Herd-based Testing for Infectious Diseases

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Abstract

The objective of herd-based testing for specific infectious diseases is to monitor for either the presence of the disease in the herd or the presence of individual animals in the herd with the disease. The information can be used to develop the appropriate control measures to prevent a disease from entering into a herd or to control a disease in the herd by reducing or eliminating the disease from the herd. The herd-based testing is part of the program for control of specific diseases within the herd and needs fit within the goals and objectives of the herd for the overall control of infectious diseases. The needs of herds for herd-based testing and types of herd-based tests used will vary depending upon whether the herd is a "closed-herd," producing its own replacements internally, or purchasing replacement animals from outside sources, whether the herd has had on-going control program and has reduced the prevalence of disease to low levels and needs to now monitor for the presence of the disease. The specific herd-based tests need to be selected based on the goals for the individual herd. For example, the objective of testing for *Leptospira hardjo-bovis* is to determine if the disease is present in the herd whereas the objective of tests for BVD may be to identify individual animals that are persistently infected.

Introduction

I would like address herd based testing for five infectious diseases of concern to dairy herds: 1) bovine virus diarrhea virus (BVDV), 2) *Neospora caninum*, 3) Johne's disease and *Mycobacterium avium* subspecies *paratuberculosis* (MAP), 4) *Leptospira borgpetersenii* serovars *hardjo bovis,* and 5) mycoplasma mastitis. The herd veterinarian and the management of the dairy operation should understand the reasons for herd based testing and develop a plan on how the information from herd based testing will be used before beginning to test the herd.

Bovine Virus Diarrhea Virus

Etiology

Bovine virus diarrhea (BVD) is caused by genetically diverse strains of a pestivirus. BVD is one of the most important infectious diseases of cattle. It has been associated with diseases of the respiratory, digestive, hematological, immunologic, neurologic and reproductive systems. The greatest economic losses associated with BVD in dairy herds are the effects of the virus on suppression of the immune system and reproductive system resulting in early embryonic death, abortions, and congenital defects. The losses associated with BVDV infection of the reproductive system will depend upon prevalence of persistent infections (PI) in the herd and the herd's vaccination program. The effect of BVDV on reproduction depends on the stage of gestation when the cow is exposed to the virus. Seronegative cows exposed to BVDV around the time of breeding or shortly after have experienced decreases in conception rate. Cows exposed to BVDV during the embryonic stage and early fetal stages (45 – 175 days of gestation) may experience either embryonic death, abortion, give birth to a calf that is persistently infected (PI) with BVDV as the result of a fetus being immunotolerant to BVDV, or birth of calves with a variety of congenital detects. Calves that are born to cows that are exposed to BVDV in late gestation are normally able to mount an effective immune response although occasionally infection may result in abortions or birth of weak calves.

Persistently infected cattle shed high amounts of BVDV and are the most important source of BVDV within dairies and between dairies. PI calves result when the developing fetus is exposed to a noncytopathic BVDV between 45 and 175 days of gestation while the fetal immune system is developing. The majority of PI calves are born to dams that either have no or low levels of immunity to BVDV at the time they were initially exposed to the virus. A small percentage of PI calves are the result of PI dams giving birth to PI offspring. Most PI calves that are born alive will die soon after birth. Unfortunately a few PI calves survive and are a reservoir of the virus and an important source of transmission of virus to herdmates. One of the most common factors associated with the introduction of BVDV is the entry of newly purchased pregnant animals, regardless of vaccination status. The dam is seropositive and has serum neutralizing titers to BVDV at the time of introduction to the premise, but is carrying a PI calf.

Control Measures

Management practices that can reduce the risk of losses associated with BVDV include biosecurity mea-

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sures, elimination of PI cattle through testing and culling, and strategic use of effective vaccines.

Testing Strategies

The choice of testing strategies and specific tests used depends upon the objectives of the veterinarian and dairy management. Testing programs can either be herd based or individual animal tests. Individual animal testing may be part of a herd based control program. The three primary reasons for testing would be to identify persistently infected individuals, ongoing herd surveillance and evaluation of a vaccination program.

Identification of persistently infected individuals

Herd testing strategies: The goal of most herd surveillance strategies is to accurately detect the presence of PI cattle within the herd. Methods most commonly used to detect PI cattle have included traditional virus isolation assays, immunoperoxidase monolayer assay, ELISA, microscopic examination of immunohistochemistry-stained skin biopsies (IHC) and polymerase chain reaction (PCR) of pooled specimens of blood or milk. With the exception of IHC, it is necessary to test cattle with positive virus-detection results twice to distinguish transient infections (TI) from PI, and the presence of maternal antibodies may result in false-negative classifications in calves under three months of age.

Use of composite bulk milk: Collect bulk milk from a group or string of cows, not to exceed 400 cows per sample. Milk samples can be either collected from a single string milked into a bulk tank or from an inline string sampler. Somatic cells from the milk are screened by PCR. Some laboratories will also do virus isolation on the bulk milk sample. It is important to have a record of the cows contributing to the string sample at the time the sample is taken. It is also important to have a means of identifying cows, such as dry cows and cows in the hospital string, that did not contribute to the bulk milk string samples. If a bulk milk sample comes back positive for BVDV, the individual cows in the positive string sample need to be sampled to identify the infected animal(s).

Whole herd testing: Whole herd testing is the most definitive method for eradicating BVDV from the herd. Blood or skin samples from all animals that are greater than four months of age, including bulls, need to be collected. Ear notches are effective for calves less than four months of age.

Calfhood testing: Calfhood testing has several advantages over whole herd testing for large dairy herds. It spreads out the cost and labor requirements for testing, which may be more manageable over a period time. In addition, calfhood testing has the advantage of implying that a calf that tests negative must have come from a BVDV negative dam. However, if a calf tests positive, the dam must also be tested to determine whether the she is persistently infected or was acutely infected during gestation. Ear notches should be collected from all aborted calves and all calves that die early in life. If samples are not collected from these calves, then the dams of these calves should be sampled.

Detecting the Infected Fetus

Purchased cattle that were pregnant when they entered the herd can act as a "Trojan horse" for BVDV. If the dams were carrying a persistently infected calf at the time of purchase, the dam can test negative at the time of entry to the herd, but this does not preclude the calf she was carrying from being PI. Hence, the calf should be tested at birth for PI status.

Ongoing herd surveillance

Sentinel animal antibody surveillance: One innovative method of monitoring BVD circulation within a group of animals is to introduce a sentinel animal to that group and monitor its antibody status utilizing the BVD serum neutralization (SN) test. The advantages of the sentinel calf system of surveillance are diagnostic efficiency, and the system should detect transmission resulting from either TI or PI exposure. The sentinel must be tested to demonstrate it is a non-PI animal and must remain unvaccinated throughout its life within the herd. One example of the use of this animal would be to introduce it to a calf cohort and test it at times critical to the transmission of BVD virus within the cohort. One strategic testing strategy could include testing the sentinel three weeks after introduction to a group, at three months of age for the calf cohort, heifer cohort prefreshening and annually once introduced to the milking herd. Utilize the BVD SN test for this evaluation. A strategy to identify herds with PI cattle based on the distribution of antibody titers from 10 vaccinated and unvaccinated Michigan dairy herds used a cutoff BVDV antibody titer value of at least 1:128 from three of five calves 9-18 months of age. The herd-level classification of 14 Michigan dairies following this strategic sampling strategy (except testing unvaccinated calves 6-12 months of age) was compared with the results of virus isolation testing of all animals within those herds. Cattle BVDV PI were detected by virus isolation in six of the 14 herds (43%). Serologic data from the sentinel calves was used to correctly identify four of the six herds with PI cattle and all eight of the herds without PI cattle (herd-level sensitivity=67%, herd-level specificity= 100%).

Tests for Detecting BVDV

1) Serology, Serum Neutralization. Serum neutralization tests have been the mainstay of BVDV test-

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ing to idientify exposure and previous infection. While there is some cross reactivity between BVDV type 1 and type 2 isolates, it is possible to have animals infected with one genotype show no serological reaction to the heterologous genotype. Thus, serum neutralization tests to both type 1 and type 2 viruses should be performed when screening animals for infection. While vaccine titers can be fairly high, titers over 512 are suggestive of infection, especially in younger animals. A four-fold or greater rise in titer is suggestive of recent infection. Serum neutralization tests cost about $5 per sample.

2) BVD Immunohistochemical (IHC) Test. The IHC method provides an antigen detection test that is done using formalin-fixed tissue. IHC staining uses monoclonal antibodies to detect viral antigens in tissue sections. IHC staining identifies the presence or absence of BVD virus in skin, thereby identifying the animals that are PI with BVDV. It is widely used on skin biopsies (ear notches) for the identification of PI animals. IHC staining of skin for detection of persistent infection with BVD has some advantages and disadvantages. Advantages are, first, colostral antibody does not affect the tissue distribution of BVD virus in persistently infected animals and does not interfere with IHC staining. Skin is easily collected from live animals. Excision biopsies or 10mm punch biopsies from the ear or neck skin have been commonly used. Biopsies must be individually identified, fixed in formalin and submitted. However, disadvantages are that IHC staining is labor intensive and relatively expensive. Skin biopsies should be placed in neutral buffered formalin before transport to the laboratory. Current charges for IHC staining of a single slide are approximately $20 to 25 per slide. Multiple skin samples (usually up six sections) can be included on a single slide to reduce costs. Interpretation of results is subjective and may be difficult in some cases. It is recommended that biopsies be sent to the laboratory within seven days of collection as intensity of staining for BVDV decreases with longer storage time.

3) PCR Detection Using Bulk Milk Samples. This test uses the sensitivity of the PCR test to screen several hundred lactating animals for PI status with a single sample. A 100-150 ml sample of bulk tank milk is sent cold, but not frozen, to the lab for testing. This test should *not* be used to assess PI status of the entire herd because most PI animals do not survive to produce milk. A negative bulk tank test tells you nothing about the non-lactating animals (dry cows) or cows not contributing to the bulk tank.

4) Bovine Viral Diarrhea Micro-Plate Herd Screen. The Micro-Plate virus isolation test is formatted to provide an economical way to screen herds for persistently infected animals. The economy of the test comes from the use of serum as the test sample and testing many samples at the same time. This test should *not* be requested on animals less than three months of age (colostral antibody interference) and should *never* be used to detect acute infections. It is also *not* appropriate to use this test for export testing or for qualifying animals for AI centers.

5) Antigen-capture ELISA. The assay is designed for detecting an envelope glycoprotein of BVDV which is secreted as an extra-cellular protein during virus replication. The assay has been validated for use in testing serum, plasma, whole blood and ear-notch tissue samples. The method for testing ear tissue samples involves a simple soaking step in buffer. The soaking buffer can then be tested with a BVDV antigen-capture ELISA in the same way as a serum sample. There is some indication that maternal antibodies in calves after receiving colostrum do not interfere with the detection of BVDV antigen from ear-notch tissue samples. This potentially allows the detection of BVDV very early in the life of the calf. The test does not distinguish between persistent infections and acute infections. Hence, any animal that tests positive to the antigen-capture ELISA should be retested to distinguish between PI and acute infections.

6) Bovine Viral Diarrhea Serum Isolation and Bovine Viral Diarrhea Serum Isolation (with IP detection). These tests are virtually identical virus isolation tests with the exception of the method used to detect the presence of the virus. Normally, the virus is detected using fluorescent antibody staining of the test cells. The test "with IP detection" uses an immuno-peroxidase system to detect the presence of BVDV.

These tests are used to detect persistently infected animals when a small number of animals are to be tested or when the level of certainty of a negative status is of paramount importance, such as exports or animals qualifying for AI centers.

7) Bovine Viral Diarrhea Whole Blood Isolation. This test is a virus isolation which uses the mononuclear cells in blood as the test sample. For acute infections, this is the most reliable sample for a BVDV diagnosis. It can also be used to detect persistently infected animals of any age. It is particularly useful for animals under three months of age when the serum isolation test is unreliable. Use a purple- or green-top Vacutainer® tube for collection of blood for this test.

Neospora caninum

Etiology

Neosporosis is a parasitic disease caused by the protozoan *N. caninum.* The definitive host is the dog, and the organism affects a variety of intermediate hosts.

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In cattle, it induces abortions, typically in the middle of the gestation period: however, abortions can occur anytime after the second month of gestation. In adult cows, abortions are the only clinical sign. When latently infected cows become pregnant, their fetuses become infected virtually every time. An important feature of *Neospora* abortions in cattle is that it may take two to four weeks for the fetus to die following infection and for the dam to recognize this and expel the fetus. The majority of fetal infections result in the birth of healthy calves with latent infections that are maintained and subsequently passed on to their fetuses, thus the cycle continues. It is estimated that 75% of dairy herds have at least one animal that is positive for *Neospora* antibodies.

Diagnosis

There are two serologic tests available to detect antibodies to *N. caninum*in in blood from cattle. The first one developed was an imunoflorescent antibody test (IFAT), which reports a titer of the level of antibody present in serum. Most labs now use an ELISA to detect antibodies to *Neospora;* this test essentially provides positive or negative results. Both tests are sensitive and specific, and clinicians can be confident in the results. Most of the tests currently available to detect *N. caninum* indicate exposure only and are therefore not necessarily diagnositic for abortion. Just because the dam is *Neospora* positive does not mean that the abortion was induced by neosporosis. To confirm that an abortion was caused by *N. caniunum*, the organism must be found in the fetal tissue, which can be difficult because most aborted fetuses are likely to be autolyzed at the time of abortion. Immunohistologic/immunohistochemical tests can identify the parasite in various fetal tissues. However, the brain and spinal cord are the most efficient and reliable method to confirm the diagnosis.

Testing for *Neopora*

At this time, the only practical application of serological testing would be as a screening test for replacement heifers. In several studies, seronegative heifers produced 2.5 lb per day more milk in their first lactation than sero-positive heifers. The abortion rate was 7.4 times greater during the first pregnancy for seropositive heifers compared to seronegative heifers. The culling rate has been 1.6 times higher for seropositive cows vs. seronegative cows. Although the economics probably justify screening, the issue of appropriate disposal of seropositive heifers arises.

Johne's Disease

Role of Johne's Testing

For Johne's disease testing to be productive and effective, the producer and veterinarian should have developed a Johne's disease farm plan and discussed a testing strategy. Anyone interested in developing a farm plan should see Johne's Disease Prevention/Control Plan for Dairy Herds, Manual for Veterinarians printed in the May 1999 issue of *The Bovine Practitioner*.

Tests for *Mycobacerium paratuberculosis*

Bacteriological Culture

Culture techniques are not standardized and laboratory proficiency varies considerably. Most diagnostic laboratories use Herrold's egg yolk agar with or without mycobactin for isolation of *M. paratuberculosis*. There is no commercial source of this medium. The isolation of *M. paratuberculosis* from clinical samples is 100% specific. In addition to its high diagnostic specificity, culture has the advantage that it detects animals excreting the organism in the feces and identifies the animals most likely to transmit infection to other animals. Animals that are shedding the organism in the feces are more likely to transmit the infection in milk, colostrum and *in utero* to the fetus. The greatest disadvantage of culture of the diagnosis of *M. paratuberculosis* is the slow growth of the organism. The conventional culture system requires 12 to 16 weeks or longer. The second disadvantage is that culture only detects those animals actively shedding the organism. Reports suggest that in naturally infected populations of cattle, roughly half of the *M. paratuberculosis* infected animals can be detected by standard laboratory culture, i.e., the sensitivity of culture is approximately 50%. The last disadvantage is that culture is relatively expensive with laboratories charge roughly $12 per sample.

Serum Antibody Detection Tests

Antibodies in serum to *M. paratuberculosis* can be detected by three readily available tests; complement fixation (CF), agar gel immunodiffusion (AGID) and enzyme-linked immunosorbent assay (ELISA). Antibody responses occur relatively late in the course of the disease but before the onset clinical signs. Thus, the timing of the antibody response limits the ability of the serum antibody tests to detect animals in the early stages of the disease. Sweeney *et al* found that the sensitivity of the commercially available ELISA kit for *M. paratuberculosis* was affected by stage of infection. The sensitivity of the ELISA for animals that were low level shedders was only 15%, whereas the sensitivity for animals with clinical signs of Johne's disease was 87%. Overall, the ELISA had a sensitivity of 45%. In other words, the ELISA will detect about half of the infected animals in most *M. paratuberculosis*-infected herds. The second most important factor affecting the sensitivity of serum based tests is the concentration of antibody to initiate a positive reaction. The ELISA is a more sensitive test than either the AGID or CF tests. The absorbed

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ELISA is commercially available as a USDA licensed diagnostic kit.

Using tests for Different Purposes

The choice of Johne's test and strategy will be client-specific and is contingent upon the objectives of the producer. Some examples of testing choices are provided.

1. Diagnosis for a cow with clinical signs of Johne's disease. The ELISA test is the most rapid and least expensive test to confirm a diagnosis of Johne's disease in individuals. Over 85% of *M. paratuberculosis*-infected cattle with diarrhea and weight loss test positive to the ELISA-test.

2. Estimation of herd prevalence of paratuberculosis. An ELISA test of all animals two years of age and older is the fastest and easiest way to determine the prevalence of paratuberculosis for the herd. The percentage of a herd that is ELISA-positive for paratuberculosis (apparent prevalence) should be doubled to get a rough estimate of the true prevalence of paratuberculosis since the sensitivity of the test is roughly 50%. Using the ELISA or any other test for paratuberculosis to estimate herd prevalence of *M. paratuberculosis* infection is only valid for herds not routinely testing and culling positive animals.

3. Paratuberculosis control. Culling test-positive animals should be part of any paratuberculosis control program. For most commercial dairy herds, a whole-herd ELISA is one of the first steps in a paratuberculosis control program. ELISA positive animals should be identified to prevent the feeding of colostrum and discard milk to calves. These animals should also be put on a "to cull" list. If these animals either have other diseases or develop a diarrhea, they should be culled.

Leptospira borgpetersenii serovars *hardjo bovis*

Control Strategies

For herds that have endemic *Leptospira* type *hardjo-bovis* infections, control strategies should involve a combination of vaccination and treatment. For herds not infected with *Leptospira* type *hardjo-bovis* the goal may be to protect the herd from becoming endemically infected through a vaccination program.

Etiology

The clinical manifestations of leptospirosis in cattle are determined by whether the strain of leptospira is the host adapted-maintenance serovar or an incidental serovar. Serovars of leptospira that are maintenance serovars in other species of mammals become incidental serovars when they infect cattle. The incidental serovars of leptospira in cattle are often associated with an acute or subacute disease that occurs during the leptospiremic phase of the infection. The chronic phase of the incidental infection is characterized by late term abortion, weak or stillborn calves, and drops in milk production. Four incidental serovars of leptospires are common to cattle—*L. pomona, L. grippotyphosa, L. canicola* and *L. icterhemmorhagiae*—can be transmitted to cattle from other carrier animals. They are found in chronically infected rats, dogs or even pigs. If these animals contaminate water or feed sources, bovines could absorb the organism orally or through a mucous membrane. In contrast to the incidental serovars, two serologically indistinguishable maintenance leptospiral serovars occur in cattle. They are genetically distinct types of serovar Hardjo that have been identified: *Leptospira interrogans* serovar *Hardjo* (type hardjo-prajitno) and *L borgpetersenii* serovar Hardjo (type hardjo-bovis). Serovar Hardjo type hardjo-bovis is common in cattle populations throughout the world; type hardjo-prajitno is isolated primarily from cattle in the United Kingdom. The most common form of acute leptospirosis occurs in dairy cows as transient pyrexia with a marked drop in milk production lasting for two to ten days. Chronic infection of the female genital tract also may be associated with infertility in cattle infected with serovar Hardjo.

Diagnosis

The objective of testing for *Leptospira* type *hardjo-bovis* is to make diagnosis for the infection status of the herd, not the individual. Since leptospira are shed intermittently, repeated sampling would be necessary for the determination of the infection status of individuals. For herds infected with *Leptospira* type *hardjo-bovis*, the usual prevalence is 20 to 30% of the individuals in the herd. Based on the 20 to 30% prevalence, sampling 15 animals will allow one to determine herd status with 95% confidence that the herd is endemically infected if one sample is positive.

Diagnostic tests for leptospirosis can be separated into serological tests and those designed to detect the organism or it's DNA in tissues or body fluids of animals. Most of the test designed to detect the organism or its DNA and do not distinguish between serovars. Each of the diagnostic procedures, for detection of the organism or for antibodies directed against the organisms, has a number of advantages and disadvantages. Some of the assays suffer from a lack of sensitivity and others are prone to specificity problems. Therefore, no single technique can be recommended for use in each clinical situation. Use of a combination of tests allows maximum sensitivity and specificity in establishing the diagnosis. Serological testing is recommended in each

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case, combined with one or more techniques to identify the organism in tissue or body fluids.

In herds that are chronically infected with *Leptospira* type *hardjo-bovis,* the mature cows in the herd seem to have developed some degree of immunity to the organism but continue to shed the organism in their urine. When first-calf heifers which are immunologically naïve enter the herd, this is there first significant exposure to the disease. The consequence is that the pregnancy rate for first-calf heifers is lower than that of cows. Normally, first-calf heifers should have higher pregnancy rates than the older cows in the herd. Whenever first-calf heifers have lower pregnancy rates than cows, *Leptospira* type *hardjo-bovis* infection should be considered in the differential and the herd tested for the disease.

Serologic Tests

The microscopic agglutination test (MAT) is the most frequently used serological test in the US. It has the advantages of being inexpensive, reasonably sensitive and widely available. The MAT involves mixing appropriate dilutions of serum with live leptospires of servars. A positive test indicates the presence of antibodies from the resulting agglutination of the leptospires. Detection of high MAT titers in combination with clinical signs consistent with leptospirosis may be adequate to make a diagnosis. This is particularly true in the case of abortions caused by incidental serovars in which the dam's agglutinating antibody titer is >1000. The situation is less clear with *Leptospira* type *hardjo-bovis*. In maintenance host infections with *Leptospira* type *hardjo-bovis*, infected animals often have very low or negative MAT titers at the time of abortion. Conversely, animals that have been vaccinated with the Pfizer's Spirovac® may have very high MAT titers that could lead to a misdiagnosis without knowledge of the vaccination history. Interpretation of MAT titers is complicated by a number of factors, including cross-reactivity between different serovars, titers induced by vaccination and the lack of consensus about what antibody titers are indicative of active infection.

Detection of Leptospires

The techniques available for the detection of leptospires in body fluids of cattle include darkfield microscopy, immunoflorescence, culture, histopathology with special stains and polymerase-chain-reaction (PCR) assays. Darkfield microscopy has been used as a rapid screening tool to identify leptospires in the urine of animals. The advantage of darkfield microscopy is speed; disadvantages include low specificity and sensitivity. The sensitivity of darkfield microscopy is low; approximately 10^5 leptospires/ml of urine must be present to be detected. Immunoflorescence can be used to identify leptospires in tissues, blood, or urine sediment. The availability of this test is increasing, and the test is rapid, has good sensitivity and can be used on frozen samples. Interpretation of immunoflorescence tests may be difficult and requires a skilled laboratory technician. The florescent antibody conjugate currently available for general use is not serovar specific; serologic examination of the animal is still required to identify the infecting serovar. There seems to be some variation between state diagnostic laboratories in their ability to detect leptospires in the urine with immunoflorescence. In the future PCR techniques may make the identification of leptospires more objective.

How to Sample

Treatment of cows with furosemide enhances the chances of finding leptospiral organism in the urine by increasing flow through the tubules of the kidney and produces a dilute urine which favors survival of the organism. The adminstration of 10 ml of furosemide intravenously facilitates collection of urine. The first dilute urine sample is collected in sterile tubes. If the urine has a slight yellow color, the sample should be discarded and a sample taken from the second or third urination. Samples should be kept cool and shipped to the lab overnight. Five to 10 ml of urine in redtop Vacutainer tubes is adequate. It's important that samples are kept cold to prevent bacterial growth. Ice packs work well. If you have to delay shipping, keep samples refrigerated. Try to avoid shipping samples that arrive at laboratories on a Saturday. Second-day air is fine as long as samples are kept cold. Freezing should be avoided as it tends to break up the leptospiral organisms. Milk from cows treated with furosemide must be withheld for 48 hours.

Sample Contamination

Samples of urine can be become contaminated with either environmental contaminants or cross-contamination between samples. For best results, the sample should be collected as cleanly as possible. It is possible to get false positives when there is cross-sample contamination. When an infected animal is shedding in excess of a million organisms per ml of urine, a small amount of urine from the heavy shedder can cross-contaminate the next sample.

Mycoplasma spp Mastitis

Since mycoplasma mastitis is caused by several species of mycoplasma organism and some of which are among the most contagious mastitis pathogens, it is a good practice to have a mycoplasma surveillance program in place, particularly as dairies become larger. Some level of milk culturing is necessary as part of a

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sound surveillance program. At a very minimum, a dairy should be screening bulk tanks on a regular basis. If a dairy has a separate bulk tank for the hospital pen, it should be cultured on a regular basis, probably weekly. When a sample of bulk tank milk is collected, it is important to have a system in place to identify the cows that contributed to the bulk, especially since the interval between collections of the sample and obtaining a final report may be up to 10 to 14 days. It is also a good practice to culture all new additions to the herd including first calf heifers either as they freshen or as lactating animals added to the herd. A feature of mycoplasma mastitis is that cows with clinical mastitis shed tremendous numbers of organism, often in excess of millions of organisms per ml of milk. This not only contribute to the risk of spread from cow to cow, but also increases the risk of contamination of samples collected from non-infected cows resulting in false positive results. In contrast to the high levels of shedding that occurs from cows with clinical mastitis, cows that are subclinical and chronically infected may shed mycoplasma intermittently. Mycoplasma isolated from bulk tank samples should be speciated to distinguish between environmental contaminates and pathogenic mycoplasma.

Guidelines for Bulk Tank Sampling

To get the most out of bulk tank milk sampling, follow these five suggestions:

1. Take samples for four or five days in a row, freeze sample following collection, and send to the frozen samples to the lab to be cultured as a composite at the lab.

2. Agitate the bulk tank for 10 to 15 minutes before taking a sample.

3. Take samples from the top of the bulk tank with a sterile syringe and needle or vial, to avoid contamination from the outlet valve.

4. Freeze the sample immediately and pack it for shipment to ensure that the sample will stay frozen until it reaches the laboratory. It is also a good idea not to ship to the laboratory after Wednesday in order to avoid long storage times such as over the weekend.

5. If lab test results are inconclusive, retake the samples or identify and sample individual cows with high somatic cell counts to provide further information.

Sample Handling

The way milk samples containing mycoplasma organisms are handled following collection affects the ability to recover the organism. Compared to directly culturing samples of milk, two factors affected the recovery of mycoplasma: 1) The longer the duration of time the sample was frozen, the greater the reduction in recovery of the organism. Milk samples should not be stored in a refrigerator following collection. Refrigerator temperature is very detrimental to the survival of mycoplasma organisms. 2) Milk samples that were thawed in a water bath at 98.6°F (37°C) have lower recovery rates than samples thawed at room temperature.

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