance (MLSβ phenotype) was performed by using the double disk diffusion method as described in M100-S14 NCCLS 2004 Informational Supplement (23).

Isolates were typed by using PFGE after DNA extraction and *SmaI* digestion (24). PFGE images were read manually by 1 investigator (B.W.), and related isolates were divided into subtypes by using an arbitrary naming system based on previously described principles (25). Further typing by DNA sequence analysis of the X region of the protein A gene (*spa* typing) and SCCmec typing were performed, as has been described, on isolates from those with clinical infections, atypical isolates from colonized horses, and a random sample of isolates from colonized horses (12,26).

Isolates from those with clinical infection were tested for the presence of the Panton-Valentine leukocidin (PVL) genes by polymerase chain reaction (PCR) and by molecular beacon with the lukF component of *pvl*. Amplification of the *pvl* gene was performed by using the following primers: LukS-PV: GGCCTTTCCCAATACAATTTGG; and LukF-PV: CCCAATCAACTTCATAATTG.

Thermal cycling consisted of initial heating at 95°C for 5 min followed by 35 cycles of denaturation (1 min at 94°C), annealing (30 s at 57°C), and extension (1 min at 72°C). The beacon experiment was carried out using the following beacon and primers: lukF beacon: 5’-6-FAM d(CGCGAAGAATTTATTGGTGTCCTATCTCGAGTCGCG)DABCYL 3’, LukF F: 5’-GCCAGTGTATTACAG AGG-3’, LukF R: CTATCCAGTGTAGTGATCC-3’.

Quantitative real-time PCR mixture contained 1x I.Q. supermix (Bio-Rad Laboratories Ltd., Hercules, CA, USA), 0.1 µmol/L each molecular beacon, 0.5 µmol/L of each primer, and DNA template. The thermal cycling program consisted of 10 min on a spectrofluorometric thermal cycler at 95°C, followed by 45 cycles of 30 s at 95°C, 30 s at 50°C, and 30 s at 72°C.

Statistical Analysis

Duration of carriage by adult horses versus foals was compared by using the Mann-Whitney test. The incidence of intermittent MRSA shedding by adult horses and foals was compared by using Fisher exact test. A p value of <0.05 was considered significant for all comparisons.

Results

In total, MRSA was isolated from 79 horses and 27 persons. Two equine isolations occurred in 2000, 5 in 2001, and 72 in 2002. Of the 79 equine cases, 27 (34%) were in horses that had been hospitalized at OVC-VTH, 41 (52%) were from 1 thoroughbred farm (farm A), and 11 (14%) were from other Ontario farms. Twenty-four of the 79 (30%) horses were adults; the remaining 55 (70%) were <1 year of age.

Clinical infections developed in 13 (16%) horses at 1 or more body sites. Incision or wound infections (n = 6), infection from intravenous catheter (n = 2), bacteremia (n = 2), pneumonia (n = 1), infection from surgical implant (n = 1), septic arthritis (n = 1), omphalophlebitis (n = 1), gluteal abscess (n = 1), and osteomyelitis (n = 1) were identified. In 2 neonatal foals, MRSA was isolated from the nares, blood, and intravenous (jugular) catheter; septic arthritis developed in 1 of the foals. The intravenous catheter was thought to be
lates from horses with clinical infections contained SCCmecIV. They werespa type 7 and did not contain PVL genes. The 2 isolates from postabortion uterine swab specimens also contained SCCmecIV and were negative for PVL genes; however, 1 wasspa type 7 and the otherspa type 235. Five isolates from colonized horses were tested, and all also contained SCCmecIV and werespa type 7. All 3 non-CMRSA-5 isolates were obtained from farm A and were similar to, but distinguishable from, CMRSA-2,spa type 2 and contained SCCmecIV.

MRSA infection or colonization was suspected of being nosocomial in 17 (63%) of 27 OVC-VTH cases and community-acquired in 3 (11%) cases. Origin of infection was unclear in the remaining 7 (26%) cases. In these cases, the nasal swab specimen was not collected on admission; MRSA was isolated from the first sample collected >72 hours after admission, and horses were admitted from farm A during a time when numerous colonized horses were on the farm.

Sixty-eight of the equine isolates were obtained during periods of organized screening at OVC-VTH or on horse farms. The remaining 11 isolates were from clinical specimens submitted directly by primary care veterinarians to a diagnostic laboratory and are excluded from prevalence calculations. In 2000, MRSA was isolated from the nasal passages of 2 (4%) of 57 horses, including the 2 initial clinical cases. In 2002, MRSA was isolated from 25 (8%) of 320 horses at OVC-VTH and 41 (13%) of 321 horses on farm A. Of the 9 other farms evaluated after identification of an infected or colonized horse at OVC-VTH, MRSA was only identified on 1 farm, where 3 (5%) of 64 of horses were colonized. MRSA was not isolated from any of 277 horses from 8 other Ontario farms.

MRSA was isolated from 27 persons; 17 (14%) of 125 of tested OVC-VTH personnel, 8 (12%) of 67 of farm A personnel, 1 owner of a horse with an MRSA wound infection and the spouse of a colonized OVC-VTH clinician. Three human isolates were obtained in 2000, and 24 were obtained in 2002. Only 1 (4%) was from a source with clinical infection, an OVC-VTH veterinarian with a tattoo site infection. That person was infected with CMRSA-5 subtype H12, a strain that contained SCCmecIV, wasspa type 7, and was PVL negative, and that strain was isolated from 2 horses that had been under that person’s care for a week before the wound infection developed. All but 1 colonized person (96%) had previous contact with 1 or more MRSA-positive horses; in 24 (89%) of 27 persons, recent contact with a horse infected with an indistinguishable subtype was documented. The colonized spouse of the colonized OVC-VTH clinician reported no contact with horses; however, isolates from both of these persons were indistinguishable from an isolate recovered from a horse under that clinician’s care. CMRSA-5 was isolated from 26 (96%) of 27 persons. One person harbored both CMRSA-5 and the CMRSA-2–like isolate in his nose at the same time. This person was a veterinarian from farm A, which was the origin of the 3 horses colonized with this strain. Nine different subtypes of CMRSA-5 were identified among human isolates.

All but 2 of the human isolates were obtained during organized screening of personnel from OVC-VTH or selected Ontario horse farms. In 2000, MRSA was isolated from 2 (10%) of 21 humans at the OVC-VTH. In 2002, MRSA was isolated from 15 (12%) of 127 persons at OVC-VTH and 8 (12%) of 68 from farm A.

Antimicrobial susceptibility testing was performed on 67 of the 72 equine and all 26 human CMRSA-5 isolates, and the 5 CMRSA-2–related strains. Five of the equine CMRSA-5 isolates were unavailable for testing. All 101 MRSA isolates tested were susceptible to ciprofloxacin, clindamycin, fusidic acid, linezolid, mupirocin, quinupristin-dalfopristin, and vancomycin. The range of oxacillin MIC was 1–32 µg/mL, and although 21.8% of strains had oxacillin MIC of ≤2 µg/mL at 24 h, all such strains grew on the NCCLS oxacillin screen agar and produced the PBPs protein. Isolates of both CMRSA-5 and CMRSA-2–related strains that were erythromycin-resistant were found to be inducibly resistant to clindamycin when challenged by using the double disk approximation test. The remaining susceptibility test results are presented in the Table.
**Discussion**

This study has identified the largest number of reported cases of clinical MRSA infection in horses and horse personnel. It also identified extensive nasal colonization in horses and horse personnel from a veterinary hospital and horse farm, nosocomial infection in a veterinary hospital setting with clinical illness in horses, and for the first time, clinical infection in 1 person working with infected horses. The subtyping information and timing of isolation provide solid evidence supporting both human-to-horse and horse-to-human transmission.

The prevalence of MRSA colonization in horses at the OVC-VTH was 4% in 2000 and 8% in 2002; however, care must be taken when interpreting these data because screening was performed during 2 periods that followed identification of clinical MRSA infection in horses at the facility. Similar limitations are present with the prevalence data regarding MRSA colonization on breeding farms, which ranged from 0% to 13% and were based on screening after identification of MRSA infection or colonization at OVC-VTH in horses from these farms. The prevalence of human colonization at OVC-VTH and 1 Ontario horse farm is of concern, particularly because of the likelihood of transmission between horses and humans on these farms. As with horses, the prevalence data in humans must be interpreted with care because of the nature of sampling. Further studies are required to determine the prevalence of MRSA infection and colonization in horses and humans at veterinary hospitals and equine farms. One well-recognized human *S. aureus* strain, CMRSA-5, a relatively uncommon isolate in Canada (11), has the ability to colonize the nose of horses and to spread between horses and between horses and persons on farms and within a veterinary hospital setting. The PFGE pattern of the CMRSA-5 isolates was similar to the PFGE patterns published in a previous report of MRSA infection in horses (13). This finding, along with the isolation of CMRSA-5 from horses in Prince Edward Island, Canada (J.S. Weese et al., unpub. data) and Colorado, USA (P. Morley, pers. comm.) that did not have any contact with colonized Ontario horses or horse personnel, further suggests that CMRSA-5 may be more disseminated in the horse population beyond Ontario.

Why MRSA has emerged in the equine population is not known. It may reflect increased exposure of horses to MRSA-infected persons, a unique ability of CMRSA-5 to colonize horses, the increasing use of certain antimicrobial drugs in veterinary medicine, or a combination of these factors. Several recent investigations in humans suggest that the fluoroquinolones themselves may actually predispose patients to infection with or carriage of MRSA (27,28). Two case-control studies examining risk factors for MRSA found a significant association between fluoroquinolone exposure and MRSA isolation or infection (4,29).

Whether fluoroquinolone use in horses has facilitated emergence of MRSA is unclear, since no current data exist on fluoroquinolone use in veterinary medicine. Enrofloxacin is widely used in some sectors of the Ontario horse population, particularly racing horses; however, it is rarely prescribed at OVC-VTH and uncommonly used on breeding farms (J.S. Weese, unpub. data).

While MRSA has been considered primarily a hospital-associated pathogen in humans (10), the increasing incidence of community-acquired infection is concerning (8,9,30,31). Similar to the equine and equine-associated cases reported here, community-acquired infection with SCCmecIV strains has been reported in humans (32–34). Most reports of community-acquired-MRSA in humans involve skin and soft tissue infections, and production of PVL has been implicated as the possible virulence factor in community-associated MRSA infection in humans (31,35). None of the isolates from clinical equine or equine-associated infections in this study contained PVL genes; however, definitive conclusions regarding the role of PVL in equine-associated infection cannot be made with the reasonably small number of clinical isolates evaluated here. Isolates in this study were also multidrug resistant, in contrast to results of many reports of community-associated MRSA in humans (36,37). Therefore, determining the origin of these isolates (community versus hospital) is not straightforward and requires further study.

The lack of proven, safe, and acceptable options of eradication of nasal colonization in horses creates potential management problems. To date, isolation of infected horses and use of barrier precautions have been employed (J.S. Weese, unpub. data). However, such methods may be difficult on farms, particularly if colonization is prolonged.

Our study has shown that MRSA infection may be an emerging disease in horses, which agrees with earlier reports (13,14). MRSA infection also may become an important nosocomial problem in the veterinary hospital setting and become endemic on horse farms, particularly in foals. Because of the extensive movement of horses, especially thoroughbreds and standardbreds, between and within Canada and the United States, MRSA colonization and infection may be more widespread than recognized. Emergence of MRSA as an equine pathogen is of additional concern because horses may be a community reservoir of MRSA and source of infection or reinfection for persons. In view of the size of the North American horse population and the frequent close contact between many persons and horses, this concern must not be dismissed. Further study is required to clarify the role of this pathogen in equine disease and transmission between horses and humans.

Dr. Weese is an associate professor in the Department of Clinical Studies, Ontario Veterinary College, University of Guelph. His current research interests include multidrug-resistant bacteria (particularly interspecies transmission of such bacteria), zoonotic diseases, and veterinary infection control.


**Table.** In vitro antimicrobial susceptibilities of 101 methicillin-resistant *Staphylococcus aureus* isolates from humans and horses*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC in µg/mL</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;16†</td>
<td>&gt;16†</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>8/152</td>
<td>&gt;8/152</td>
</tr>
</tbody>
</table>

*MIC, minimum inhibitory concentration; TMP/SMX, trimethoprim-sulfamethoxazole; NA, no intermediate MIC category in NCCLS guidelines.
†All strains were susceptible to gentamicin at a concentration of 500 µg/mL.
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Chronic wasting disease (CWD) of deer and elk is endemic in a tri-corner area of Colorado, Wyoming, and Nebraska, and new foci of CWD have been detected in other parts of the United States. Although detection in some areas may be related to increased surveillance, introduction of CWD due to translocation or natural migration of animals may account for some new foci of infection. Increasing spread of CWD has raised concerns about the potential for increasing human exposure to the CWD agent. The foodborne transmission of bovine spongiform encephalopathy to humans indicates that the species barrier may not completely protect humans from animal prion diseases. Conversion of human prion protein by CWD-associated prions has been demonstrated in an in vitro cell-free experiment, but limited investigations have not identified strong evidence for CWD transmission to humans. More epidemiologic and laboratory studies are needed to monitor the possibility of such transmissions.

Chronic wasting disease (CWD) is classified as a transmissible spongiform encephalopathy (TSE), or prion disease, along with other animal diseases, such as scrapie and bovine spongiform encephalopathy. The only known natural hosts for CWD are deer (Odocoileus species) and Rocky Mountain elk (Cervus elaphus nelsoni) (1,2). CWD and other TSEs are believed to be caused by a pathogenic effect on neurons of an abnormal isoform of a host-encoded glycoprotein, the prion protein. The pathogenic form of this protein appears to be devoid of nucleic acids and supports its own amplification in the host. TSEs in animals primarily occur by transmitting the etiologic agent within a species, either naturally or through domestic husbandry practices. In contrast, most such encephalopathies in humans occur as a sporadic disease with no identifiable source of infection or as a familial disease linked with mutations of the prion protein gene (3). A notable exception among the human TSEs is the variant form of Creutzfeldt-Jakob disease (vCJD), which is believed to have resulted from the foodborne transmission of bovine spongiform encephalopathy (BSE) to humans (4,5).

CWD was first identified as a fatal wasting syndrome of captive mule deer in the late 1960s in research facilities in Colorado and was recognized as a TSE in 1978 (6,7). Subsequently, this wasting disease was identified in mule deer in a research facility in Wyoming and in captive elk in both the Colorado and Wyoming facilities (6–8). The disease was first recognized in the wild in 1981, when it was diagnosed in a free-ranging elk in Colorado (1,9). By the mid-1990s, CWD had been diagnosed among free-ranging deer and elk in a contiguous area in northeastern Colorado and southeastern Wyoming, where subsequent surveillance studies confirmed it to be endemic (10). Epidemic modeling suggested that this wasting disease might have been present among free-ranging animals in some portions of the disease-endemic area several decades before it was initially recognized (10). On the basis of hunter-harvested animal surveillance, the overall prevalence of the disease in this area from 1996 through 1999 was estimated at approximately 5% in mule deer, 2% in white-tailed deer, and <1% in elk (10). In 2000, surveillance data indicated that the disease-endemic focus extended eastward into adjacent areas of Nebraska (1,11), and ongoing surveillance continues to redefine the limits of this focus.

Clinical manifestations of CWD include weight loss over weeks or months, behavioral changes, excessive salivation, difficulty swallowing, polydipsia, and polyuria (1,6–8). In some animals, ataxia and head tremors may occur. Most animals with the disease die within several months of illness onset, sometimes from aspiration pneumonia. In rare cases,
illness may last for \(\approx\) 1 year. In captive cervids, most cases occur in animals 2–7 years of age; however, the disease has been reported in cervids as young as 17 months and as old as >15 years of age (1). This disease can be highly transmissible within captive deer and elk populations. A prevalence of >90% was reported among mule deer in facilities where the disease has been endemic for >2 years (2,6,7,12). The mode of transmission among deer and elk is not fully understood; however, evidence supports lateral transmission through direct animal-to-animal contact or as a result of indirect exposure to the causative agent in the environment, including contaminated feed and water sources (12).

**Geographic Distribution of Chronic Wasting Disease**

The geographic extent of CWD has changed dramatically since 1996 (2). Two largely independent and simultaneous epidemics, one in free-ranging deer and elk and another in the captive elk and deer industry, appear to represent the main framework for explaining the disease’s current distribution (2). More extensive and coordinated surveillance has provided a clearer picture of its distribution over the last few years. Since 2000, the disease in free-ranging cervids has been increasingly identified outside of the original CWD-endemic areas of Colorado and Wyoming (Figure). The observed distribution seems to be related in part to natural movement of deer and elk and to commercial movement of infected animals to areas far from the disease-endemic zone. Considerable attention has been given to recent increases in the geographic spread of the disease, which in some areas is likely a result of increased surveillance rather than evidence of explosive geographic spread.

No single original event or source links all wasting disease foci documented to date. Given the disease’s insidious nature and the apparent duration (at least several decades) of epidemics among captive and free-ranging cervids, gaps in knowledge about its spread and distribution are not surprising, particularly within the captive deer and elk industry. However, our current knowledge cannot explain some of the distinct foci of CWD among free-ranging animals (e.g., in New Mexico and Utah). Thus, unidentified risk factors may be contributing to the occurrence of CWD among free-ranging and captive cervid populations in some areas.

**Chronic Wasting Disease in Free-ranging Deer and Elk**

In 2000, surveillance of hunter-harvested deer first detected the occurrence of CWD in counties in southwestern Nebraska, adjacent to the previously recognized areas of Colorado and Wyoming that are endemic for this disease (Figure) (1,11). It was reported subsequently in other Nebraska counties, including among deer and elk in a commercial, large enclosure surrounded by a fence in northwestern Nebraska, where the prevalence of CWD was >50% (11). Free-ranging deer from areas surrounding the enclosure also tested positive for the disease but at substantially lower rates. In 2001, CWD in a free-ranging deer was identified in the southwestern part of South Dakota along the Nebraska border close to an area where the disease had been reported among captive elk (13). Since then, additional CWD-positive free-ranging deer and elk have been identified in southwestern South Dakota.

CWD in free-ranging cervids was first reported east of the Mississippi River in Wisconsin among white-tailed deer harvested in the 2001 hunting season (14). Subsequent surveillance indicated that this CWD epidemic focus was limited to several counties in the south-central region of Wisconsin, although a second focus spanning the Illinois border was also detected (15). The absence of evidence for a widespread occurrence of CWD and its low prevalence, despite a highly dense deer population, indicate that the disease probably was recently introduced into Wisconsin. Because the distance from the CWD-endemic area of Colorado-Wyoming effectively precludes eastward migration of animals as a logical source of infection, CWD in Wisconsin was more likely introduced...
by an imported infected cervid or some other unidentified source (14). The proximity of the Wisconsin-Illinois focus to a white-tailed deer farm with infected animals appears to support this explanation, as highlighted by the report of CWD in a previously captive white-tailed deer approximately 7 months after it escaped into the wild in southern Wisconsin (14). The disease among the captive deer herd from which the white-tailed deer escaped was demonstrated earlier, when a still-captive deer tested positive for the disease. The captive source herd was held in a facility 30–50 km from the Illinois location where CWD was recently identified in a free-ranging deer (16). In 2002, the Wisconsin Department of Natural Resources launched an ambitious culling program by providing special hunting permits to eliminate the disease in a designated “eradication zone” around the areas where it was detected (15,17). Whether such aggressive management will succeed in eliminating free-ranging foci of CWD remains to be determined.

In Colorado, the Continental Divide initially appeared to have prevented natural expansion of CWD into the western part of the state. However, in 2002, the disease was confirmed for the first time in several free-ranging deer harvested in western Colorado in an area surrounding a commercial enclosure, where entrapped mule deer tested positive for CWD. Aggressive culling of deer and elk surrounding the enclosure was initiated to prevent further spread of the disease in the western slope of Colorado. Through the 2002 hunting season, CWD-positive deer and elk continued to be identified outside of the previously defined disease-endemic area, primarily in northwestern Colorado (18). This northwestern focus appears to be discontinuous from the previously identified CWD-endemic area, although surveys conducted in 2002 demonstrated that the western and southern boundaries of that area were wider than previously believed. The ultimate source of this wasting disease in northwestern Colorado remains unidentified.

In 2002, samples from an emaciated, free-ranging mule deer found in White Sands, New Mexico, tested positive for CWD (1,19). No cervids have been held in captivity close to the area where the New Mexico deer was found, and the origin of the disease in this deer remains unknown. In addition, CWD-positive, free-ranging deer have been identified in Wyoming west to over the Continental Divide from the known CWD-endemic zone (20). In 2003, a mature buck deer harvested in the fall of 2002 in northeastern Utah tested positive for the disease (21); additional cases have since been found in central and eastern Utah (Figure). These cases provide additional evidence for the potential spread of this wasting disease in the wild.

In Canada, CWD was first detected in free-ranging cervids (two mule deer) in 2001 in Saskatchewan; a few additional deer tested positive in 2002 and 2003 (22). Saskatchewan Environment has implemented a herd-reduction program using “control permits” to prevent further spread of the disease among free-ranging cervids.

**Chronic Wasting Disease in Captive Deer and Elk**

CWD was first recognized in the captive elk industry in Saskatchewan in 1996, but subsequent investigations indicated that the most likely source of Canadian cases was captive elk imported from South Dakota prior to 1989 (2,22). Since 1996, surveillance has detected infected animals on more than 25 elk farms in Colorado, Kansas, Minnesota, Montana, Nebraska, Oklahoma, South Dakota, and Alberta, Canada, and the Republic of Korea (1,14,23,24). CWD in most of these farms was identified in the past 5 years. In 2002, the disease was detected in white-tailed deer on farms in Alberta and Wisconsin (23,25). More extensive and uniform surveillance in captive white-tailed deer is needed to determine the full extent of the disease in this industry.

Captive herds with a CWD-infected cervid are often depopulated both in Canada and the United States. Carcasses of depopulated animals are incinerated or buried in accordance with local regulations. Meat from depopulated animals has not been allowed to enter the human food and animal feed supply.

**Transmission to Other Animals**

Concerns have been raised about the possible transmission of the CWD agent to domestic animals, such as cattle and sheep, which may come in contact with infected deer and elk or CWD-contaminated environments. If such transmissions were to occur, they would potentially increase the extent and frequency of human exposure to the CWD agent. In addition, passage of the agent through a secondary host could alter its infectious properties, increasing its potential for becoming more pathogenic to humans. This phenomenon may have occurred with BSE when a strain of scrapie, a possible original source of the BSE outbreak, changed its pathogenic properties for becoming more pathogenic to humans. However, the exact origin of BSE remains unknown.

Although CWD does not appear to occur naturally outside the cervid family, it has been transmitted experimentally by intracerebral injection to a number of animals, including laboratory mice, ferrets, mink, squirrel monkeys, and goats (1,26). In an experimental study, the CWD agent was transmitted to 3 of 13 intracerebrally injected cattle after an incubation period of 22 to 27 months (27). The susceptibility of cattle intracerebrally challenged with the agent of this disease was substantially less than that observed after intracerebral scrapie challenge: nine of nine cattle succumbed to scrapie challenge after intracerebral injection (28). In ongoing experimental studies, after >6 years of observation, no prion disease has
developed in 11 cattle orally challenged with the CWD agent or 24 cattle living with infected deer herds (E.S. Williams and M.W. Miller, unpub. data) (1). In addition, domestic cattle, sheep, and goat residing in research facilities in close contact with infected cervids did not develop a prion disease.

Analysis by immunohistochemical studies of the tissue distribution of prions in CWD-infected cervids identified the agent in the brain, spinal cord, eyes, peripheral nerves, and lymphoreticular tissues (Table 1) (29,30). Distribution of the CWD agent outside of the brain seems to be less widespread in elk than in deer (2). Involvement of the tonsils and peripheral nerves early in the course of experimental and natural prion infection suggests the possible involvement of the lymphoreticular and peripheral nervous systems in the pathogenesis and transmission of the disease (2,12,30,31).

**Risk for Transmission to Humans**

**Epidemiologic Studies**

The increasing detection of CWD in a wider geographic area and the presumed foodborne transmission of BSE to humans, resulting in cases of vCJD, have raised concerns about the possible zoonotic transmission of CWD (32). In the late 1990s, such concerns were heightened by the occurrence of CJD among three patients 30 years of age who were deer hunters or ate deer and elk meat harvested by family members (Table 2). However, epidemiologic and laboratory investigations of these case-patients indicated no strong evidence for a causal link between CWD and their CJD illness (33). None of the patients were reported to have hunted deer or eaten deer meat harvested in the CWD-endemic areas of Colorado and Wyoming. Such a history in unusually young CJD patients, if present, would have supported a causal link with CWD. Moreover, the testing of brain tissues from >1,000 deer and elk harvested from areas where the patients hunted or their venison originated did not show any evidence of CWD (33). In addition, the lack of homogeneity in the clinicopathologic manifestation and codon 129 of the prion protein gene among the three patients suggested that their illnesses could not be explained by exposure to the same prion strain. In vCJD, homogeneity of the genotype at codon 129 and the clinical and pathologic phenotype were attributed to the patients’ exposure to the same prion strain, the agent of BSE.

In 2001, the case of a 25-year-old man who reportedly died of a prion disease after an illness lasting ~22 months was investigated (Table 2). Although this man had hunted deer only rarely, his grandfather hunted deer and elk throughout much of the 1980s and 1990s and regularly shared the venison with the case-patient’s family. The grandfather primarily hunted in southeastern Wyoming, around the known CWD-endemic area. The case-patient’s illness began with a seizure and progressed to fatigue, poor concentration, and depression. Memory loss, ataxia, speech abnormalities, combative behavior, and recurrent seizures also developed. Histopathologic, immunohistochemical, and Western blot testing of brain autopsy samples confirmed a prion disease diagnosis. Analysis of the prion protein gene indicated a P102L mutation coupled with valine at the polymorphic codon 129 in the mutant allele, confirming a diagnosis of Gerstmann-Sträussler-Scheinker syndrome (GSS). This case-patient was unusually young even for a person with a GSS P102L mutation. It remains unknown whether the possible exposure of the case-patient to CWD-infected venison potentially contributed to the early onset of his prion disease.

In 2001, two additional CJD patients 26 and 28 years of age were reported from a single state (Table 2) (34). The patients grew up in adjacent counties and had illness onset within several months of each other. As a result of this fact and their unusually young age, a possible environmental source of infection, including exposure to CWD-infected venison, was considered. One of the patients died after an illness lasting 5–6 months that was characterized by progressive aphasia, memory loss, social withdrawal, vision disturbances, and seizure activity leading to status epilepticus and induced coma. Histopathologic, immunohistochemical, and Western blot testing of brain biopsy and autopsy samples confirmed a CJD diagnosis. The patient’s disease phenotype corresponded to the MM2 sporadic CJD subtype reported by Parchi et al. (35). This patient did not hunt, and family members provided no history of regularly eating venison. The patient may have occasionally eaten venison originating from the Upper Peninsula of Michigan while away from home during his college years. However, ongoing surveillance has not detected CWD in Michigan deer (36).

The second patient died from an illness lasting ≤16 months. The patient’s illness began with behavioral changes, including unusual outbursts of anger and depression. Confusion, memory loss, gait disturbances, incontinence, headaches, and photophobia also developed. Western blot analysis of frozen brain biopsy tissue confirmed a prion disease diagnosis. Immunohistochemical analysis of brain tissue obtained after the patient’s death showed prion deposition consistent with GSS. A prion protein gene analysis could not be performed because appropriate samples were lacking. However, prion protein gene analysis of a blood sample from one of the patient’s parents indicated a GSS P102L mutation. The patient did not hunt but may have eaten venison from Michigan once when he was 1–2 years old. The GSS diagnosis greatly reduced the likelihood that the two patients reported from adjacent counties had disease with a common origin.

Recently, rare neurologic disorders resulting in the deaths of three men who participated in “wild game feasts” in a cabin owned by one of the decedents created concern about the possible relationship of their illnesses with CWD (Table 2) (37). Two of the patients reportedly died of CJD, and the third died from Pick’s disease. More than 50 persons were
identified as possibly participating in these feasts; the three patients were the only participants reported to have died of a degenerative neurologic disorder. Reanalysis of autopsy brain tissues from the three patients at the National Prion Disease Pathology Surveillance Center indicated that two of them had no evidence of a prion disease by immunohistochemical analysis. CJD was confirmed in the third patient, who had clinicopathologic, codon 129, and prion characteristics similar to the most common sporadic CJD subtype (MM1/MV1) (35). This patient participated in the feasts only once, perhaps in the mid-1980s. In addition, the investigation found no evidence that the deer and elk meat served during the feasts originated from the known CWD-endemic areas of Colorado and Wyoming.

In 2003, CJD in two deer and elk hunters (54 and 66 years of age) was reported (38). The report implied that the patients had striking neuropathologic similarities and that their illness may represent a new entity in the spectrum of prion diseases. A third patient (63 years of age), who was also purported to have been a big game hunter, was subsequently reported from the same area. However, none of the three patients were reported to have eaten venison from the CWD-endemic areas of the western United States. The 66-year-old patient hunted most of his life in Washington State. Although information about the 54-year-old patient was limited, there was no evidence that he hunted in CWD-endemic areas. The third patient was not a hunter but ate venison harvested from Pennsylvania and Washington. The neuropathologic changes, Western blot profile, and genotype at codon 129 of the three patients each fit the MM1, VV1, or VV2 sporadic CJD subtype, indicating absence of phenotypic similarity among the cases or atypical neuropathologic features (35).

To date, only two nonfamilial CJD cases with a positive history of exposure to venison obtained from the known CWD-endemic areas have been reported. One of the patients was a 61-year-old woman who grew up in an area where this disease is known to be endemic, and she ate venison harvested locally. She died in 2000, and analysis of autopsy brain tissues from the three patients at the National Prion Disease Pathology Surveillance Center indicated that the patient’s CJD phenotype fit the MM1 subtype, with no atypical neuropathologic features. The second patient was a 66-year-old man who was reported to have eaten venison from two deer harvested in a CWD-endemic area. Both deer tested negative for CWD, and the patient’s illness was consistent with the MM1 CJD phenotype.

Despite the decades-long endemicity of CWD in Colorado and Wyoming, the incidence of CJD and the age distribution of CJD case-patients in these two states are similar to those seen in other parts of the United States. From 1979 to 2000, 67 CJD cases from Colorado and 7 from Wyoming were reported to the national multiple cause-of-death database. The average annual age-adjusted CJD death rate was 1.2 per million persons in Colorado and 0.8 in Wyoming. The proportion of CJD patients who died before age 55 in Colorado (13.4%) was similar to that of the national (10.2%). The only CJD case-patient <30 years of age in Colorado had iatrogenic CJD linked to receipt of human growth hormone injections. CJD was not reported in persons <55 years of age in Wyoming during the 22-year surveillance period.

**Laboratory Studies**

The possible interspecies transmission of prions can be assessed with laboratory methods. In BSE and vCJD, several laboratory studies provided crucial evidence that helped establish a causal link between the two diseases (39–41). These studies characterized the molecular similarities of the agents causing BSE and vCJD and determined the lesion profile and incubation period patterns of different panels of mice inoculated by the two agents. Limited laboratory studies have been performed to molecularly characterize CWD-associated prions and to compare them with prions from human case-patients and other species. Strain typing studies involving wild-type inbred mice indicated that the CWD agent from a mule deer produced incubation-period and brain-lesion profiles different from those produced by the agents causing BSE and scrapie (39,42). These same strain-typing techniques had identified the similarities of the etiologic agents of BSE and vCJD, providing strong laboratory evidence for a link between the two diseases.

In human prion diseases, two major types of the proteinase-K–resistant prion protein fragment have been identified on the basis of their molecular size by one-dimensional immunoblot analysis: type 1 migrating at 21 kDa and type 2 at 19 kDa (35). N-terminal protein sequencing indicated that the cleavage site of the type 1 fragment is generally at residue 82 and that of type 2 is at residue 97 (43). Prion strain diversity is believed to be encoded in the three-dimensional conformation of the protein, which determines the cleavage site and molecular size of proteinase-K–treated prion fragment, indicating that the difference in molecular size may correlate with strain differences. However, one-dimensional immunoblot analysis may not identify more subtle differences that may influence the conformation of different prion strains. Analysis of the glycoform ratios of prion fragments and application of a two-dimensional immunoblot may help further identify these subtle differences. On one-dimensional immunoblot analysis, the prion fragment from several CWD-infected deer and elk migrated to 21 kDa, corresponding to the type 1 pattern. This specific type has been identified in most cases of sporadic CJD in the United States. However, the deer and elk prion fragment differs from that in sporadic CJD cases in the glycoform ratio. In the CWD-associated prion fragment, the diglycosylated form was predominant, but in the CJD-associated prions, the monoglycosylated form was predominant. Preliminary analysis using two-dimensional immunoblot indicated that the CWD-associated prion frag-
Conclusions

The lack of evidence of a link between CWD transmission and unusual cases of CJD, despite several epidemiologic investigations, and the absence of an increase in CJD incidence in Colorado and Wyoming suggest that the risk, if any, of transmission of CWD to humans is low. Although the in vitro studies indicating inefficient conversion of human prion protein by CWD-associated prions raise the possibility of low-level transmission of CWD to humans, no human cases of prion disease with strong evidence of a link with CWD have been identified. However, the transmission of BSE to humans and the resulting vCJD indicate that, provided sufficient exposure, the species barrier may not completely protect humans from animal prion diseases. Because CWD has occurred in a limited geographic area for decades, an adequate number of people may not have been exposed to the CWD agent to result in a clinically recognizable human disease. The level and frequency of human exposure to the CWD agent may increase with the spread of CWD in the United States. Because the number of studies seeking evidence for CWD transmission to humans is limited, more epidemiologic and laboratory studies should be conducted to monitor the possibility of such transmissions. Studies involving transgenic mice expressing human and cervid prion protein are in progress to further assess the potential for the CWD agent to cause human disease. Epidemiologic studies have also been initiated to identify human cases of prion disease among persons with an increased risk for exposure to potentially CWD-infected deer or elk meat (47). If such cases are identified, laboratory data showing similarities of the etiologic agent to that of the CWD agent would strengthen the conclusion for a causal link.

Surveillance for human prion diseases, particularly in areas where CWD has been detected, remains important to effectively monitor the possible transmission of CWD to humans. Because of the long incubation period associated with prion diseases, convincing negative results from epidemiologic and experimental laboratory studies would likely require years of follow-up. In the meantime, to minimize the risk for exposure to the CWD agent, hunters should consult with their state wildlife agencies to identify areas where CWD occurs and continue to follow advice provided by public health and wildlife agencies. Hunters should avoid eating meat from deer and elk that look sick or test positive for CWD. They should wear gloves when field-dressing carcasses, bone-out the meat from the animal, and minimize handling of brain and spinal cord tissues. As a precaution, hunters should avoid eating deer and elk tissues known to harbor the CWD agent (e.g., brain, spinal cord, eyes, spleen, tonsils, lymph nodes) from areas where CWD has been identified.

Acknowledgments

We thank Claudia Chesley for editorial assistance and state and local health departments for facilitating and participating in the investigation of individual case-patients.

Dr. Belay is a medical epidemiologist in the Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention (CDC); he coordinates the CDC prion disease surveillance and research activities. His research areas
of interest include the interspecies transmission of prion diseases, Kawasaki syndrome, and Reye syndrome.

References


Table 1. Deer tissues tested for the CWD agent by animal bioassay or immunohistochemical studies

<table>
<thead>
<tr>
<th>Tissues positive for CWD agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
</tr>
<tr>
<td>Pituitary gland</td>
</tr>
<tr>
<td>Spinal cord</td>
</tr>
<tr>
<td>Eyes (optic nerve, ganglion cells, retina)</td>
</tr>
<tr>
<td>Tonsils</td>
</tr>
<tr>
<td>Lymphoid tissues (e.g., gut-associated, retropharyngeal, posterior nasal septum)</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Peripheral nerves (e.g., brachial plexus, sciatic nerve, vagosympathetic trunk)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissues negative for CWD agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal root ganglia</td>
</tr>
<tr>
<td>Parotid and mandibular salivary glands, tongue, esophagus, small intestine, colon</td>
</tr>
<tr>
<td>Thymus</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Kidneys, urinary bladder, ovary, uterus, testis, epididymis, placentomes</td>
</tr>
<tr>
<td>Myocardium, Purkinje fibers, arteries, veins</td>
</tr>
<tr>
<td>Trachea, bronchi, bronchioles, aleveolar parenchyma</td>
</tr>
<tr>
<td>Bone marrow</td>
</tr>
<tr>
<td>Thyroid gland, adrenal gland</td>
</tr>
<tr>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Skin</td>
</tr>
</tbody>
</table>

*CWD, chronic wasting disease*
Table 2. Creutzfeldt-Jakob disease patients investigated for a possible causal link of their illness with chronic wasting disease of deer and elk, United States

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age at death (y)</th>
<th>Year of death</th>
<th>Codon 129</th>
<th>Western blot</th>
<th>Final diagnosis</th>
<th>Eating of venison from CWD-endemic area</th>
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<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2002</td>
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<td>nd</td>
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<sup>a</sup>CWD, chronic wasting disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; CJD, Creutzfeldt-Jakob disease; nd, not done.

<sup>b</sup>Immunohistochemical analysis of postmortem brain tissue was consistent with GSS, and a GSS 102 mutation was confirmed in the family.

<sup>c</sup>Investigated as part of a cluster of three case-patients who participated in “wild game feasts” in a cabin owned by one of the decedents.

<sup>d</sup>Patient grew up in an area known to be endemic for CWD and ate venison harvested locally; however, the CJD phenotype fits the most common form of sporadic CJD.

<sup>e</sup>Patient may have been successful in harvesting two deer since 1996 from a CWD-endemic area, but both deer tested negative for CWD.
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