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Effective Use of the Diagnostic Laboratory in Dairy Practice

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Today's dairy practitioner is challenged to provide rapid and accurate diagnosis for his clients. Diagnostic medicine is becoming more complex, and proper use of diagnostic procedures and facilities is sound medicine. The practitioner needs to competently use his practice's laboratory as well as public and private referral diagnostic laboratories. This article deals with information that should improve the quality of diagnostic laboratory procedures.

GENERAL STRATEGIES

A dairy practitioner is a client of the referral diagnostic laboratories that he or she uses, so it is to the practitioner's advantage to learn as much as possible about these laboratories. It is important to have a clear and complete knowledge of the laboratories that are available and an understanding of the services they can provide. A visit to prospective laboratories is a useful way to accomplish this. The practitioner is able to see the scope of a given laboratory's operation and can develop individual contact with the laboratory personnel and begin to establish valuable personal relationships. If a visit is not possible, a telephone call to the laboratory director or other key individuals in the laboratory may be useful. He should ask for all written material available on the operation of the laboratory. Information regarding tests and services, turn-around time on submissions, hours of laboratory operation, availability of forms or manuals, and costs of services is extremely useful. Such information should be collected in written form and put in a convenient place in the practice where both lay and professional personnel can find it for rapid

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reference. Also, a system for periodic (at least annual) updating of the reference material should be set up.

A practitioner should consider the diagnostic laboratory's quality control program. Each laboratory should have planned means to periodically check its laboratory services or tests to detect errors. Some laboratories periodically send known samples to their own facility to check accuracy. Other laboratories encourage referring veterinarians to submit multiple samples from a single source. The laboratory then reimburses the veterinarian for all but one of the samples (as a means of quality control). Some veterinarians periodically send multiple samples of a single source material to two or more laboratories in order to gauge a laboratory's consistency and reliability. Such costs are borne by the veterinarian but are worthwhile if inconsistencies or mistakes are found. If they are found, the practitioner should inform the laboratory involved. Diagnostic laboratories usually will welcome knowing about these problems.

If a practitioner uses a diagnostic laboratory that provides telephone reports, he should be sure to train all personnel to write down such reports, including the patient's identification, owner's name, the results, and the name and telephone number of the individual at the laboratory who provided the test results in case further clarification is needed. An error made on a casually taken telephone report can lead to costly problems.

The practitioner should pay particular attention to getting to know the laboratory personnel. They have special training, experience, interests, and contacts with other diagnostic laboratories, research facilities, or veterinary colleges. Such personnel will often provide valuable assistance beyond routine services.

Once the practitioner has good knowledge of the diagnostic laboratory procedures and personnel he is using or plans to use, he is ready to take maximal advantage of such services for himself and his clients. The following considerations relate to use of proper diagnostic techniques in order to improve diagnostic accuracy. There are a number of services and tests available that may be useful to the dairy practitioner. Those most commonly used can be categorized into the areas of microbiology, pathology, clinical pathology, parasitology, and toxicology.

MICROBIOLOGY

Materials submitted for microbiologic evaluation can give misleading test results. Four general points to increase the value of submissions for microbiologic examination are considered here.

1. Animals that have been dead more than six hours in warm or hot environments are usually unreliable subjects to use for microbiologic isolations. Clostridial, coliform, and Bacillus overgrowth occurs rapidly, so that isolation of these organisms is likely to be significant only if the carcass is fresh. Many viruses are destroyed in

autolyzed tissues. Samples for virus isolation should be collected from live animals whenever possible. Cattle in the early acute stages of a viral disease will most likely provide suitable samples, whereas cattle in convalescent stages usually will not.

2. Tissues submitted should be packed individually in sterile containers and well labeled.

3. The use of transport media deserves special consideration. Some bacteria and viruses store and transport well in tissues or fluids kept refrigerated. However, samples taken by dry swabs often do not. Anaerobes require use of anaerobic culture media* in order to survive even brief storage or shipment. Questions regarding transport media are best answered by consulting with the microbiology laboratory. Some bacterial transport media contain thioglycollate, which is harmful to viruses. Conversely, some viral transport media contain antibiotics that inhibit or kill bacteria. In some cases, it may be more prudent to submit animals directly to the microbiology laboratory to allow for rapid collection of microbiologic samples or to have field samples delivered directly to the laboratory.

4. Histologic examination may yield rapid evidence to establish the clinical diagnosis and provide the information necessary for the selection of appropriate treatment. In the case of fungal infections, the histopathologist, with the aid of special staining techniques, can often diagnose the etiologic agent more rapidly than the mycologist can culture and identify the organism.

Following are some considerations that are useful for interpreting microbiologic data of specific diseases.

Enteric Disease

Recently voided feces or rectal swabs for attempted isolation of coliforms, Salmonella or Clostridium species, *Mycobacterium paratuberculosis*, parvoviruses, coronaviruses, herpes viruses (IBR), pestivirus (BVD), or rotaviruses are often taken. Establishing that one of these infectious agents was a cause of a disease in dairy cattle can be strengthened by some of the following procedures.

1. Postmortem examination and culture of fresh portions of segments of intestine or stomach with lesions is useful. Isolation of *Escherichia coli* in nearly pure culture is most significant in scrapings from the upper intestine (duodenum, jejunum) and has less significance farther down the digestive tract, since *E. coli* are normally found there. These *E. coli* isolates need further characterization by animal inoculation or biochemical evaluation to determine if they are pathogenic. Salmonella isolated from hemorrhagic intestines and associated lymph nodes strengthens the likelihood that this isolate is causing the enteritis. Ileal or colonic lymph nodes often contain organisms in cases of salmonellosis.

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*Anaerobic Culturette (only one of several available), Marion Scientific Corp., Kansas City, Missouri

2. Use of the Gram stain is helpful in determining the biologic significance of an enteric isolate. In fresh bovine intestine, about one-fourth to one-third of the organisms are gram-positive and the rest are gram-negative. The ratio of Gram stain types allows an assessment of whether a given isolate could be the cause of the disease. Clostridial enterotoxemias usually show 80 to 90 per cent of the organisms in the congested or hemorrhagic areas to be gram-positive; in colibacillosis, *E. coli* organisms will comprise 90 to 95 per cent of the bacteria present and are gram-negative. Cattle that have been dead for more than a few hours often have rapid clostridial overgrowth, so that the normal Gram stain ratio cannot be expected.
3. Histopathologic and/or electron microscopic examination may show the pathogens and characteristic lesions they cause. The tissue changes are particularly diagnostic for *Mycobacterium paratuberculosis* infections and some viral infections. Other methods of detection such as serology, immunofluorescence, hemadsorption, or enzyme-linked immunosorbent assay (ELISA) may be useful in making specific diagnoses.
4. Animal toxicity studies can be used to assess pathogenicity of enteric isolates. Suckling mice and guinea pigs are used when such confirmation is necessary.

Respiratory Disease

Nasal swabs are often used to obtain isolates for diagnosis of respiratory disease. Nasal swab isolates are not well correlated to individual lower respiratory tract infections. When dairy cattle are housed in dusty or dirty surroundings, they can have massive numbers of nonpathogenic or opportunistic organisms impinged in the nasal sinuses which have no connection with any pulmonary infection. Tracheal swabs and washes are more accurate ways of culturing the bovine respiratory tract. Infectious agents that infect the airways will be carried upward by the mucociliary apparatus. Isolates obtained from bovine lung tissue collected at necropsy are most significant if the following procedures are used.

1. Whenever possible, euthanatize animals for necropsies for pulmonary microbiologic isolation. Animals that have been dead for more than six hours do not provide reliable specimens.
2. Take tissue for culture from the lower lung (secondary bronchioles or alveoli). This tissue is normally sterile. Because lungs are so porous, extra effort should be made to handle the lung aseptically when collecting swab samples or lung for culturing.
3. Culture the bronchial lymph nodes if the animal has been dead for more than six hours. The bronchial lymph nodes are more reliable to culture than are the lungs.
4. If there is an acute pleuritis, culture the pleura and associated fluids as they will often contain pathogenic organisms.
5. Culture lung tissue at the transition from normal to acute lesions. Such areas usually reflect the more important infection present. Chronic lesions will often be contaminated with a myriad of organisms, many of which are strictly opportunistic or saprophytic. Pulmonary ab-

scesses may contain secondary invaders that do not necessarily relate to the primary infection.

Mastitis

Always try to collect milk samples for culture before cattle with mastitis are treated with antibiotics. If the cow has been treated, inform the laboratory. By use of special media, organisms from treated quarters can sometimes be grown, whereas the organisms from milk containing antibiotics will usually not grow if directly applied to culture plates. Do not collect milk samples from wet udders. The chance of direct water or aerosol contamination from the skin of the udder is too great. If the udder is dirty, wash it and dry it well. Use 70 per cent ethanol swabs to wipe the two far teats, then the closest teats. Pay attention to the teat meatus in the cleaning procedure. Collect milk from the two closest teats first. Discard the first stream of milk from the teat as it will have slight contamination of environmental organisms in the meatus. Sterile screw-top vials are the best containers to use. Stoppered test tubes or large containers with large openings, such as plastic bags or wide mouth jars, are more likely to be contaminated. Hold the screw-top vials nearly horizontally to keep debris from falling into the opening. Refrigerate milk samples (2 to 4°C). Freezing milk samples for shipment in cases of subclinical mastitis is not recommended. If storage of samples will exceed one week, samples may be frozen. Although freezing destroys large numbers of organisms in most cases of subclinical and clinical mastitis, there are still sufficient viable organisms to assure cultural isolation. One exception might be *E. coli* mastitis in which the organism is sometimes difficult to isolate under any circumstance.

Reproductive Infections

Abortion. Few other conditions evoke as much concern in a dairy herd as one or more abortions. An abortion is a pathophysiologic event that may be caused by a number of factors other than infectious disease. Usually, less than 25 per cent of bovine abortions have detectable infectious causes. Because of the variety of possibilities, the dairy practitioner needs to consider all possible etiologies and should examine the dam, placenta, fetus, and the area the dam was in prior to abortion. Consider feeding and water sources or unusual features of the management or environment that could possibly cause an abortion. The dam's prior reproductive and vaccination history should be reviewed and submitted with the materials sent to the diagnostic laboratory. If an infectious cause is suspected, the following procedures are suggested to maximize detection of an infectious cause of an abortion.

1. As much placental tissue and uterine discharge as can be collected should be submitted in individual sterile containers. Refrigerate the tissues during storage and shipment. Bovine chlamydial, mycotic, and

some viral and bacterial abortions can be diagnosed directly from the placenta. Also submit placental tissue, including 6 mm (¼ in.) sections of cotyledons in 10 per cent neutral buffered formalin for histologic evaluation. Isolation of an infectious agent from the placenta only does not establish pathogenicity, but demonstration of histologic involvement and isolation may do so.

2. Submit the intact fetus as soon as possible. Refrigerate it; do not freeze it. If a fetus cannot be submitted intact, ship in separate containers, intact abomasum and contents (tie off the esophagus and duodenum), liver, lung, kidney, spleen, and adrenal glands. Also send samples of these tissues fixed in 10 per cent neutral buffered formalin.
3. Collect urine from the dam. The urine should be collected midstream in a clean or preferably sterile container. Fix a portion of this urine by adding 1.5 ml of 10 per cent formalin to 20 ml of urine. Also submit refrigerated fresh urine if it can be cultured within six hours or less. These specimens can be used to detect the presence of leptospirae in order to establish whether the dam is a shedder or not. Diagnosis of leptospiral abortion cannot be made on the basis of the urine shedding alone, but if the dam is a shedder, further examination of the fetus and dairy herd may establish the cause of the abortion and herd infection, respectively. Since shedding is intermittent, urine and serum should be sampled from at least 10 cows (aborted and in-contact) initially, in order to increase the likelihood of detecting at least one shedder.
4. Collect serum from the dam at the time of abortion and about two weeks later so that paired samples are obtained for submission and evaluation. A rising serum titer for a given infectious agent following abortion is circumstantial evidence of its involvement in the abortion. Unfortunately, a rising titer may not been seen if the abortion follows infection by a few weeks or more. Interpretation of serum titers alone has the most value with brucellosis and possibly leptospirosis. As a single indicator of bovine abortion for other common infectious diseases, it has less value. Hence, interpret serologic data with microbiologic isolation and cytologic and histologic evaluations whenever possible.

Postpartum Infection. Uterine contamination following parturition is normal, yet it is surprising how few cows develop a uterine infection leading to infertility. Culturing of the uterus requires some sort of double-tube swab* to obtain a reliable sample. *Corynebacterium pyogenes* is often isolated, but is only likely to be a primary pathogen if there is a pyometra or endometritis and if the organisms are found in high concentration and in relatively pure culture. Often, *C. pyogenes* is found with other organisms such as *E. coli* and *Staphylococcus aureus* in a mixed infection of the uterus following parturition. In such cases, *C. pyogenes* is not likely pathogenic.

Infertility. In dairy herds experiencing low fertility, campylobacteriosis (vibriosis) and trichomoniasis should be considered as possible causes. Isolation of *Campylobacter fetus* and *Trichomonas fetus* from the preputial area of bulls establishes the presence of this bacterium

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*Tiegland Uterine Swabs (modified), A. J. Buck & Sons, Inc., Cockeysville, Maryland.

and parasite in dairy herds. The presence of *C. fetus*, *T. fetus*, or specific antibodies for *C. fetus* in the vagina or cervix also establishes exposure of the herd female population, but does not prove pathogenicity.

Two varieties of *C. fetus* are important in bovine reproductive infections. *C. fetus* (var. *intestinalis*) is often found in the bovine digestive tract and occasionally in the vagina, but it is less frequently a cause of infertility or abortion in cattle. *C. fetus* (var. *fetus*) is more likely to be pathogenic. Both *C. fetus* (var. *fetus*) and *C. fetus* (var. *intestinalis*) can be found in fertile adult cattle that are immune to the organisms. If these organisms can be detected in utero in the presence of a salpingitis, endometritis, or in placental or fetal tissue from an abortion, this constitutes good evidence of pathogenicity. Fresh preparations of uterine or placental (cotyledonary) fluids or fetal stomach contents can be examined with stained preparations or by dark field microscopy. The presence of fast moving, flagellated organisms in this material or in stained organisms resembling *C . fetus* is circumstantial evidence of infection with *C. fetus*. However, culturing is necessary to positively identify these organisms. Special care needs to be taken when materials are submitted to the diagnostic laboratory for culture. *C. fetus* stores and transports poorly in presently available transport media and is susceptible to sunlight and drying, so speed (less than 24 hours) is necessary for delivery of viable samples for culturing. *Trichomonas fetus* in uterine pus samples remains viable and motile for 48 hours or longer.

Infectious Arthritis

In live animals, aseptically prepare the skin over joints and obtain synovial fluid using a sterile needle and syringe. The same procedure can be used for dead cattle. Alternatively, the entire intact joint can be submitted for culture. It is recommended that three or more dry smears be made from the synovial fluid samples, and both a Gram stain and a Wright-Giemsa stain be made. These stained slides may be made in the practice laboratory, or the dried smear slides can be fixed in absolute methyl alcohol for five minutes and sent to the diagnostic laboratory. The Gram stain allows assessment of the bacterial numbers and the Gram stain type, while the Wright-Giemsa stain will stain white blood cells responding to the infection. Both of these staining procedures will help confirm if an isolate is causing an infectious arthritis by permitting detection of many bacteria and inflammatory cells, respectively. Absence of inflammatory cells and bacteria in the smears suggests the isolate obtained was a contaminant of the synovial fluid sample. The presence of white and red blood cells without evidence of infectious agents would suggest a noninfectious arthritis (traumatic or degenerative).

Infectious Keratitis

A presumptive diagnosis of infectious bovine keratitis or keratoconjunctivitis is usually made on the basis of clinical signs.

Ocular swabs may be taken for attempted isolation of *Moraxella bovis* and cultural confirmation of this diagnosis. *M. bovis* is best isolated from early cases of the disease. It is a difficult organism to transport because of its susceptibility to drying and poor storage viability. If swabs are shipped, collect from six or more animals and expect to get a good proportion of false negatives. Often, the most successful way to isolate *M. bovis* is to directly streak the swab on an ox blood agar plate at the farm or within two hours of collection. Some older cattle are intermittent shedders and will have an avirulent *M. bovis* strain in their ocular or nasal secretions. *C. pyogenes* is often found as a secondary invader in cases of infectious keratitis. Staphylococcal and streptococcal organisms may be isolated from the bovine eye; these are often secondary invaders.

Abscesses

Skin, muscle, lung, mammary glands, bones, and joints may become abscessed in cattle. Collect pus near the abscess wall since the greatest number of viable organisms are usually here; pus in the center of the abscess may contain no viable organisms. Tissue for culture, granules, and/or pus, collected in sterile containers after adequate sterilization of the skin, should be submitted to the laboratory together with biopsy material or tissue specimens fixed in 10 per cent neutral buffered formalin for histopathologic examination.

Skin Lesions

Skin isolates require careful interpretation. The skin usually yields isolates of varying pathogenicity. Table 1 provides information relative to the isolation of specific infectious agents from bovine skin.

Myositis and Septicemias

Clostridia can cause various acute musculoskeletal and septicemic diseases in cattle. These organisms may also be postmortem contaminants. Hence, be conservative in making a diagnosis of blackleg, malignant edema, or hemorrhagic disease (icterohemoglobinuria) in dairy cattle unless the animal is necropsied within a few hours after death.

1. In cases of sudden death, always consider anthrax. Certain carnivorous birds and mammals are asymptomatic carriers and may bring *Bacillus anthracis* into an area formerly free of the organisms.
2. *Clostridium novyi (oedematiens)* is a common postmortem contaminant in cattle and *Clostridium septicum* may be also. *Cl. septicum* can also cause malignant edema, so it is important to decide whether the degree of autolysis and lesions warrant this diagnosis. Isolation of *Clostridium chauvoei* (blackleg) is more diagnostically significant since the organisms are less likely to be postmortem contaminants. Submit thick sections of hemorrhagic muscle or connective tissue, whole bones, blood, liver, and spleen in individual sterile containers. Anaerobic transport media or swabs may be used for fluids (blood,

Table 1. *Summary of Common Infectious Diseases in Dairy Cattle and Methods of Sample Collection*

PATHOGENIC ORGANISMS	SAMPLES TO SUBMIT	SPECIAL CONSIDERATION
ENTERIC DISEASE *E. coli** Parvoviruses* Coronaviruses* Rotaviruses* Salmonella serotypes *Clostridium perfringens* (Type C or E) *Mycobacterium paratuberculosis* *Campylobacter jejuni* Mycotoxicoses Pestivirus (BVD) Herpesvirus (IBR) *Mycobacterium bovis*	Live animal: recently voided feces or fecal swab Dead animal: upper intestinal contents; fresh and fixed tissue containing lesions; mesenteric lymph nodes; fresh and fixed ileocecal valve for *M. paratuberculosis*	(1) Organisms such as Salmonella can be present in healthy carrier animals. (2) Toxins due to *Cl. perfringens* type C or E are very labile. Must collect ileal contents from a recently dead animal and get to laboratory within a few hours.
RESPIRATORY DISEASE *Pasteurella multocida* *Pasteurella haemolytica* *Streptococcus pneumoniae*† *Mycoplasma mycoides* *Mycobacterium bovis* *Aspergillus fumigatus* Bovine adenoviruses PI_3 virus Mucosal disease virus Herpesvirus (IBR) *Hemophilus somnus*	Live animal: tracheal swabs and washes Dead animal: lung lesions and/or tissue from lower lung; bronchial lymph nodes; pleural fluid and pleural lesions	(1) Nasal swab isolates are not well correlated with lower respiratory tract infections. (2) Samples should be taken within six hours of death. (3) Take samples from acute rather than chronic lesions.
MASTITIS *Staphylococcus aureus* *Streptococcus agalactiae, dysgalactiae,* or *uberis* *E. coli* Other coliforms *Nocardia* spp. *Pseudomonas aeruginosa* *Corynebacterium pyogenes* *Mycoplasma* spp. Fungi or Yeasts *Mycobacterium bovis*	Unpreserved fresh milk kept in refrigerator	(1) Take great care in collecting the samples to ensure that the organisms cultured are not external contaminants. (2) Collect milk from cows before treatment with antibiotics.
REPRODUCTIVE INFECTIONS **Abortion** *Brucella abortus* *Campylobacter fetus* var. *fetus* var. *intestinalis* *Chlamydia* spp. *Listeria monocytogenes* Leptospira serotypes *Aspergillus fumigatus* *Mortierella wolfii* Herpesvirus (IBR) Indirectly any organism causing a febrile condition	Fetal material: (a) intact fetus if possible; refrigerate, but do not freeze; OR (b) intact abomasum and contents; fresh and fixed lung, liver, kidney and spleen Maternal specimens: placental tissue including cotyledons fixed and fresh; uterine discharge; 20 ml urine with 1.5 ml of 10% formalin; serum sample at time of abortion and two weeks later	(1) Require both fetal and maternal samples whenever possible (2) A diagnosis of leptospiral abortion should be made on a herd rather than an individual cow basis. Collect paired serum and urine samples from aborted and in-contact animals. (3) Always submit fixed and fresh material. For example, a diagnosis of fungal abortion requires isolation and histopathology.

Table 1. *Summary of Common Infectious Diseases in Dairy Cattle and Methods of Sample Collection (Continued)*

PATHOGENIC ORGANISMS	SAMPLES TO SUBMIT	SPECIAL CONSIDERATION
Postpartum Infection (metritis) See the organisms involved in abortion Secondary invaders: *Corynebacterium pyogenes, Staphylococcus aureus*	Uterine discharge in a double-tube swab	Generally find a mixed infection present.
Infertility		
Campylobacter fetus (var. *fetus*) *Trichomonas fetus* *Corynebacterium pyogenes*	Uterine discharge or vaginal/cervical mucus; OR semen and preputial washings (saline) from bulls	Infertility unlikely to be due to these venereal pathogens if artificial insemination is used.
INFECTIOUS ARTHRITIS		
Staph. aureus *Erysipelothrix rhusiopathiae* *E. coli* and *Salmonella* *Corynebacterium pyogenes* *Brucella abortus* Streptococci Mycoplasmas *Chlamydia* spp.	Live animal: Aseptically taken joint fluid Dead animal: Aseptically taken joint fluid or unopened joint.	A dry smear made from the fluid upon collection is useful.
INFECTIOUS KERATITIS		
Moraxella bovis IBR and BVD viruses Adenoviruses Mycoplasmas	Preference: (a) Place swab in lower conjunctival sac and allow it to become saturated; plate directly on ox blood agar; OR (b) place swab in a little saline and get to laboratory within a few hours.	Recovery of *M. bovis* is difficult, so collect from about 10 animals.
ABSCESSES		
Actinobacillus lignieresii *Actinomyces bovis* *Mycobacterium bovis* or *avium* *Corynebacterium pyogenes* or *equi* *Staphylococcus aureus* *Nocardia asteroides* *Fusobacterium* spp.	Collect pus near the abscess wall and include scrapings of the wall itself.	Pus at the center of an abscess often does not contain viable organisms.
SKIN LESIONS		
Bovine papilloma	A few whole warts and adjacent normal skin, fixed in 10% neutral buffered formalin	Take warts that are not brittle; these may be necrotic.
Bovine mamillitis	Aspirate vesicle fluid if possible (the best sample) Skin biopsy of lesions on teats. Must have *early* untreated lesions. Fix some lesions in 10% neutral buffered formalin, submit some fresh tissue; refrigerate	Histologic examination may detect intranuclear inclusions, epidermal degeneration. Virus can be isolated in cell cultures (in bovine kidney cells).
Pseudocowpox	Same as for bovine mamillitis.	Histologic examination may reveal intracytoplasmic inclusions; can cause human lesions (milker's nodules); can be isolated in cell culture.

Table 1. *Summary of Common Infectious Diseases in Dairy Cattle and Methods of Sample Collection (Continued)*

PATHOGENIC ORGANISMS	SAMPLES TO SUBMIT	SPECIAL CONSIDERATION
Bovine ringworm (Trichophyton spp.)	*Pluck hair* and submit skin scrapings; take skin biopsy near edge of lesions; fix in 10% neutral buffered formalin.	Request culture and morphologic identification. Submit hair and skin scrapings in *paper* envelope.
Streptothricosis *(Dermatophilus congolensis)*	Scab from affected areas.	Methylene blue or Gram stains of scab will reveal characteristic filaments. Submit scab in a paper envelope.
Cutaneous "TB"	Skin biopsy in 10% neutral buffered formalin.	Request acid-test stain.
MYOSITIS AND SEPTICEMIAS		
Clostridium novyi, chauvoei, or *septicum* *Clostridium haemolyticum* *Bacillus anthracis*	*Malignant edema:* rib containing bone marrow; piece of affected muscle. *Hemorrhagic disease:* rib containing bone marrow; liver lesion, fixed and fresh. *Anthrax:* dry, thin, blood smears from ear vein.	Collect specimens as soon after death as possible.
NERVOUS SIGNS		
Listeria monocytogenes *Clostridium tetani* Malignant catarrhal fever virus *Haemophilus somnus*‡ *Chlamydia* spp. *Pasteurella multocida* (calves)	Cerebrospinal fluid; brain from dead animal	(1) Tetanus can occur after parturition. Diagnosis best made on clinical findings.
Rabies	Submit entire head	Submit to local health department in most states, *not* the diagnostic laboratory.
INFECTIONS OF URINARY TRACT		
Corynebacterium renale Leptospira serotypes	*Pyelonephritis:* fresh urine; fresh and fixed kidney *Leptospirosis:* 20 ml urine in 1 to ½ ml 10% formalin; fresh and fixed kidney	
STOMATITIS AND/OR LARYNGITIS		
Fusobacterium necrophorum (calf diphtheria) IBR and BVD viruses Bovine papular stomatitis virus Bluetongue virus Vesicular stomatitis virus Foot and mouth disease virus	Biopsy: fixed and fresh scrapings in transport medium	If the lesions are vesicular, it might be wise to consider the exotic vesicular viral diseases.

*Young animals only.
†Septicemia in calves.
‡Infectious thromboembolic meningoencephalitis.

hemorrhagic exudates) or for swabbing of solid tissue. Keep and ship samples refrigerated or frozen. Histologic presence of clostridial organisms in areas of hemorrhagic and purulent inflammation, and fluorescent antibody techniques are additional ways to confirm specific infection. Laboratory animal (guinea pig) inoculation also is useful to show virulence of clostridial isolates.

3. When *Cl. haemolyticum* causes disease, there are almost always liver infarcts in which the organisms can be found. There is usually a hemoglobinuria. Sometimes the organisms can be cultured from the urine or blood.
4. The marrow of intact long bones is a good tissue to culture for bacterial organisms that have caused septicemic disease, and it has the advantage of not being contaminated by postmortem bacteria that normally grow by extension through soft tissue.

Other Diseases

In addition to the previously discussed diseases, Table 1 provides information on infections of the nervous, urinary, and upper digestive systems.

GROSS AND HISTOLOGIC PATHOLOGY

Gross Pathology

A dairy practitioner should perform his own necropsies whenever possible. The farm necropsy often provides the most rapid solution to a health problem. The opportunity to talk with the dairyman directly and to examine the environment, feed, water and associated livestock may provide important clues to a diagnosis. The farm necropsy gives the veterinarian a chance to show the dairyman lesions and will reinforce the need for proper veterinary care and preventive medicine better than any amount of discussion. A farm necropsy often will eliminate much of the cost of labor, time, and vehicular use necessary to bring an animal to a diagnostic laboratory. In some cases, it is less expensive for the practitioner to do the necropsy on the farm than in the diagnostic laboratory.

Time and labor can be minimized by use of lay assistance and hydraulic or mechanical lifting units found on most dairy farms. A few good surgical instruments, water, and a good source of light generally allow necropsies to be done in a civilized fashion on the farm. A meat saw, a pair of heavy-duty tree trimmers, and an ax are useful additional tools to use. The meat saw can be used to cut the skull and other bones. The tree trimmers are excellent for cutting ribs. An ax or hatchet will allow the dorsal portions of vertebrae to be cut away for rapid removal of the spinal cord. The necropsy should be done away from contact with other cattle or their environment in the event that infectious agents are present. There also should be proper disposal of the carcass.

The veterinary practitioner has sufficient formal education to do a good routine necropsy and by investing some time and effort, he can

further improve his necropsy skills. This may be done through short courses, reading, going to the diagnostic laboratory and assisting with the necropsies there, conversing with pathologists, and regularly performing routine necropsies.

Avoid submitting "mystery death" cases. These cases always lack a good history or clinical involvement. Clinical histories are usually limited to statements such as "sudden death" or "ate fine yesterday, dead this morning." The dead animal is usually observed very little by anyone—much less examined or treated. When this carcass is removed from its environment and necropsied in a diagnostic laboratory, the probability of establishing a diagnosis often decreases rather than increases.

There are at least two specific situations in which a practitioner should request a referral necropsy. The first is when field procedures, including field necropsy, have been used and have failed to establish a diagnosis. The second is when an animal needs to be necropsied in the diagnostic laboratory in order to very rapidly obtain materials for further evaluation (for example, taking tissue samples for virologic isolation from a euthanatized calf).

When submitting a bovine carcass to a diagnostic laboratory, provide a complete history. The veterinarian should supply any information or records he has regarding treatment or examination of the submitted animal or herd mates. The person who delivers the carcass should be someone who is familiar with the animal so that the diagnostic laboratory personnel are able to ask about the animal's clinical signs, eating habits, and so forth just prior to death.

When there is a choice, submit animals that represent the median clinical conditions, not extreme examples. For economic reasons, dairymen have a tendency to volunteer submission of the poorest animal in a group. If reliable virologic or bacteriologic isolations are to be made, select live animals that are in the acute phase of infection or animals that have been dead less than six hours, if possible. Cattle that have been dead beyond 12 hours are unlikely to be reliable for microbiologic purposes, particularly if digestive, respiratory, or reproductive tract cultures are to be attempted. In warm or hot weather, try to slow autolysis. If refrigeration is not possible, the carcass should be placed in as cool a place as possible. Use ice packs or run cold water over the body surface to help cool down the carcass. Opening the abdominal cavity and placing containerized ice inside is an effective way to cool down a carcass. However, it also contaminates this cavity and should not be used if microbiologic samples from the serosal surfaces of this cavity are expected to be taken. In winter, do not freeze carcasses; this creates tissue artifacts that hinder histologic evaluation.

Histopathology

In order to avoid certain common errors, some basic procedures need to be followed by dairy practitioners when submitting tissues for histopathologic evaluation. Some of these errors lead to incorrect

diagnoses or to no diagnosis at all. The following errors are most often noted: (1) improper sectioning, (2) improper fixation, (3) tissues in poor condition before fixation, (4) little or no clinical history provided, (5) improper packaging, (6) improper labeling, and (7) submission of inappropriate tissue.

Improper Sectioning. The accuracy of histologic evaluation cannot be better than the accuracy of sampling. Try to submit entire lesions with normal adjacent tissue. If this is not possible, submit two or three sections representing the lesion and normal adjacent tissue. Some practitioners believe the central portion of a lesion is most diagnostic. This may not be true because the central area can be necrotic due to the loss of blood supply. More often, the periphery of a lesion has the most active lesions. Submission of the adjacent normal tissue allows for evaluation and comparison with normal tissue.

Lesions are often submitted in large, thick pieces. This can result in poor fixation of the submitted tissue and can lead to a loss of the diagnosis if the primary lesion is centrally located in the submitted section. Most fixatives do not penetrate rapidly beyond 3 mm in the solid tissue. Hence, a section should not be more than 6 mm (¼ in.) thick. There are no limitations in the other dimensions. Large lesions can be submitted by making a series of 6 mm incomplete slices through the tissue. The brain may be one exception to this procedure. Many pathologists prefer that the brain be fixed whole for five to ten days before submission and submitted whole.

Improper Fixation. A common error made with fixation solutions is to use too little volume compared to the bulk of tissue being fixed. Tissue should be fixed in 10 to 20 times its volume of fixative solution. The container holding the fixative and tissue should allow the tissues to lie separately and flat so that no crushing, bending, or folding occurs. Floating tissues (lung, fatty tissues) may be properly fixed by using wads of paper towels to press the floating tissues down into the fixture solution. Once tissues are fixed, the tissues can be removed from large volumes of fixative and shipped in smaller, lighter containers. Shipment of fixed tissues using two plastic bags and a wad of gauze soaked in the fixative is effective, economical, and safe; large glass containers are expensive and fragile. Narrow-necked bottles make it difficult to remove fixed tissues from the bottle without damaging them.

The consideration of type of fixative is important. General purpose needs are best handled by using 10 per cent *buffered* neutral formalin. Unbuffered formalin should not be used because it causes acid formalin hematin precipitate in stained sections, which confuses histologic evaluation. Zenker's acidic fixative is excellent for bone marrow and lymph node biopsies. Zenker's solution is also excellent for preservation of cytoplasmic inclusions such as Negri bodies, noted in rabies. Zenker's solution is a dichromate fixative that must be used in submission of tumors containing chromaffin, such as pheochromocytomas, which are occasionally seen in older cattle. Bouin's solution has been recommended for use in infectious bovine rhino-

tracheitis cases, since it preserves IBR viral inclusions in tissues much better than do formalin fixatives. Use of either Zenker's solution or Bouin's solution requires that the tissues be removed from the fixative after 4 to 24 hours, depending upon tissue size, so that the tissue does not overfix, become brittle, and section improperly. Tissues fixed in Zenker's solution should be rinsed in water for the same length of time as they were fixed and then placed in 80 per cent ethanol for storage and shipping. Tissues fixed in Bouin's solution should be rinsed in several changes of 50 per cent ethanol and shipped and stored in 70 per cent ethanol.

Tissues in Poor Condition before Fixation. Unfortunately, many tissues are submitted for histologic evaluation in such poor condition that no good diagnosis is possible. The more common causes in order of frequency are autolysis, handling artifacts, freezing, and water damage.

Autolysis is unavoidable, but it can be minimized (see section on Gross Pathology). After collection, fix tissues as soon as possible; refrigerate them if necessary, but do not freeze tissues that are to be used for histologic evaluation. Freezing distorts tissues and greatly decreases the chances of finding subtle lesions.

A dairy practitioner should keep some good instruments in his office or vehicle for necropsy use. Old, dull, poorly working instruments often are used for necropsies; do not use them. Tissues collected for histologic submission with such faulty instruments are excessively compressed, torn, and distorted. This alters the architecture and staining of the tissue, decreasing the possibility of making a good evaluation. Handle tissue for histologic evaluation as you would for a surgical procedure.

Washing the tissue is useful to remove extraneous materials such as ingesta, bone chips, and dirt that impair sectioning of tissue blocks in preparation of histologic slides. The wash solution should be either physiologic saline or some of the fixative being used. Plain water causes osmotic artifacts if the water has a chance to make long contact with the tissue.

Little or No Clinical History Provided. When infectious, metabolic, immunologic, or toxicologic problems have occurred, a complete history will help the pathologist understand the reason for the tissue responses and the pathogenesis of the lesions noted. There are a limited number of ways in which bovine tissue reacts to injury. The tissues submitted represent a small portion of the total animal and only one point in time. History helps fill in much of the missing information such as the patient data, incidence, duration and distribution of the condition or lesions in the herd, and what treatment was given. These facts allow the pathologist to focus on examining certain organs or parts of organs for specific lesions in much the same way a clinician uses a history in examining a patient.

Use a standardized submission form. Many diagnostic laboratories provide such forms. If not, you can easily construct one of your own. Such a form should indicate the name, address, and telephone

number(s) of the practice, name of the referring veterinarian, age, sex, breed, and identification of the animal, name and address of the owner of the herd, tissue submitted, location of lesions, clinical history, treatment, necropsy and/or clinical records, date and time of animal death, date and time of necropsy, and date of submission. Much of such a form can be filled out by lay personnel to save the practitioner time. Such forms remind the practitioner to provide information he might otherwise forget.

Improper Packaging. Most dairy practitioners submit tissues for histologic evaluation in medicine bottles of various sizes. As already mentioned, these containers are fragile, heavy to mail, and difficult to use if narrow-mouthed. If containers break, the tissues can dry out, and specimens may get mixed up or lost. In addition, the United States Postal Service has regulations on tissue shipments (request Publication 2, Packaging for Mailing). Double waterproof containers surrounded by absorbent material sufficient to take up all liquid is required. Double sealed plastic bags are light, inexpensive, and strong if enclosed in shock-absorbing absorbent material. Slides of blood, impression or body fluid smears should be placed in small microscope boxes, not in envelopes or folders. Automatic canceling machines used by the Post Office consistently crush glass slides sent by envelope into small unusable pieces.

Improper Labeling. Labels should be applied to the specimen container. Labels that are not obscured by fixative can also be placed within the container to assure no mix-up in the laboratory. Usually stiff paper and pencil are not affected by fixatives. Some inks run in the presence of fixative; check this before labeling. Submission sheets are best sent inside a separate waterproof envelope or plastic bag to avoid any possible damage to the submission records.

Table 2. *Tissue Samples to Submit for Histologic Evaluation of Some Common Dairy Cattle Diseases*

SPECIFIC DISEASES	TISSUES
Aujesky's Disease (Pseudorabies)	Cerebrum, lung, spleen
Blackleg, Malignant Edema	Skeletal muscle, lung, tongue
Bovine Virus Diarrhea	Affected GI organs, spleen, tonsil, lymph nodes, kidney
Campylobacteriosis (Vibriosis)	Fetus, fetal stomach contents (must be fresh when fixed)
Coccidiosis	Intestine, colon
Infectious Bovine Rhinotracheitis	Lung, turbinate, spleen, adrenal glands, brain, affected GI organs, fetus
Johne's Disease	Terminal ileum and large intestine with attached lymph nodes, uterus
Leptospirosis	Kidney (must be fresh when fixed)
Listeriosis	Brain, fetal liver, liver, spleen, mesenteric lymph nodes

Submission of Inappropriate Tissue. Sometimes important tissues are omitted and a histologic diagnosis is not possible. This may occur because of unavailability of the tissues or a lack of time or knowledge in collecting the important tissues. Before the start of a necropsy, write down which tissues to collect, adding other tissues as the necropsy is performed. This will prevent important tissues being forgotten. If neither the history nor the necropsy reveals a cause of death, submit at least the following tissue specimens: brain, kidney, liver, spleen, heart, lungs, abomasum, small intestine, colon, and lymph nodes. Usually one of these tissues will have histologic lesions.

Table 2 includes some of the more common problems and conditions a dairy practitioner might see and lists appropriate tissues to submit for histologic evaluation.

CLINICAL PATHOLOGY

The dairy practitioner may use his own or local laboratory facilities for routine clinical pathology work or may elect to use a diagnostic laboratory that provides clinical pathologic services.

The materials most commonly submitted to a clinical pathology laboratory are blood or serum. Unfortunately, they often are submitted poorly. The following points need to be considered.

Blood for Complete Blood Counts

1. Be gentle with cattle during blood collection. Severely excited cattle often have elevated white blood cell counts (15,000 to 25,000 per cu mm and a transient neutrophilia).
2. Blood should be obtained by clean puncture of the jugular vein, if possible. In order to avoid damage to red blood cells and hemolysis, do not pass blood through smaller than a 20 gauge needle.
3. Ethylene diaminotetracetate (EDTA) is the anticoagulant of choice for CBCs. Heparin is not acceptable because it interferes with some leukocyte stains. Slowly agitate the tube containing EDTA as the blood is obtained from the jugular vein. If *any* clots appear, the results of a CBC or platelet count will not be reliable. Obtain blood in siliconized sterile glass tubes.
4. Make fresh blood smears (three or more per sample) within 15 minutes after blood collection if possible, but definitely before three hours of storage. Air dry and fix in absolute methanol for five minutes if the slides are to be held more than a few days before staining or shipping.
5. Refrigerate the blood sample immediately. Platelet counts and fibrinogen levels can also be determined on blood submitted as described for the CBCs.
6. Interpretation of single bovine blood submissions is difficult because of this species' normally wide variability in white blood cell counts. Use two or more CBCs to make prognostications on an individual's progress.

Blood for Examination for Parasites

1. Uncoagulated EDTA blood samples can be used, and three or more blood smears should also be made.

2. Peripheral blood (such as from a nicked ear capillary) is recommended as a source because blood parasites and damaged erythrocytes tend to collect in capillary beds.

Serum for Blood Chemistries or Serology

1. Obtain 10 to 30 ml of blood from the jugular vein as previously described or from the median coccygeal (tail) vein *aseptically* and *gently* to avoid hemolysis.

2. Store the blood sample in a sterile container. Remember that bacteria can grow in contaminated blood or serum and very rapidly change the serum chemical values.

3. Allow the blood to clot for 30 minutes or more at room temperature (about 23°C) in a glass container, then centrifuge if necessary to settle out the red blood cells. Owing to the thermolability of some enzymes (such as LDH, SGOT, and SGPT), serum for enzyme determinations should be collected by centrifugation rather than by standing at room temperature for longer than 30 minutes. Such serum also needs excellent refrigeration during storage and shipment. Pour off the serum into a sterile container. *Do not* ship clotted blood with the clot still in the container. Such samples are not satisfactory because of the white blood cell metabolism and hemolysis that occurs during storage and shipment, resulting in inaccurate values for glucose, potassium, sodium, magnesium, and phosphate tests.

4. Label tubes, not just the stoppers or caps.

5. When interpreting blood chemistries, remember that comparison of values from one animal over time will result in a more accurate prognostication than single values from many animals because of individual variations (diet, age, etc.).

6. For nonregulatory serology from a dairy herd, submission of 10 to 12 random samples is sufficient to diagnose herd infection. Values of titers should not be compared between laboratories, as each laboratory uses slightly different procedures. Paired samples obtained during acute and convalescent phases of suspected infection from 10 to 12 animals are most useful. Interpretation of single samples should be made with advice from the laboratory personnel.

Cytologic Preparations

Submit and ship fluids (abdominal, thoracic, synovial, etc.) in refrigerated sterile EDTA stoppered tubes. Slides should also be made. Smear such fluids on a dry, clean slide as for a blood smear. Solid tissues that are sectioned for impression smears should be blotted first if there is considerable blood on the cut surface to remove extraneous blood cells. Following blotting, make firm contact with dry clean slides for impression smears. This procedure will increase the number of affected tissue cells that will stick to the slide and aid

diagnosis. The slides can be air-dried, fixed with absolute methanol, and submitted for cytologic evaluation.

Urine

Refrigerate bovine urine following collection and during shipment. If more than six hours elapse between collection and evaluation, add one drop of 37 to 40 per cent formalin per 30 ml of urine to preserve casts, red and white blood cells, renal epithelial cells, and bacteria that may be present.

PARASITOLOGY

There is considerable interest by dairymen and dairy practitioners in checking and monitoring external and internal parasite levels in dairy cattle, often as part of a herd health program. This section reviews the more common procedures used for parasitologic evaluation, and covers procedural problems and interpretation of results that are important to dairy practitioners.

INTERNAL PARASITES

Enteric Parasites

Dairymen often encounter problems in controlling gastroenteric parasites; however, nematodes and trematodes in tissues other than the gastrointestinal tract may also be present in dairy herds. A dairy practitioner can check the collective effectiveness of pasture management, housing sanitation, and deworming programs by using a combination of direct fecal examination, flotation and sedimentation methods, copraculture, and necropsy examination.

Direct Fecal Examination. Mixing a small amount of feces with saline and examining this mixture microscopically is a simple and effective screening procedure when high numbers of coccidial oocysts, worm eggs, or lungworm larvae are present. For low or moderate level parasitisms or when quantitation is desired, some of the following procedures are recommended.

Flotation Procedures. These procedures can concentrate and allow detection of most nematode eggs and coccidial oocysts. Solutions of either sugar or salt can be used, although some of the more concentrated solutions will lyse coccidia and some nematode eggs on standing. Flotation methods are not useful for isolating and identifying trematode (fluke) eggs unless the specific gravity of the sugar or salt solution is over 1.30. If trematodes are suspected, the dairy practitioner should be sure that the flotation methods used will incorporate flotation solutions that are dense enough. Nematode larvae can be detected but are not quantitated by these procedures.

Sedimentation Procedures. In these methods, water is mixed with the fecal material and screen filtered. Worm eggs, coccidial oocysts,

and larvae settle to the bottom and can be identified in the sediment of such preparations.

Copraculture. In dairy practice, there are circumstances in which copraculture or fecal culturing is useful. Copraculture allows the nematode eggs to embryonate and develop to larvae, which are then collected and identified. This procedure will allow identification of Cooperia, Haemonchus, Trichostrongylus, Ostertagia, and Oesophagostomum, which are quite difficult to differentiate by egg examination. The differentiation of these nematode species in cattle can be important during anthelmintic therapy. Cattle infected with Cooperia need twice the dose of thiabendazole for most effective results. For herd therapy, this is expensive unless it is necessary. Doing copraculture is a cost effective way to determine whether or not a species of Cooperia is the predominant source of infection for a given group of cattle; Haemonchus in dairy cattle is rare. If Haemonchus organisms are present, however, it is important to know since they often develop resistance to many anthelmintics.

Necropsy Examination. A gross necropsy as a parasitologic examination is the most accurate way to detect enteric parasites. The contents from the abomasum, small intestine, cecum, and colon can be collected and screened for adult worms, larvae, and eggs, or oocysts. Tissues of the digestive tract can also be sectioned and examined histologically for coccidia; careful gross examination often reveals nematodes or other parasites involved with the mucosa. The liver can also be examined for flukes. An additional advantage is that quantitation of parasite burden can be made directly from the observed number of worms.

Regardless of the specific procedures used, some consideration must be made for fecal collection. A plastic sleeve can be used to collect feces rectally from larger stock. A tongue depressor will usually be effective to collect the feces rectally in young stock. For shipment or storage, refrigerate the feces. Do not freeze feces as freezing will destroy many of the coccidial oocysts and nematode and trematode eggs and larvae. Feces can also be preserved in 10 per cent formalin. Fixation kills eggs and larvae, so it should not be done for copraculture or larval collection procedures but is satisfactory for examination or collection of eggs.

Interpretation of Fecal Egg or Larval Counts

Negative Results. A single negative or low level egg or larval fecal concentration does not establish the lack of significant enteric parasitism. Repeat the procedure at least two more times. If three or more examinations are made from one animal or a group of animals without detection of significant fecal egg or larval concentration, the possibility is low that enteric parasitism is a problem at that point in time. Scheduling fecal examinations should be based on climate, pasture management, previous worming, and prior fecal examination results. For calves grazing for the first time, pasture-derived infections in the spring are usually not manifested by high egg counts until about eight to 12 weeks after pasturing. Lungworm infections may be

detected much sooner. Examine the feces in mid-summer to check pasture management and late in fall or before calves are brought into the barn or feeding area. Treat as necessary. If worming is necessary, recheck feces four to eight weeks later to assess therapy and be aware of the possibility for reinfection. For adult cattle, check heifers one month prior to lactation. Examine sufficient cows one to three weeks following parturition so that the milking herd is constantly monitored. The periparturient period is a time when adult nematodes emerge in greater numbers and possibly increase egg shedding due to periparturient and lactational stress on the cow. If any cows have moderate to high egg counts, worm every cow and heifer just prior to parturition.

Positive Results. While some fecal egg or larval count procedures are quantitative, care must be taken in interpreting such counts. Egg counts of 100 eggs or less per gram of feces are usually considered low; 100 to 500 eggs per gram of feces, moderate; and over 500 eggs per gram of feces, high. However, the number of eggs or larvae in a given fecal sample does not correlate precisely with the actual level of parasitism. Some of the reasons for the lack of this correlation are as follows:

1. Because of dilution, the water content of the feces can greatly affect the egg or larval concentration.
2. Seasonal effects are noted in cattle. When cattle are on new green pasture, there may be a stimulation of the adult female nematodes to produce greater number of eggs.
3. Older cattle have immune mechanisms that inhibit appearance of adult nematodes and possibly their ability to lay eggs, so an egg count in older cattle tends to underestimate actual nematode numbers.
4. Cattle that are stressed by factors such as parturition or transportation may temporarily lose some of their immunity to enteric parasites, resulting in a rapid but transient increase in fecal egg concentrations.
5. Certain nematodes (Ostertagia) of cattle tend not to produce constant high numbers of eggs, whereas others (Haemonchus) normally produce large numbers. Hence, the total egg numbers may be related to the type of nematodes infecting the cattle.
6. There are daily variations in fecal egg concentrations for some enteric parasites. Also, the bovine hepatobiliary trematode, *Fasciola hepatica*, generally causes higher fecal egg concentrations during mid-day.
7. The female worms have cyclic periods of egg release, and the concentration of eggs in fecal material may vary accordingly.
8. In primary infections, few adult female worms are present, so fecal egg counts would be negligible yet the parasite load could be very significant. Immature parasites may occur in sufficient number to cause clinical disease prior to the appearance of eggs, larvae, or oocysts in feces.

Considering the above factors collectively, two general rules are suggested when making interpretations of fecal egg or larval counts: (1) Compare fecal egg and larval counts directly with each other *only* if the same procedure was used, preferably by the same technicians, in animals of the same age, on the same diet, and when fecal samples

were taken at the same time. (2) If any one of these conditions varies, do not interpret the egg or larval counts to be significantly different unless there is at least 10 times the difference between the egg counts.

Pulmonary Parasites

The lungworm of major significance in dairy cattle in the United States is *Dictyocaulus viviparous*. This nematode can be detected by tracheal wash and microscopic examination for larvae, sedimentation of feces (Baermann apparatus), or necropsy examination of the lungs.

1. Tracheal wash procedures and microscopic examination of the wash fluid are usually done in the practice laboratory. If the tracheal wash is mixed with equal volumes of 10 per cent formalin, the wash can be submitted to a diagnostic laboratory for larval identification. If there is a moderate to heavy lungworm infection, the wash fluid is usually diagnostic.

2. Fresh feces from dairy cattle can be submitted for a special sedimentation procedure. This procedure, using a Baermann apparatus, allows larvae to gravitate out of the feces through a gauze and mesh filter. The larvae settle to the bottom of the apparatus and are collected there. Other sedimentation procedures will collect larvae, but the Baermann apparatus concentrates the lungworm larvae in a small volume of fluid. All larvae found *in fresh* feces are presumed to be larvae of the lungworm Dictyocaulus. If feces used for sedimentation procedures has lain on the ground for even a short period, it will be contaminated by ground nematode larvae and larvae from enteric nematode eggs that have hatched. These larvae will confuse the diagnosis since all larvae from fresh feces would be counted as lungworm larvae, whether or not they actually were.

3. Necropsy examination of lungs is an effective method of diagnosing lungworms. The middle airways of the dorsocaudal lung usually contain numerous Dictyocaulus larvae if the animal has a moderate infection. Sectioning into the subpleural alveolar spaces in those areas and compressing the small airways will cause lungworm larvae to appear from the cut airways.

EXTERNAL PARASITES

When there is a problem with external parasites, young stock generally are more severely affected. Look carefully in the warm, thin-skinned areas for lice. Use a good light source, especially on dark pigmented cattle. External parasites can be fixed in 10 per cent formalin or 70 per cent ethanol and submitted to a diagnostic laboratory for identification.

Skin areas infested with mites need to be scraped with a sharp blade near the periphery of the most recent lesions until some blood is

noted. Take scrapings from at least three locations. Glycerine or mineral oil works well as a viscous vehicle to hold the material on the knife blade. A disposable scalpel blade is handy to collect such materials. The scalpel blade can be placed in a capped glass vial or stoppered test tube for submission. Skin sections can also be submitted in 10 per cent neutral buffered formalin for histologic evaluation.

TOXICOLOGY

Submission of toxicologic samples may require special consideration. It is important for the veterinarian to be sure that he has direct knowledge of how, what, and from where samples sent to a toxicology laboratory were obtained. Legal questions may occur that require the veterinarian to be thorough in history taking, sampling, packaging, labeling, preservation, and submission procedures. When serious financial losses have occurred, it is prudent to collect at least double sets of materials; send one set to the toxicology laboratory and freeze other sets in a locked freezer for future reference. With some toxicants (such as organophosphorus insecticides) it it also wise to obtain equivalent samples of urine, blood, ruminal contents, and ingesta, for example, from herd mates in order to be able to show a difference between toxicant levels seen in normal animals due to background contamination and toxicant levels in sick or dying cattle. Telephoning ahead to a toxicology laboratory for information and to inform the personnel there of what is to be sent is highly recommended.

When cattle die suddenly without any observed illness, there is a tendency to blame "poisoning" as the cause. Before suspecting a toxicosis, consider the history and complete at least one necropsy. Single deaths are usually due to peracute infections, physical factors, or organic causes rather than toxicoses. So it is sensible that animals dying rapidly be necropsied carefully and soon after death to eliminate more likely causes. If no gross lesions are present, tissues should be taken for histopathologic, microbiologic, and toxicologic evaluation.

History-Taking

Taking and submitting an accurate and detailed history including all clinical signs, necropsy findings, times of death, lengths of illnesses, percentage morbidity and mortality, treatments, environmental factors, water and feed sources, and consideration of exposure to toxicants is critical to the establishment of a toxicologic diagnosis. If it is unclear as to what toxicant(s) may be involved, call the toxicology laboratory personnel to obtain suggestions for toxicants that might fit the history given. Do not send a request to "check for all poisons." Such a request is unrealistic. The cost of time, materials, and equip-

ment for such a "shotgun" approach is not acceptable to the laboratory.

Animal Toxicity Studies

Some toxicology laboratories can do laboratory animal toxicity screening if appropriate arrangements are made in advance. Feeding suspect materials to an appropriate laboratory animal sometimes can detect nonspecific toxicity. Once a material is identified as biologically toxic, it can be used to check for a specific toxicant. The practitioner should contact the laboratory personnel to find out if such screening services exist, their cost, when they could be used, and how long such tests take to complete.

Sampling

When the toxicant causing illness or death in a dairy herd is unknown, it is difficult to establish a diagnosis. Laboratory animal toxicity screening procedures and/or analyses may need to be done on a specific sample submitted; hence, there is a potential need for large sample quantities. Table 3 provides a comprehensive sample group in ample quantities to submit when the toxicant is unknown.

The chances of making a specific etiologic diagnosis in a toxicologic problem are vastly increased if the type of toxicant can be identified in the field. Table 4 summarizes some specific toxicants that have been found in dairy cattle and the types and amounts of samples to take for submission.

Containers

Before beginning collection of materials, acquire *clean, inert* containers. Plastic Zip-lock bags are fine except for certain organic toxicants. In such cases, plastic containers can contaminate the samples and confuse the analysis. Aluminum foil is a good emergency material to use to wrap solid materials that can later be placed in glass or plastic containers.

Table 3. *Quantities of Samples to Submit for Toxicologic Study*

Feed	1–4 kg
Forage, silage	2–4 kg
Water	1 L
Reticulum contents	500 gm
Rumen contents	500 gm
Abomasum or small intestine contents	500 gm
Tissue samples: liver, kidney, brain, body fat, lung, heart, bone, spleen	200 gm each (also fixed thin sections of each in 10% neutral buffered formalin)
Urine, milk (all 4 quarters)	250 ml each
Blood, heparinized blood	50 ml
EDTA blood	50 ml
Serum	20 ml

Table 4. *Sampling for Toxicologic Evaluation*

TOXICANTS	MATERIAL OR TISSUE TO COLLECT FROM LIVE ANIMALS	MATERIAL OR TISSUE TO COLLECT FROM DEAD ANIMALS	SPECIAL NOTES—PRESERVATION
Aflatoxin	200 gm feed	100 gm liver	Place feed, liver in sealed bags, freeze
Arsenic	100 gm feed	100 gm liver 100 gm kidney	
Carbamates	100 gm feed, 100 ml urine, 20 ml heparinized blood	100 gm liver	Provide normal (control) blood, urine, ruminal fluid from herd mates, refrigerate blood, freeze tissues
Chlorinated Hydrocarbons	100 gm feed, 30 ml EDTA blood, 100 ml milk from each quarter	100 gm liver 200 gm fat 50 gm spleen	Wrap in aluminum foil, place in glass (*not* plastic), freeze, refrigerate blood
Chlorophenols, Herbicides and Fungicides, Triazines	30 ml heparinized blood	100 gm liver, body fat, kidney	Same as for chlorinated hydrocarbons
Cyanide	30 ml EDTA blood, 15 ml serum, 100 ml water, 200 ml ruminal fluid, 50 ml urine	200 gm abomasal contents 100 gm liver	Freeze, in airtight container, refrigerate blood
Diethylstilbestrol	200 gm feed, 20 ml heparinized blood	200 gm ruminal contents	Freeze, refrigerate blood
Ethylene Glycol	30 ml heparinized blood, 20 ml serum	kidney	Kidney in formalin, refrigerate blood and serum
Fluorides	100 gm feed, 100 ml water, 100 gm forage	20 gm bone	Freeze bone
Herbicides (Diquat, Paraquat, 2-4-D, etc.)	30 ml heparinized blood, 100 gm weeds or forage, 100 ml urine	200 gm ingesta	Freeze ingesta, urine, weeds; refrigerate blood
Iron	20 ml heparinized blood	50 gm spleen 100 gm liver 100 gm kidney	Freeze tissues, refrigerate blood
Lead (heavy metals)	20 ml heparinized blood, 20 gm paint, motor oil, ceramic dishes, feed, etc., 50 ml urine	100 gm liver, kidney, bone	Refrigerate blood, freeze tissues, place duplicate tissues in 10% buffered neutral formalin
Mycotoxin	200 gm feed, 200 ml urine	200 gm liver and kidney	Freeze tissues; seal feed in airtight containers
Nitrate/Nitrite	100 ml water, 100 gm forage-silage, 100 gm plants	200 gm ruminal contents	Freeze all materials as soon as possible

Table 4. *Sampling for Toxicologic Evaluation (Continued)*

TOXICANTS	MATERIAL OR TISSUE TO COLLECT FROM LIVE ANIMALS	MATERIAL OR TISSUE TO COLLECT FROM DEAD ANIMALS	SPECIAL NOTES—PRESERVATION
Nitrogen Oxides	200 ml heparinized blood, 250 ml air		Collect air with bicycle pump, pumping air into a balloon
Ochratoxin	100 ml urine, 100 gm feed	100 gm liver 100 gm kidney	Freeze Freeze
Organophosphorous Insecticide	20 ml heparinized blood, 100 ml urine	100 gm abomasal contents, 100 gm ruminal contents	Normal (control) blood or urine, ruminal fluid from herd mates are useful for comparison
Oxalates	8 *whole* fresh plants	kidney	*Do not* cut up plants; put section of one kidney in 10% buffered formalin
Poisonous Plants	100 gm whole plants presumed ingested	200 gm ruminal contents, 100 gm liver and kidney	Freeze; formalin fix tissues
Polychlorinated Biphenyls (PBP)	200 gm feed, 20 ml water, 200 gm silo scrapings	100 gm body fat, 250 ml milk, 100 gm liver	Wrap materials in aluminum foil, place in glass; *do not* use plastic containers
Selenium	100 gm feed, 20 ml heparinized blood, 10 ml urine	100 gm ruminal contents	Refrigerate blood, freeze other materials
Urea	30 ml EDTA or heparinized blood, 20 ml serum, 50 ml urine, 200 ml ruminal fluid	200 gm ruminal contents	Freeze materials as rapidly as possible; materials must be taken from animals under 1 hour dead, otherwise urea is metabolized by rumen microbes.

Labeling

Proper labeling can determine the outcome of many legal cases. Do not rely on tags or labeling caps or covers that can be mixed up. Place inert labels (paper with pencil writing) within samples or label the containers *directly*. Keep a written list of the type and amount of material collected and the time and place of collection. Collection of duplicate samples may be helpful for later reference.

Preservation

Freezing is the safest way to store and ship toxicologic samples. Use of dry ice or well packed, insulated chests with ice, or ice substitutes is necessary to assure materials arrive frozen. Some samples can be sent in formalin or methyl alcohol. Blood samples are best sent refrigerated to prevent hemolysis. For specific preservation

requirements, refer to laboratory manuals, Table 4, or talk with the laboratory personnel.

CONCLUSION

The dairy practitioner plays the most important role in the success or failure of diagnostic medicine. It is hoped that this article provides the dairy practitioner useful information for both submission of material and interpretation of procedures performed by diagnostic laboratories. *A Handbook for Veterinarians,* prepared by the Department of Veterinary Science Animal Disease Research and Diagnostic Laboratory of South Dakota State University, appropriately summarizes the relationship between the practitioner and the diagnostic laboratory.

"Laboratory examinations can provide valuable assistance to the practitioner and his clients. It is an aid and tool for the veterinarian. It should be remembered, however, that it does not replace a thorough clinical evaluation and a thorough investigation of the problem. The laboratory can provide answers and indications but it is not a substitute for thought, analysis, observation, education, experience, and common sense. Use the laboratory to support clinical studies and then evaluate each situation using all the information available, including laboratory results. The final diagnosis and therapy must be determined and applied in the field."

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