# PEER REVIEWED

# Trends in export of dairy Holstein heifers from the United States

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#### Abstract

A general overview of the export and testing requirements is given for bred female dairy cattle from the United States (US) to Turkey, Mexico, and Russia, the top three export markets. Disease-by-disease serologic test results are presented for 7,339 Holstein dairy heifers tested in quarantine in southeastern Chester County, Pennsylvania for export to Turkey from June 2010 to October 2011. Management practices to decrease the prevalence of diseases of importance to the export market are reviewed. Prevalence rates of serologically positive heifers tested during the quarantine period using the enzyme linked-immunosorbent assay were: bluetongue virus (BTV) 4.9%, Mycobacterium avium subsp paratuberculosis (MAP) 0.65%, and bovine leukemia virus (BLV) 5.7%. Prevalence rate of bovine viral diarrhea virus - persistently infected (BVDV PI) heifers using pooled polymerase chain reaction was 0.68%. Because of the high prevalence of BLV in US cattle, most if not all heifers are routinely screened for BLV prior to purchase and entry into quarantine. Some of the heifers in this data set originating from endemic BTV states were pre-screened for BTV. Because of pre-screening for BLV prior to purchase for export, the prevalence of BLV reported was lower than would be expected in the general population of heifers in the US. The prevalence of BTV would depend on whether heifers came from BTV free or endemic states. Dairy herds wanting to market heifers for export should apply management practices known to decrease the prevalence of BTV, BLV, MAP, and BVDV PI.

Key words: dairy heifer, export, Turkey

#### Résumé

On présente un survol des conditions d'exportation et de testage des vaches laitières en reproduction des États-Unis pour les trois plus grands marchés d'exportation, soit la Turquie, le Mexique et la Russie.

Les résultats de tests sérologiques sont présentés maladie par maladie chez 7339 taures laitières Holstein testées en quarantaine dans le sud-est du comté de Chester en Pennsylvanie avant leur exportation vers la Turquie de Juin 2010 à Octobre 2011. Les pratiques de régie ayant pour but de réduire la prévalence des maladies importantes dans le cadre du marché de l'exportation sont discutées. On a documenté les taux suivants de prévalence de taures positives à la sérologie durant la période de quarantaine par dosage d'immunoadsorption par enzyme liée: le virus de la fièvre catarrhale (4.9%), *Mycobacterium avium* subsp *paratuberculosis* (0.65%) et le virus de la leucémie bovine (5.7%). Le taux de prévalence pour le virus de la diarrhée virale bovine chez les taures immunotolérantes était de 0.68% suite au test de la réaction en chaîne de la polymérase. En raison de la forte prévalence du virus de la leucémie bovine aux États-Unis, la plupart des taures sont testées de façon routinière pour ce virus avant l'achat et la quarantaine. Quelques-unes des taures dans nos données provenant d'états où le virus est endémique ont été pré-testées pour le virus. En raison de ces pré-tests pour le virus avant l'achat pour l'exportation, la prévalence du virus de la leucémie bovine que l'on rapporte est probablement moins élevée que celle attendue dans la grande population des taures aux États-Unis. La prévalence du virus de la leucémie bovine varierait selon que les taures proviennent d'états où le virus n'est pas présent ou endémique. Les fermes laitières qui veulent exporter leurs taures devraient mettre en place des pratiques de régie qui peuvent décroître la prévalence du virus de la fièvre catarrhale, du virus de la leucémie bovine, du virus de la diarrhée virale bovine chez les animaux immunotolérants et de Mycobacterium avium subsp paratuberculosis.

#### Introduction

World demand for United States (US) dairy cows and heifers has increased. Exports of bred female dairy cattle rose from 8,835 in 2007 to 37,356 in 2010.<sup>12</sup> This trend continued into 2011, with US exports of bred female dairy cattle totaling 66,620 from January through November 2011, representing an economic value of \$198 million.<sup>12</sup> Of that number, Turkey was the major importer with 47,806, followed by Mexico at 10,528, and Russia with 5,084.<sup>12</sup> The majority of bred female dairy cattle exported were dairy heifers.

Dairy heifers exported to Turkey come from the west, midwest, and mid-Atlantic and leave the US from east coast ports in Wilmington, Delaware and Eastport, Maine and enter the Turkish port of Izmir via the Mediterranean Sea. Animals are transported in special ships with appropriate ventilation, manure management, and feed storage necessary for the two to three week trip.

#### **Export Requirements**

Specific requirements for export of cattle from the US to foreign countries are listed on the Animal Plant and Health Inspection Services (APHIS) website.<sup>4</sup> The accredited veterinarian performing the health tests and preparing the health certificates should consult with the federal veterinarian endorsing the health papers to ensure a thorough understanding of the requirements. Ultimately, it is the responsibility of the accredited veterinarian to know the current regulations, which are always changing.

Cattle selected for export must first go to a quarantine facility approved by APHIS veterinarians for the time period specified by the importing country. During the quarantine period, depending on the importing country's specifications, cattle may undergo the following: tested for tuberculosis (TB); blood samples collected for required serological testing; and receive vaccines, antibiotics, and parasiticides.

The most common disease test requirements reported for export of US purebred dairy females are brucellosis, tuberculosis, and enzootic bovine leukosis (EBL).<sup>24</sup> The following is a general overview of the export and testing requirements pertaining to US-bred female dairy cattle being exported to Turkey, Mexico, and Russia, the top three markets identified for export of dairy heifers through August 2011.

#### **Certification Statements**

#### Country, States

Table 1 lists the diseases for which freedom-of-disease certification statements are required on the health certificate for either the US as a whole, individual states, herd, premise, or administrative territory of origin. The US is recognized by the World Organization for Animal Health as being free of foot and mouth disease, rinderpest, contagious bovine pleuropneumonia, heartwater disease, and as a country of controlled risk for bovine spongiform encephalopathy (BSE).

Turkey requires exported animals to originate from herds free of brucellosis and TB, and that states from

Disease	Turkey	Mexico	Russia
Foot and Mouth	Yes		Yes
Rinderpest	Yes		Yes
Contagious bovine pleuropneumonia	Yes		Yes
Heartwater	Yes		
Controlled risk for bovine spongiform encephalopathy	Yes	Yes	Yes
Vesicular stomatitis	Yes		Yes
Bluetongue	Yes		Yes
Tuberculosis	Yes	Yes	Yes
Brucellosis	Yes	Yes	Yes
Enzootic bovine leukosis	Yes		Yes
Johne's disease			Yes
Trichomoniasis	Yes		
Vibriofetus	Yes		
Leptospirosis	Yes		Yes
Infectious bovine rhinotracheitis	Yes		
Bovine virus diarrhea–mucosal disease	Yes		
Neosporosis	Yes		
Parainfluenza-3	Yes		
Besnoitiosis			Yes
Anthrax			Yes

**Table 1.** Certification statements for freedom of diseases required for either the United States as a whole, individual states, herd, premise or administrative territory of origin.

which the cattle originate be free of vesicular stomatitis (VS) for 12 months prior to export. Mexico requires that animals originate from brucellosis certified-free herds or states. Russia requires that animals originate from states that have been free of brucellosis, TB, and VS for the last 12 months prior to export.

As of November 2011, cattle to be exported to Turkey must have resided in states free of bluetongue virus (BTV) for 40 days prior to export. Prior to November 2011, the requirement was 60 days. Russia requires exported cattle either to originate from premises free of BTV for the last two years, or that the premises be BTV free for the last three months and the animals test negative for BTV during the quarantine period.

# Herds of Origin

Turkey, Mexico, and Russia all require that animals originate from premises and herds which are free of certain contagious diseases. The accredited veterinarian handling the export shipment must have herd of origin statements signed by the herd owner and veterinarian certifying that herds from which the animals originate are free of the specified contagious diseases.

Turkey requires that export animals originate from farms which have been clinically free of EBL, trichomoniasis, *Campylobacter fetus*, leptospirosis, infectious bovine rhinotracheitis (IBR/IPV), bovine virus diarrhea/mucosal disease (BVD/MD), neosporosis, and parainfluenza-3 (PI3) for the last 12 months. Russia requires that exported animals originate from premises and administrative territories that are free of paratuberculosis (MAP) for the last three years; besnoitiosis during the last 12 months; leptospirosis during the last three months; anthrax during the last 20 days on the premises; and that animals originate from premises where in the last three years no cases of EBL were detected.

# **Exported Animals**

# Health

Table 2 lists health certification, vaccination, antibiotic, and anthelmintic requirements pertaining to the cattle to be exported. Cattle selected for export must have a permanent identification system enabling them to be traced to the herd of origin. Mexico requires that exported cattle be born in the US or legally imported from North America, be free of ectoparasites, and come from areas free of the Boophilus tick causing piroplasmosis. Mexico requires that animals be inspected and found free of clinical signs of IBR, leptospirosis, and piroplasmosis. All cattle must be clinically healthy and free of contagious diseases, including warts, ringworm, and bovine keratoconjunctivitis, upon inspection prior to export. Russia requires that animals not be treated with natural or synthetic estrogens, hormonal substances, and thyreostatic preparations.

# Testing Requirements

Turkey, Mexico, and Russia all require TB and serology testing for certain diseases (Table 2). Both Turkey and Mexico require animals be negative for bovine TB within 60 days prior to export, using an individual intradermal tuberculin caudal fold test that uses 0.1 ml of purified protein derivative (PPD) tuberculin. Russia requires a negative TB test during its 21-day mandated quarantine period.

Turkey requires negative serological blood tests for brucellosis, MAP, BLV, BVDV PI, and BTV during its mandated quarantine period. As of November 2011, the mandated quarantine period changed to 21 days from 30 days. Mexico does not require an official quarantine period, but requires a negative brucellosis test within 30 days of export. Russia requires negative tests for brucellosis and MAP; two negative tests for BLV at least 30 days apart (the last test taken during quarantine), and negative serological tests for BTV (if freedom statement cannot be made) during the mandated 21-day quarantine period.

Both Turkey and Mexico require testing for campylobacteriosis and trichomoniasis in non-virgin bulls and non-artificially inseminated females. Russia only requires campylobacteriosis and trichomoniasis testing for bulls. Turkey specifies using the direct microscopic examination of a genital smear to test for trichomoniasis and campylobacteriosis. The accredited veterinarian can send the samples to an accredited laboratory or do the testing for campylobacteriosis and trichomoniasis. The accredited veterinarian should consult an accredited laboratory for the appropriate handling and testing of the samples.

# Vaccination and Antibiotic Requirements

Turkey, Mexico, and Russia all require vaccinations be given to animals being exported. Turkey requires either a negative microagglutination test or vaccination for leptospirosis during the quarantine period and one treatment with 9.1 mg/lb (20 mg/kg) of long-acting oxytetracycline, given within 14 days of shipment. Universally, heifers exported to Turkey from the US are vaccinated for leptospirosis and given one treatment of oxytetracycline in lieu of serologic testing for leptospirosis serovars. Mexico requires vaccination for leptospirosis between 10 and 90 days prior to export. Russia requires two antibiotic treatments for leptospirosis 14 days apart, but does not specify which treatment to use.

Turkey requires vaccination for bovine keratoconjunctivitis, pasteurellosis (*Pasteurella multocida* and *Mannheimia haemolytica*), IBR, PI3, bovine respiratory syncytial virus (BRSV), and BVD (types I & II) during

Table 2.	Health certification,	vaccination,	antibiotic and	anthelmintic	requirements f	for Holstein	dairy heifers to
be export	ed to Turkey, Mexico,	or Russia.					

	Turkey	Mexico	Russia
Quarantine period:	21-days <sup>1</sup>		21-days
Permanent ID enabling search to herd of origin	Yes	Yes	Yes
Born in the United States or legally imported from North America		Yes	
Clinically healthy and free of contagious diseases upon inspection			
prior to export	Yes	Yes	Yes
Not treated with certain hormones			Yes
Free of ectoparasites		Yes	
Not from Boophilus tick area		Yes	
Free of clinical signs of infectious bovine rhinotracheitis, leptospirosis,			
and piroplasmosis		Yes	
Tested negative for:			
Brucellosis	Yes	Yes	Yes
Tuberculosis	Yes	Yes	Yes
Johne's disease	Yes		Yes
Bluetongue	Yes		Yes
Enzootic bovine leukosis	Yes		Yes
Bovine viral diarrhea – persistent infection	Yes		
Campylobacteriosis	$Yes^2$	$Yes^2$	Yes <sup>3</sup>
Trichomoniasis	$Yes^2$	$Yes^2$	Yes <sup>3</sup>
Vaccinated for:			
Bovine keratoconjunctivitis	Yes		
Pasteurellosis	Yes		
Infectious bovine rhinotracheitis	Yes	Yes	Yes
Parainfluenza-3	Yes		Yes
Bovine viral diarrhea	Yes		Yes
Bovine respiratory syncytial virus	Yes		Yes
Leptospirosis	Yes	Yes	
Treated for:			
Endo- and ectoparasites	Yes		
Endoparasites			Yes
Antibiotics for leptospirosis	Yes, once		Yes, twice

<sup>1</sup>Changed to 21-day from 30-day quarantine period as of November 2011 <sup>2</sup>Testing required only for non-virgin bulls and non-artificially inseminated heifers <sup>3</sup>Testing required only in bulls

the quarantine period. An additional requirement is that animals be vaccinated with killed vaccines for IBR, PI3, BRSV, BVD (types I & II), and leptospirosis at least twice with the interval of not more than six months, with the last vaccination given during export quarantine. Mexico requires vaccination for IBR using an intranasal vaccine, and leptospirosis 10 to 90 days prior to export. Russia requires that animals be vaccinated for IBR, PI3, BRSV, and BVD (not specified whether killed or modified-live vaccines) not earlier than six months, and at least two weeks, prior to export. The herd owner and herd veterinarian from where the animals originate must certify on the herd of origin statements that the animals have received the required vaccines prior to entering quarantine.

#### Endo- and Ectoparasites

Turkey requires exported animals be treated against endo- and ectoparasites at least 10 days prior to export. Russia requires deworming during the quarantine period.

The objectives of this study are to report the disease-by-disease serologic test results for dairy heifers tested during export quarantine for export to Turkey, and to give an overview of management practices to decrease the prevalence of these diseases.

#### Methods

From June 2010 through October 2011, blood samples were taken for serology testing from 7,339 Holstein dairy heifers during their 30-day quarantine period at two approved export quarantine facilities in southeastern Chester County, Pennsylvania prior to export to Turkey. The heifers were purchased from states free of VS for the past 12 months, originated from herds free of brucellosis and TB, and clinically free for the last 12 months of EBL, trichomoniasis, vibriofetus, leptospirosis, IBR, BVD/MD, neosporosis, and PI3. The heifers were between 17 and 24 months of age, and confirmed pregnant. They came from herds in California, Delaware, Idaho, Indiana, Maine, Maryland, Missouri, New Hampshire, New York, Ohio, Oklahoma, Pennsylvania, South Dakota, Vermont, Virginia, and Wisconsin.

The heifers spent 60 days in BTV-free states prior to export to Turkey. Most if not all of the dairy heifers were pre-screened for BLV, and a portion of the heifers originating from BTV endemic states were pre-screened for BTV prior to purchase and entering the 30-day quarantine period. Table 3 provides an example of the chronology of testing and processing procedures administered to heifers during export quarantine for Turkey. The heifers were tested for BTV, MAP, and BLV by serology using an enzyme linked-immunosorbent assay (ELISA) test, and for BVDV PI by polymerase chain reaction (PCR) pooling 10 blood samples at the South Dakota State University (SDSU) laboratory. The heifers were tested by the tube agglutination test for brucellosis at SDSU, and tested for TB by injecting 0.1 ml PPD tuberculin intradermally in the caudal tail fold. The TB tests were read in 72 +/- 6 hours. Cost for all serological and PCR testing was approximately \$18 per heifer.

#### Results

Prevalence rates of serologically positive heifers in Table 4 were as follows: 4.9% BTV, 0.65% MAP, 5.7% BLV, 0.68% BVDV PI, and 0.33% of heifers were positive at 1:50 on the brucellosis tube agglutination test, but later confirmed negative by the Rivanol test. A small proportion (0.34%) of heifers were classified as "suspect" by the caudal fold test for TB and were subsequently confirmed negative by the comparative cervical test. Only heifers negative on all the tests are eligible for export to Turkey.

#### Discussion

Serology data reported in this paper were for Holstein dairy heifers purchased for export to Turkey. BLV and BTV had the highest serological positive rate at 5.7% and 4.9%, respectively. Because of the high prevalence of BLV in US cattle, most if not all heifers are pre-screened for BLV prior to purchase for export. The BLV prevalence would be considerably higher if not for pre-screening. A portion of heifers originating from

**Table 3.** An example of the chronology of testing procedures and processes administered to Holstein dairy heifers during export quarantine for Turkey.

Day	Procedures	
0	Heifers enter 30-day quarantine <sup>1</sup>	2
7	1. Test for tuberculosis (TB)	
	2. Collect blood for serology	
	3. Pregnancy check	
	4. Treat for endo- and ectoparasites	
	5. Vaccinate for respiratory diseases and leptospirosis	
	6. Treat ringworm and keratoconjunctivitis	
	7. Remove warts and extra teats	
10	Read TB tests at 72 hrs	
12	Remove heifers with positive serology results	
17	1. Treat with 9.1 mg/lb (20 mg/kg) oxytetracyline for leptospirosis	
	2. Vaccinate for keratoconjunctivitis	
	3. Test non-artificially bred heifers for trichomoniasis and vibriofetus	
	4. Treat ringworm and keratoconjunctivitis	
26-28	Health papers endorsed by APHIS veterinarian	
	Inspection of heifers by APHIS veterinarian	
31	Heifers eligible for transport	

<sup>1</sup>Quarantine period changed from 30 days to 21 days as of November 2011.

Date	Tested	$TB^1$ suspect	<b>Brucellosis<sup>2</sup></b>	$BTV^3$	MAP <sup>4</sup>	BLV <sup>5</sup>	BVDV PI <sup>6</sup>
6/22/10	187	0	1	0	4	9	0
10/15/10	499	2	0	13	1	39	0
2/9/11	352	0	0	6	1	11	1
2/9/11	300	0	0	22	0	28	0
4/1/11	540	3	1	167	2	22	0
4/14/11	325	3	1	17	0	13	0
4/29/11	541	2	1	9	4	43	11
6/8/11	153	0	0	1	1	11	0
6/8/11	326	1	1	5	3	16	2
7/6/11	589	0	2	4	0	12	1
7/7/11	307	1	2	8	4	32	1
7/7/11	302	0	3	6	1	10	0
7/8/11	613	1	1	17	3	47	0
8/17/11	719	5	9	11	5	58	11
10/5/11	500	2	1	6	7	17	3
10/6/11	532	3	1	60	11	21	11
10/13/11	554	2	0	8	1	31	9
Total	7339	25	24	360	48	420	50

**Table 4.** Prevalence rates and 95% confidence intervals for tuberculosis suspects and serologically positive Holstein

<sup>1</sup>TB – Tuberculosis – injection of 0.1 mL PPD tuberculin in caudal tail fold

0.34%

0.23 - 0.50

<sup>2</sup>Brucellosis – tube agglutination test at 1:50

<sup>3</sup>BTV – Bluetongue virus – ELISA test

<sup>4</sup>MAP - Mycobacterium avium subsp paratuberculosis – ELISA test (Biocor Kit using an average of the optical densities of the negative controls at 0.1 as cutoff)

4.9%

4.4-5.4

0.33%

0.22 - 0.49

<sup>5</sup>BLV – Bovine leukemia virus – ELISA test

<sup>6</sup>BVDV PI – Bovine viral diarrhea virus – persistently infected – PCR test

<sup>7</sup>CI – 95% confidence interval

Percent

95% CI7

BTV-endemic states would have been pre-screened for BTV. The prevalence of heifers positive for BVDV PI and MAP was low. As expected, all heifers were negative for brucellosis and TB after confirmatory tests on any suspects. Rather than testing for leptospirosis by the microagglutination test, all heifers were treated with oxytetracycline at 9.1 mg/lb (20 mg/kg) within 14 days prior to export, as positive leptospirosis titers would be prevalent either due to vaccination or prior exposure.

Turkey is purchasing heifers confirmed pregnant between two and six months of gestation. Heifers greater than seven months pregnant should not be shipped, as they might calve early enroute. Heifers pregnant by artificial insemination do not need further testing during the quarantine period for campylobacter or trichomoniasis. Herds using clean-up bulls on their heifers should make sure the bulls are negative for trichomoniasis and campylobacter. Ideally, dairy heifers should be properly vaccinated as heifer calves for IBR, PI3, BVD, BRSV, and leptospirosis, and receive a booster vaccine prior to entering quarantine. Otherwise, in addition to being vaccinated for pasteurellosis and keratoconjunctivitis, heifers would have to be vaccinated twice for IBR, PI3, BVD, BRSV, and leptospirosis during the quarantine period.

0.65%

0.5-0.9

5.7%

5.2-6.3

0.68%

0.5-0.9

Dairy operations wanting to market heifers for export should focus on decreasing the prevalence of BTV, BLV, MAP and BVDV PI in their herds by applying management practices known to decrease the prevalence.

#### Bluetongue

Bluetongue virus is a gastrointestinal orbivirus transmitted by Culicoides spp (biting midges) to both domestic and wild ruminants. The disease is not contagious and is only spread by competent vectors of Culicoides spp. BTV causes vasculitis resulting in disseminated intravascular coagulopathy. Sheep typically have clinical signs of facial and pulmonary edema, oral ulcers, lameness, and pneumonia with nasal discharge.<sup>17</sup> Cattle in the US typically have mild or unapparent infections.17

The US does periodic serologic surveys to determine the status of states with respect to BTV. The accredited veterinarian should consult the federal area veterinarian in charge to determine the BTV status of a particular state. Within the US, the New England states and northern states from Maine west to Montana, as well as those states extending as far south as Maryland and Pennsylvania, are considered BTV free because they are free of the BTV vector species.<sup>9</sup>

Worldwide there are 24 serotypes of BTV, five of which (BTV2, 10, 11, 13, and 17) are endemic to the United States.<sup>1</sup> The vector of BTV serotypes 10, 11, 13, and 17 is *Culicoides sonorensis*, which persists in the middle, southern, and western US but not in the Northeast. *Culicoides variipennis*, the probable vector of epizootic haemorrhagic disease, a similar disease to BTV but antigenically different and primarily affecting wild ruminants such as white-tail deer, exists in the northeastern states and is thought to compete with *Culicoides sonorensis*.<sup>9</sup> The vector of BTV serotype 2 is *Culicoides insignis* found in Florida.<sup>9</sup>

Since 1999, additional isolates of non-endemic BTV have been isolated in the US, but none of these has caused disease outbreaks.<sup>1</sup> Before 1998, BTV was an exotic disease in Europe, but from 1998 through 2005, at least six BTV strains belonging to five serotypes (BTV1, 2, 4, 9, and 16) were identified in the Mediterranean Basin.<sup>1</sup> BTV8, identified in 2006, caused 30,000 outbreaks of BTV in the northern part of Europe.<sup>1</sup>

Since 1980, live cattle have not been exported from the US to the EU primarily because of BTV restrictions on US cattle.<sup>9</sup> However, there is agreement that live cattle can be exported from BTV-infected countries by following recognized testing and quarantine procedures.<sup>9</sup> The US does not consider seropositive cattle to be a disease or trade risk, as scientific evidence suggests that vectors are responsible for the movement of BTV rather than viremic livestock.<sup>9</sup> Not all *Culicoides* spp can transmit BTV, so regulatory agencies only need to consider those *Culicoides* spp that are competent for transmission of BTV.<sup>22</sup>

BTV has never been isolated in resident cattle from BTV-free areas in the US.<sup>9</sup> The US does not require BTV testing of cattle for importation from BTV-infected countries such as Australia after 60 days of quarantine in vector-free isolation prior to import.

As of November 2011, Turkey requires that cattle be serologically negative for BTV and spend 40 days in BTV-free states prior to export.<sup>9</sup> This requirement has enabled cattle negative on serology to BTV to be exported even if they originate from BTV-endemic areas in the US.

Presumably, application of fly repellants to both the premises and dairy heifers in BTV endemic areas would decrease the prevalence of BTV, but research is lacking on the efficacy of any repellents to control the vectors. It is economically advantageous to screen heifers from known endemic BTV areas in the US prior to purchase for export. Typically, testing for BTV by an ELISA test would cost \$4 to \$5 per test.

# **Bovine Leukemia Virus**

Enzootic bovine leukosis is a neoplastic disease of lymphocyte-forming tissue in cattle caused by the retrovirus BLV. Once animals are infected, they are infected for life. Peak incidence occurs in adult cattle between four and eight years. The virus produces fatal tumors in a small percentage (< 5%) of infected cattle,<sup>10</sup> resulting in carcass condemnation at harvest. Organs most frequently affected are the lymph nodes, heart, abomasum, uterus, kidney, spinal cord, and eyes.

BLV is common in the US dairy industry, and costs the industry through both clinical disease and the negative impact on international trade. The financial loss from clinical EBL in an individual herd is usually low unless the herd has a very high prevalence of BLV resulting in clinical cases of EBL. BLV-infected cows do not have lower milk production, lower reproductive efficiency or less longevity in the herd compared to noninfected cows,<sup>14</sup> but herds with seropositive cows produced 3% less milk than herds with no positive cows.<sup>23</sup> Pollari *et al*<sup>26</sup> reported that older BLV-infected cows, while Rhodes *et al*<sup>28</sup> reported no difference in culling between BLV serologically positive or negative cows.

A serological study in 1975 found animals with BLV antibodies in 66% of herds tested.<sup>6</sup> Between 1975 and 1980, the annual rate of positive sera for BLV in cattle submitted to tests prior to export was between 13 and 19%.<sup>27</sup> This rate most likely represented the rate in heifers as they were the main cattle group exported.<sup>27</sup> Of dairy cows tested, serological prevalence rates varied in different regions from 14 to 48%.<sup>27</sup> A 1981 serological study of the prevalence of BLV in Florida found 47.8% of 7,768 dairy cattle were positive for BLV on the agar gel immunodiffusion (AGID) test.<sup>7</sup>

The most recent measurements of the extent of BLV infection in the US were the 1996 and 2007 National Animal Health Monitoring System (NAHMS) reports. The 1996 study reported that 89% percent of US dairy operations had cattle seropositive for BLV using the AGID test, and 74.8% of the dairy operations were estimated to have a within-herd prevalence of at least 25%.<sup>19</sup> The 2007 dairy study results showed that 83.9% of US dairies were positive for BLV using the ELISA assay on bulk-tank milk.<sup>19</sup> Between November 2008 and April 2009, 17 to 24% of Holstein heifers not screened previously for BLV were positive to BLV during pre-export eligibility testing for export to Russia.<sup>16</sup>

In our data, 5.7% of heifers were positive for BLV in blood samples taken during the 30-day quarantine period for export to Turkey, but most if not all were previously screened negative for BLV prior to the quarantine period. This suggests a continual high rate of infection in dairy herds. Seroconversion observed in heifers tested negative prior to quarantine is most likely explained by either normal seroconversion in newly infected animals, or use of the AGID test as compared to the more sensitive ELISA test prior to export. Our data is in agreement with Martin,<sup>16</sup> who reported a large number of positive seroconversions of BLV during the quarantine period in heifers screened negative prior to entry into quarantine for export to Russia.

The most significant mechanism of infection for BLV is the horizontal transmission of blood from an infected animal with BLV. The risk of *in utero* infections is low and estimated at 3%.<sup>11</sup> An infected cow might transmit BLV to her calf through colostrum or milk, although not often because colostral antibody concentration is usually high enough to prevent infection by the oral route.<sup>18</sup>

Herd-level control measures to decrease horizontal transmission of blood between infected cattle include using bloodless dehorning methods, sanitizing ear taggers between heifers, and using individual needles and palpation sleeves. In one herd a BLV control program using these herd-level control measures reduced the prevalence of BLV from 44% to 17% during a two-year period.<sup>29</sup> In high prevalence herds, segregation of infected cows in addition to using control measures to decrease horizontal transmission of blood, would be effective strategies for producers who wanted to move toward a BLV-negative status.<sup>30</sup>

#### **Johne's Disease**

Johne's disease is a chronic granulomatous gastrointestinal disease of cattle caused by the bacteria *Mycobacterium avium* subsp *paratuberculosis*. The NAHMS Dairy 2007 report using environmental culture of MAP to determine infection status reported that 68% of US dairy herds had infected animals.<sup>21</sup> The incubation period is typically five years from the time of infection to clinical disease, and calves are usually infected around birth when they are exposed to cow manure with MAP.<sup>8</sup>

The disease can be controlled by effective heifer husbandry practices that limit the contact of heifers with adult manure in combination with diagnostic testing to remove the most infectious shedders in the herd.<sup>8</sup> Separating calves from cows and their manure after calving decreases the risk of newborn calves ingesting MAP.<sup>8</sup> Calves should be fed colostrum from only MAP negative cows. Colostrum should be collected under hygienic conditions and unpasteurized milk should not be fed to calves.

Calves also should be managed to prevent exposure to manure from cows. Cows that are heavy shedders

(either by ELISA or fecal PCR or culture) should be culled when practical.<sup>8</sup> The prevalence of heifers positive serologically for MAP in this report was 0.65%. The prevalence was expected to be low, since the incubation period for disease is so lengthy and the animals chosen for export were young.

### **Bovine Viral Diarrhea Virus**

Infection with bovine viral diarrhea virus (BVDV), a pestivirus, causes financial loss in dairy herds primarily through reproductive problems, loss of milk production, and decreased animal health.<sup>13</sup> BVDV can cause either transient or persistent infections (PI). A PI animal is created when a fetus is exposed to a non-cytopathic BVDV while *in utero* prior to development of a competent immune system, generally described as less than 125 days of gestation.<sup>5</sup> The PI cattle continually shed large amounts of BVDV during their lifetime. BVDV is generally introduced in a herd through purchase of PI cattle or PI negative cows pregnant with PI fetuses.

The 2007 NAHMS survey used PCR testing for BVDV of bulk-tank milk to determine infection status and found BVDV on more than 10% of large dairies (greater than 500 cows) and 1.7% of all operations.<sup>20</sup> Prevalence of BVDV PI calves was 0.33% in two stocker operations using confirmatory immunohistochemical (IHC) testing.<sup>15</sup> A prevalence of 0.68% for BVDV PI was found in our data using the reverse-transcriptase polymerase chain reaction (RT-PCR) without confirmatory IHC testing. The prevalence of BVDV PI in our data was likely skewed higher because confirmatory testing with IHC on skin biopsy samples was not done. Presumably, some of the BVDV PI heifers identified would have been false positives. Heifers positive on PCR for BVDV PI are not eligible for export to Turkey.

The continued prevalence of BVDV PI in cattle suggests rigorous control programs are necessary. Control for BVD is centered on annual herd vaccination and eliminating BVDV PI cattle, because they are viremic and continually shedding BVDV, making more BVDV PI calves *in utero*.

Initially all cows and calves in a herd should be tested for BVDV PI. Various tests are available to identify PI cattle. The RT-PCR is generally used to test large numbers of pooled samples. The sensitivity and specificity of the assay for pooled samples was 100% and 89.7%, respectively.<sup>15</sup> RT-PCR cannot differentiate transient infections or recent vaccination with modifiedlive vaccines from true BVDV PI cattle, so all positive RT-PCR samples should be confirmed with IHC on skin biopsy samples.<sup>15</sup>

Bulk-tank milk samples can be used to screen milking herds for BVDV using PCR. Calves from pregnant females during the testing period should be tested at birth by IHC on skin biopsy samples. All PI animals should be slaughtered and any incoming cattle should be tested. $^{25}$ 

#### **Tuberculosis**

Tuberculosis is a contagious disease caused by the bacteria *Mycobacterium bovis*, which can be transmitted from livestock to humans. The disease has almost been eliminated from livestock in the US; however, it persists in white-tail deer populations in parts of Michigan and Minnesota. The tuberculosis classification status of each state can be found on the APHIS website.<sup>3</sup> Heifers classified as "suspect" and subsequently confirmed negative by the comparative cervical test are still not eligible for export.

#### **Brucellosis**

Brucellosis is a contagious and zoonotic disease, caused by the bacteria Brucella abortus, causing reproductive loss in cattle and a variety of syndromes in humans, including undulant fever. The US is approaching brucellosis-free status. The brucellosis classification status of each state can be found on the APHIS website.<sup>2</sup> As of November 2011, all states were classified as free from bovine brucellosis. Brucellosis is still found in wild elk and bison at the Greater Yellowstone Area National Park. The ultimate goal for APHIS is to develop a strategy to identify and mitigate the risk caused by brucellosis in wildlife and to develop a surveillance strategy to document national disease freedom. Heifers testing positive on the tube agglutination test and subsequently confirmed negative on the Rivanol test are not eligible for export.

#### Conclusion

Certain diseases can have significant health and economic impacts related to animal health, productivity, and trade. With world demand for US dairy cows and heifers on the rise, as referenced by Turkey, Mexico, and Russia, dairy herds interested in gaining entry to the export market should focus on decreasing the prevalence of BTV, BLV, MAP, and BVDV-PI by applying appropriate management practices. Specific health requirements for export of cattle from the US to foreign countries are listed on the APHIS website.<sup>4</sup> The accredited veterinarian performing the health tests and preparing the health certificates should consult with the federal veterinarian endorsing the health papers to ensure a thorough understanding of the requirements. Ultimately, it is the responsibility of the accredited veterinarian to know the current regulations, which are always subject to change.

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