Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Veterinary Clinical Microbiology: Part II

Continued from 12(20)

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Sample Submission

Veterinary practices, unlike most medical practices restricted to human patients, often are at some distance from the diagnostic testing center. Use of transport media is essential under these conditions if the veterinarian is to successfully diagnose infectious diseases. Because the choice and quality of the specimen received by the diagnostic testing center is the major, uncontrolled and limiting factor in the laboratory's performance, the discussion in this section is perhaps the most important for the laboratory performing testing on animal samples.

Several considerations affect the choice of swabs. Dacron, calcium alginate, and rayon are readily available. Swabs that contain cotton wool are not desirable for transport of specimens because of inhibitory substances in the swab fibers. In the special case of anaerobic cultures the swab should be maintained in a tube filled with nitrogen so that oxygen cannot be trapped in the swab's fibers.

Table 2 lists several types of transport media available to the veterinarian for microbiological cultures. The best transport medium for aerobic bacterial cultures is Amie's transport medium (ATM). ATM comes packaged in vial or swab form, is a gel material containing agar, and will not dry out during transit to the diagnostic laboratory. ATM is non-nutritive so that pathogens are not overgrown by resident flora while in transit. ATM supports the growth of both mycoplasma and ureaplasma. ATM is also available with added charcoal (ATMC), which further helps to detoxify metabolic byproducts of bacterial respiration, and thus insures the viability of bacteria submitted for culture. In contrast, another commonly used transport medium base, Stuarts (STM), is a fluid. Specimens in transit for more than 24 to 48 hours can potentially dry out with subsequent loss of fastidious pathogens, such as *Pasteurellae*. Many other types of transport and sampling devices are available including

Accu-CulShure swab aerobic, anaerobic, cytology transport device for nasopharyngeal and cervical/uterine cultures from large and small animals; has a guarded sheath to prevent normal flora contamination while sampling is done; has Cary-Blair as transport medium.

Tiegland swab, aerobic bacteria; guarded swab for equine uterine sampling—does not include transport medium.

Artificial insemination pipette with syringe used to obtain vaginal mucus from cattle for serology and culturing.

Bartlett pipette with a rubber bulb used to collect preputial mucus from bulls for culturing *Tritrichomonas foetus* and *Campylobacter fetus* spp. *venerealis*.

Syringe with needle cap, used for anaerobic cultures if submitted within a few hours to the laboratory.

Yellow top sterile vacutainer tube without additive, sterile tube used to submit centesis samples, urine, CSF, milk to lab.

Diagnostic specimens should always be shipped or mailed by the fastest means possible. Commercial couriers for "next day delivery" should be used if possible. Many laboratories have their own courier networks for multiple daily local and regional pickups. In all cases, specimens must be safely pack-

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TABLE 2. Types of transport media used by veterinary laboratories

Medium	Comments
Amie's transport medium w/ and w/o charcoal	All-purpose aerobic bacterial transport
Stuarts transport medium	Aerobic bacteria
Cary-Blair	Anaerobic bacteria, non-nutritive salmonella, campylobacter, shigella
Thioglycollate	Used by some people for transport, but not recommended for this purpose; can be used as enrichment medium
Campy thiol	*Campylobacter jejuni* transport and enrichment broth
Selenite broth	Salmonellae (not recommended for *S. choleraesuis*); can be used as transport and as selective enrichment
GN broth	Gram-negative fecal pathogens (salmonellae, shigellae)
Diamond's medium	Enrichment transport medium for *Tritrichomonas foetus*
Clark's or Weybridge medium	Enrichment transport medium for *Campylobacter fetus* spp. *venerealis*
Chlamydia medium	Chlamydia transport (sucrose phosphate glutamate buffer with antibiotics)
Viral medium	Viral transport (buffered tissue culture medium with protein stabilizer and antibiotics)
Physiological buffered saline	Transport enrichment for *Yersinia enterocolitica;* used to moisten sterile gauze pads when submitting biopsy samples

aged with absorbent materials surrounding tubes or jars or swabs in leak-proof containers in case of breakage. Many specimens, especially tissues and sera, should be packaged with freezer packs to maintain cold temperatures during shipment.

Choice of Specimen

Veterinary microbiology laboratories receive most specimens from live animals. Specimens include swabs of fluids or tissues, fluids, or biopsied tissues. From dead animals laboratories receive necropsy tissues or swabs of these tissues, plus aborted fetuses. Depending on the clinical situation, selection of an appropriate sample can greatly aid the clinician in determining the cause of a clinical problem.

Respiratory Tract Samples

From the upper respiratory tract (nasal passages, paranasal sinuses, nasopharynx) the veterinarian will use a guarded swab device (for large animals, e.g., Accu-CulShure) to protect the specimen from resident microbial flora. The specimen of choice for diagnosis of URT disease from large animal species is a deep nasopharyngeal swab collected using a guarded swab device. For small animals (cats, dogs) the specimen of choice is a deep nasal culture of areas of hyperemia/inflammation taken with an otoscope speculum and small swab. Occasionally, swabs of nasal exudates reveal serious infections such as cryptococcosis or aspergillosis in cats and dogs, respectively. In general, it is not appropriate to swab the superficial nares from either large or small animals because of contamination with the resident flora. Pharyngeal swabs of dogs and cats should be used only for the diagnosis of tonsilitis or pharyngitis, usually caused by non-group A beta-hemolytic streptococci.

For the diagnosis of lower respiratory tract disease (bronchi, bronchioles, alveoli, pulmonary interstitium, pleura, pleural cavity), the specimen of choice is a properly collected transtracheal aspirate or wash. If the veterinarian collects the tracheal aspirate or wash through an endoscopic tube, the resulting culture will probably be contaminated with oral flora, making laboratory interpretation more difficult. Thoracentesis samples must be obtained from a surgically prepared site; in addition to bacterial (aerobic and anaerobic) and fungal cultures, appropriate cytological examinations should be performed with special stains.

Laboratory Diagnosis of Respiratory Disease

The veterinarian should submit a swab soaked in the fluid from the tracheal aspirate in Amie's transport medium and a yellow top Vacutainer tube of the fluid both for bacterial and fungal cultures. A tube of fluid collected in EDTA should also be sent for cytology examination. Swabs of nasal exudate, swabs from deep nasal cavities taken with an otoscope speculum, and sinus flushes should be submitted in Amie's transport medium with charcoal as well as an EDTA tube for cytology examination.

In establishing a differential diagnosis, the veterinarian uses the history of the animal, radiographs, and any

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clinical pathology examinations performed (cytology, hemogram, etc.) on site or at a diagnostic lab. The veterinarian will then use culture results including bacterial, mycoplasma, fungal cultures, viral FA or isolation, plus serology assays along with interpretation of results by the laboratory diagnostician in arriving at a final diagnosis. Often, follow-up cultures are obtained by the veterinarian to monitor the progress of a case and to assess whether antibiotic resistance has developed.

Anaerobic Bacterial Cultures

Until recently, anaerobic culturing has not been widely used in veterinary medicine. However, many well-characterized diseases have been studied, such as footrot in ruminants, foot abscesses, soft tissue abscesses, myositis cases due to injections, enterotoxemias, and the classic syndromes (blackleg, malignant edema, etc.). Table 3 lists the major veterinary-related anaerobic infections. With the availability of transport media and improved equipment, such as gas pack jars and commercially available rapid identification systems, even small veterinary laboratories can perform reasonable identification procedures for major anaerobic pathogens.

In general, specimens containing anaerobes should be kept at room temperature, except for surgical or necropsy tissues which must be chilled in transit to slow down the autolysis that occurs in tissues after death. If possible, anaerobic specimens should be submitted as fluids in a commercially available evacuated anaerobic vial (if transport of longer than a day is necessary) or in a capped syringe if delivered within hours or at least the same day to the lab. If there is not enough fluid to inject into the evacuated tube, an anaerobic transport medium and swab may be used to collect the specimen. It is always wise to submit a few smears of the specimen for Gram stain and fluorescent antibody testing.

Because of the time and expense of performing anaerobic bacterial cultures, specimens being considered for anaerobic cultures should be chosen wisely (Table 4). Many anaerobic infections are the result of endogenous bacteria gaining access to an otherwise sterile site. Any time a mucous membrane surface is breached, there is a high likelihood of establishing an anaerobic infection. Anaerobic infections are usually mixed microflora and the Gram stain reflects this fact.

TABLE 3. Anaerobic bacterial infections in animals

Disease	Organism
Footrot (sheep)	*Bacteroides nodosus* *Fusobacterium necrophorum*
Foot abscess (sheep)	*F. necrophorum* *Actinomyces pyogenes*
Myositis/wound infections (various species)	*Bacteroides* sp. *Clostridium chauvoei* *Clostridium septicum* *Clostridium perfringens* *Fusobacterium* sp. anaerobic cocci
Enterotoxemias: lamb dysentery (lambs, foals, calves, adult sheep and goats)	*C. perfringens* type B
Struck (adult sheep)	*C. perfringens* type C
Necrotic enteritis (lambs, calves, piglets)	*C. perfringens* type C
Pulpy kidney, enterotoxemia (lamb, adult sheep, cattle, goats)	*C. perfringens* type D
Blackleg (even-toed ungulates, rarely pigs)	*C. chauvoei* *C. septicum* *Clostridium sordellii* (rare)
Malignant edema (cattle, sheep, pigs, horses)	*C. septicum*
Braxy (sheep)	*C. septicum*
Bacillary hemoglobinuria (cattle)	*C. hemolyticum*
Infectious necrotic hepatitis/ Black Disease (sheep, cattle)	*Clostridium novyii* type B
Gas gangrene (cattle)	*C. novyii* type B *C. perfringens*
Big Head (rams)	*C. novyii* type A
Botulism (many species)	*C. botulinum* (types A–E)
Tetanus	*C. tetani*

Blood Cultures

In cases of suspect bacteremia (fever, chills), septicemia, and endocarditis the veterinarian will submit blood for bacterial culture. Veterinary laboratories frequently have a preferred blood culture bottle system that they supply to their clients. Bacterial cultures are done in a set of one aerobic and one anaerobic bottle. Statistically if three sets of blood cultures are drawn over a 24-h period, bacteremia should be detected. The interval between sets must be decided by the veterinarian depending on the condition of the patient and the urgency to institute empirical antimicrobial therapy.

The veterinarian should use a blood culture bottle that has sodium polyanethol sulfonate (SPS) but not EDTA, heparin, or oxylate as the anticoagulant because these chemicals may inhibit or kill bacteria. As stated above, the skin should be prepared before the venipuncture is performed; blood should not be drawn from an indwelling venous catheter. Under field conditions veterinarians often cannot adequately prepare the venipuncture site; therefore, laboratories processing veterinary blood cultures often are faced with interpreting what appear to be contaminants from the skin that have grown (or overgrown) a probable pathogen in the blood bottle.

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TABLE 4. Specimens for anaerobic cultures

Do accept for culture[a]

- Transtracheal aspirates
- Centesis samples from surgically prepared sites and normally sterile body sites
  - urinary bladder (cysto)
  - blood cultures
  - thoracic/pleural cavity
  - peritoneal cavity
  - pericardial cavity
  - CSF
  - joints
- Fistulous tracts
- Abscesses
- Deep wound and aspirates from other soft tissues
- Endometrial swabs
- Surgical specimens obtained from normally sterile body sites

Do not accept for culture

- Saliva or nasopharyngeal swabs (except for tooth root abscess cultures)
- Gingival swabs
- Bronchoscopy samples
- Vaginal or cervical swabs
- Skin or superficial wounds
- Gastric washes
- Urine (free catch or catheter)
- Feces, intestinal tract (except for clostridial and treponemal cultures)

[a] The centesis samples must come from surgically prepared sites; the uterine samples must be taken through a guarded swab device; for fistulous tracts the skin should first be decontaminated and then a syringe used to obtain the specimen; surgical specimens—the deeper the better; wounds should be debrided before swabbing for culture.

Urine

Specimens from suspect urinary tract disease are a common, if not the most common, culture performed by most veterinary microbiology laboratories. Regardless of the species of animal from which the urine is collected, all urine specimens should be refrigerated within 1 h after the specimen is obtained so that accurate colony counts may be obtained. Urine may be presented to the laboratory: (i) in a refrigerated, sterile yellow top vacutainer without additive; (ii) already streaked onto a MacConkey/Trypticase Soy blood agar biplate (using a disposable, calibrated 1 μl loop, tape the plate shut and send to the lab via courier to arrive the same day as the specimen was taken); (iii) in boric acid so that the colony counts are preserved without the need for refrigeration; or, as (iv) a swab of urine in Amie's transport medium with charcoal if colony counts are not requested. The specimen of choice is a properly collected cystocentesis specimen that is cultured quantitatively for bacteria. Any bacteria or fungus isolated from a properly collected centesis specimen should be identified and reported. Free catch or catheterized specimens must be quantitated using a calibrated loop for plating the specimen onto agar. The traditional interpretations of human urinary cultures are not valid for specimens from dogs and cats, because these animals often have urinary tract infections in the absence of high colony counts. Colony counts do serve, however, to give the clinician some idea of the degree of contamination and the significance of bacteria isolated from noncentesis specimens. Because small animals with urinary tract infections, especially cats, often have low colony counts of bacteria in urine (below 10^3 cfu/ml), veterinary laboratories have found it useful to enrich specimens of urine from cats in thioglycollate broth, so that bacteriuria can be detected. This is particularly important if the laboratory uses a calibrated 1 μl loop for plating urines. The threshold for this loop is 10^3 cfu/ml and if only the loop were used without enrichment the culture might show no bacterial growth.

Milk

Milk specimens from dairy cows and milking goats are most commonly submitted. On occasion, horses or dogs with suspect mastitis are cultured and laboratories dealing with clients caring for zoo animals should expect specimens from many other exotic species for cultures. Dairy herds usually are on a mastitis control program carried out through state extension services. Modern mastitis control programs seek to prevent and control mastitis in cattle by monitoring/surveillance cultures and many other activities conducted by a team that assesses all aspects of farm management such as nutrition, milking routines, and maintenance of milking equipment. In any case, milk should be presented to the laboratory in a sterile container such as a yellow top vacutainer tube that has been kept chilled. Most labs culture for the common mastitis pathogens such as *E. coli*, *Staphylococcus aureus*, streptococci, and on occasion other microorganisms like *Prototheca* or *Nocardia*. When cattle are cultured for diseases that are federally regulated, such as brucellosis (*Brucella abortus*), the laboratories must be certified by the federal government to perform this type of culture. Individual teat samples are recommended for culture from animals rather than composite samples so that suspect pathogens are not diluted out by milk from teats that are not infected.

Reproductive Tract

Specimens from the reproductive tract should be taken with a guarded culture device, such as the Accu-CulShure swab or Tiegland swab. In veterinary medicine the species commonly cultured for reproductive problems are cattle, horses, dogs, and cats. Cultures are commonly submitted for aerobic bacteria, mycoplasma, ureaplasma, and sometimes for fungi, and anaerobic bacteria. Veterinary laboratories deal with three areas of reproduction: abortions, infertility problems, and prebreeding examinations. Most laboratories have set protocols for these

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problems which may include an abortion kit that contains a styrofoam mailer box, all the transport materials, and instructions to properly take and ship specimens for histopathology, bacterial and viral cultures, or fluorescent antibody tests, and also for serology from serum (acute and convalescent) of the dam and fetal heart blood.

The best bacterial transport medium for reproductive cultures is Amie's transport medium without charcoal; mycoplasmas and ureaplasmas may easily be recovered from Amie's.

Prebreeding cultures are performed most often on both male and female horses and dogs. In a prebreeding workup, in addition to the physical examination, the veterinarian will submit preputial washes, urethral swabs, and semen from males for cultures and uterine/cervical cultures, fluid for cytology, and perhaps a biopsy for histopathology and theriogenology evaluations from females. It is beyond the scope of this article to discuss all of the interpretations of prebreeding culture results. Readers are urged to contact a veterinary diagnostic laboratory and theriogenologist for additional information on this topic.

Only federally certified veterinary labs may perform cultures for *Taylorella equigenitalis* from horses either for the diagnosis of clinical disease or for screening of animals destined for export or import. *Taylorella* causes contagious equine metritis (CEM), a disease that is uncommon in the United States at the present time (USA outbreaks have occurred linked to imported horses but the outbreaks have been controlled and eliminated).

Fecal/Gastrointestinal Tract

Veterinary laboratories receive specimens from virtually any species of mammal, bird, fish, reptile, and amphibian for the diagnosis of intestinal tract diseases. Each of these species has its own resident intestinal flora to be considered in the interpretation of culture results. The types of specimens suitable for culture include feces, rectal swabs, rectal mucosal biopsies, loops of intestines tied off with string, and cloacal swabs.

Appropriate transport media are: Amie's transport medium with charcoal for *Salmonella* spp., *E. coli*, *Aeromonas* spp., *Plesiomonas*, and *Vibrio;* Campy thiol for *Campylobacter* spp., PBS at 4°C Yersinia and Cary-Blair Clostridium for Clostridium.

Although *Shigella* spp. are rarely encountered in veterinary medicine except for in specimens from nonhuman primates, GN broth may be used as a transport medium.

It is important to include a patient history, including animal contacts, risk factors such as travel, visits to shows or fairs, feed changes, water source, feed source, etc. If clostridial toxin assays are needed, it is important to contact the lab first and then to submit fresh feces or intestinal contents chilled in a leakproof container. Smears of feces for Gram stains are also useful to submit to the diagnostic laboratory to assess quickly the presence of bacterial overgrowth or yeast. One should also consider viral and parasitic etiologies for diarrheal disease. Acute and convalescent sera for serological evaluation may be submitted also. While poultry have a gram negative intestinal flora, most psittacine or seed-eating birds do not and the isolation of any gram-negative organisms from these exotic birds should be reported to the clinician.

Veterinary laboratories use a wide variety of enrichment and direct plated media for the isolation of intestinal pathogens. It is important to note that *Salmonella choleraesuis* from pig specimens does not grow well in selenite broth enrichment. Also, horse feces should be inoculated onto CNA blood agar (colistin, nalidixic acid) for the isolation of *Rhodococcus equi*, a cause of bronchopneumonia and enteric disease in horses.

Fungal Cultures

Depending on the area of the country in which a veterinarian practices, one will see a variety of fungal infections in the veterinary diagnostic laboratory. Veterinary labs see many dermatophyte infections including those caused by *Microsporum* and *Trichophyton*. Systemic fungal infections including those caused by *Cryptococcus* and *Aspergillus* are not uncommon. In addition, horses with guttoral pouch mycosis due to *Aspergillus* and *Candida* overgrowth in animals receiving oral antibiotics are seen, and frequently infections with *Coccidioides*, *Blastomyces*, and *Histoplasma* may occur. Bacteria such as *Dermatophilus congolensis* and *Nocardia* are also commonly isolated.

Other than the use of commercially available dermatophyte test medium to screen for dermatophytes, veterinarians should not be encouraged to perform fungal cultures in their office laboratory because of the danger of growing a virulent fungus and contaminating the hospital environment. Veterinary diagnostic labs have biological safety cabinets, media, and personnel with the experience and expertise to safely isolate and identify the fungal etiologies of infection. However, veterinarians should perform as many direct microscopic studies as possible and send specimens to a reference lab for cultures, cytology, and histopathology. By performing lactophenol cotton blue and KOH mounts of hair and skin scrapings, respectively, and Giemsa and acid-fast stains of exudates and fluids, much presumptive valuable information may be obtained while the reference lab is evaluating the mailed specimens. Samples should be mailed in pill envelopes or petri dishes, but not in sealed Vacutainer tubes, since a sealed tube may trap moisture and allow saprophytic fungi and bacteria to overgrow the specimen. Two biopsy specimens should be sent, one for culture in a sterile tube covered with sterile physiological saline, and one in 10% buffered formalin for histopathology. Also, in the case of suspect systemic fungal infections paired sera may be sent for fungal serology assays.

New Trends in Veterinary Medicine and the Effects on Veterinary Diagnostic Laboratories

Many changes are occurring in the client population base of veterinary diagnostic laboratories. In the not too distant past most labs dealt with food and fiber animals. Today, in addition to the traditional species, we are receiving many more samples from pleasure horses and small horse breeding operations. There has also

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been an increase in pet goats and dairy goat operations. New world camelids, e.g., llamas and alpacas, and many exotic species of mammals and birds are becoming common. Each of these species has its own set of diagnostic problems, "normal flora" and bacterial pathogens to consider. Therefore, we are constantly updating our information, our recommendations, and our interpretations of culture results on a case by case basis. The veterinary literature, texts, and specialty meetings all provide a forum for sharing common problems and information.

As is the case in human medical laboratories, veterinary medicine is experiencing a growth in the types of diagnostic kits available for rapid diagnostic testing. Kits are now available for use in veterinary offices and in the field. The veterinarian must understand the limitations of the kits and the information that they provide and see that appropriate controls are always used. In some cases veterinary labs are at the mercy of the "kit" and cannot offer a test unless a kit is available for that particular disease agent. The evaluation and certification of kits is a matter of controversy that will probably continue as long as most labs are limited in the amount of time and dollars available in their budgets to conduct research and develop reagents for disease diagnosis.

Identification of Veterinary Pathogens

Veterinary diagnostic laboratories are faced with identifying bacteria and fungi from a great variety of species of mammals, birds, reptiles, amphibians, and environmental species. The commercially available kits and identification systems often will not identify veterinary bacterial isolates accurately because their data base is composed primarily of information about bacteria from humans, not animals. Thus, veterinary labs use a combination of conventional biochemical media and tests, commercial strips, and commercial automated systems to accomplish the task of bacterial identification.

For dogs and cats, many of the pathogens isolated will be familiar to the laboratory performing bacteriologic evaluation of human specimens; however, some isolates such as *Staphyloccus intermedius* might be misidentified as *Staphylococcus aureus* or dismissed simply as "Staph sp." unless the laboratory is alert to the differential characteristics of this common canine pathogen. Similarly, organisms such as *Pasteurella multocida* may be difficult to identify. Fortunately, the databases for most of the automated systems include many of the common veterinary pathogens, a fact that allows laboratories with these capabilities to perform identification of isolates from veterinary samples routinely.

An example of the problems facing a veterinary diagnostic laboratory working with samples from horses follows in the form of a partial list of common pathogens that are considered in the differential diagnosis of equine bacterial (and viral) disease problems.

Foal septicemia and "joint ill" problems
- *E. coli*
- *Actinobacillus equuli*
- *Klebsiella pneumoniae*
- *Streptococcus* spp. (Group C)
- *Rhodococcus equi*
- *Salmonella*

Foal pneumonias
- All of the above including *Bordetella bronchiseptica*, *Staphylococcus* spp. (coagulase positive), and *Pseudomonas* spp.

Adult respiratory problems including pneumonias
- Equine adenovirus 1
- African horse sickness virus
- Equine vial arteritis
- Equine herpesvirus 1 (EHV-1, equine rhinopneumonitis)
- Equine influenza virus 1 and 2
- Equine rhinovirus-1 (ERV-1)
- *Streptococcus equi*, *S. zooepidemicus*, *S. equisimilis* (all group C streptococci)
- *Pasteurella* spp.
- *E. coli*

Equine pre-breeding pathogens
- *E. coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- Streptococci group C
- *Candida albicans*

Equine abortions
- Equine herpes virus 1 (EHV 1)
- Equine arteritis virus (EVA)
- streptococci group C
- *Salmonella*
- *E. coli*, *Klebsiella* spp.
- *Actinobacillus equuli*
- *Rhodococcus* equi
- *Staphylococcus* spp. (coagulase +)
- *Leptospira* spp.
- *Aspergillus* spp.

Adult acute diarrhea
- *Salmonellae*
- Colitis X (hemorrhagic edematous colon)-Clostridia?
- *Rhodococcus equi*
- *Ehrlichia equi* (Potomac Horse Fever)

Adult chronic diarrhea
- Mycobacterium avium and *M. paratuberculosis* (granulomatous enteritis)
- *Rhodococcus equi*
- *Salmonella*
- Strongyle larval migrans

Foal diarrhea
- *E. coli*
- Rotavirus
- *Salmonella*
- *Clostridium perfringens* (types B, C, D)
- *Clostridium difficile*
- *Rhodococcus equi*
- Coronavirus
- Adenovirus types 1 and 2
- parvovirus-like agent
- *Strongyloides westerii*
- *Strongylus* spp.
- *Parascaris equorum*

Actinobacillus and *Rhodococcus* spp. may present the most problems for laboratories not routinely dealing with veterinary clients. Actinobacilli are gram-negative coccobacilli and rods, oxidase positive, catalase positive, nonmotile, nitrates are reduced to nitrites, indole negative, and most are urease positive and grow on MacConkey agar. Most of the colonies of actinobacilli become sticky on blood agar and are hard to remove. *Rhodococcus equi* are gram-positive coccoid (sometimes rods) that grow well on blood agar and are easily isolated on Columbia CNA (colistin nalidixic acid) agar as very mucoid, slightly pink colonies that seem to flow over the agar. *Rhodo-*

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coccus is catalase positive and urease positive. In addition, all group C streptococci from horses must be identified to species to rule out the pathogen *S. equi*.

Similar differences exist in the differential diagnosis of diseases in other animals. The list of pertinent references will direct the reader to texts that have identification schemes appropriate for identification of veterinary isolates. The reader is also encouraged to contact any of the larger veterinary diagnostic reference centers for assistance in identifying bacteria from particular diagnostic cases.

Selected References

The American Association of Veterinary Laboratory Diagnosticians (AAVLD), PO Box 6023, Columbia, MO 65205 (telephone: 314 882-6811) has many publications dealing with the diagnosis of abortions, mycoplasma and Salmonella/Arizonae infections. In addition, this group publishes a newsletter and a list of AAVLD accredited veterinary laboratories.

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Case Report

Pyridoxal-dependent *Gardnerella vaginalis* Isolated from a Vaginal Fluid

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Nutritionally variant streptococci are viridans streptococci that grow only on media supplemented with various forms of vitamin B_6. These bacteria are sometimes called nutritionally deficient, satelliting and symbiotic streptococci, or vitamin B_6-, thiol-, and pyridoxal-dependent streptococci. Thiol compounds (i.e., L-cysteine) and some forms of vitamin B_6 (i.e., pyridoxal hydrochloride or pyridoxamine dihydrochloride but not pyridoxine) will support the growth of these nutritionally variant bacteria. Pyridoxal-dependent streptococci have been isolated from blood, usually in patients with subacute bacterial endocarditis, from vaginal or mouth secretions, and from wounds of various sites (1, 2, 3, 4). On occasion, other pyridoxal-dependent bacteria have been reported, e.g., group A β-hemolytic streptococci (5). After repeated subculture, these bacteria become adapted to conventional media but are still stimulated by pyridoxal or pyridoxamine.

A strain of *Gardnerella vaginalis* isolated from a vaginal specimen grew on primary culture only satelliting around a *Candida albicans* strain isolated on the same plate of vaginalis agar. The gram-variable pleomorphic rod grew on subculture as a β-hemolytic, pinpoint colony on vaginalis agar after 24 to 48 h of incubation in 4% CO_2 only around *C. albicans* colonies. The clinical history of the patient from whom this strain was isolated is not known.

This strain was oxidase- and catalase-negative and produced acid from glucose and starch but not from mannitol by the rapid test of Greenwood et al. (6, 7). This isolate was identified as *G. vaginalis*, which was confirmed by the Laboratoire de Santé publique du Québec, Sainte-Anne de Bellevue.

The growth of this isolate was tested on supplemented and unsupplemented media. The unsupplemented media consisted of trypticase soy agar with 5% sheep blood and vaginalis agar [Columbia agar base and proteose peptone No. 3 (Difco Laboratories, Detroit, Mich.)] with 5% human blood. Supplemented blood and vaginalis agars were studied with .001% pyridoxal hydrochloride and .001% pyridoxamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.). A 0.001 ml aliquot of a McFarland 0.5 suspension in brain heart infusion broth diluted 1/10 was used as the inoculum. All media were incubated for 24 to 48 h at 35°C in 4% CO_2. The amount of growth semi-quantitated as light (+), moderate (++) or heavy (+++) if the growth occurred on less than half, half, or on more than half of the plate, respectively.

As shown in Table 1, our strain became adapted in vaginalis agar after many subcultures, growing lightly and moderately on this medium after 24 and 48 h, respectively. This isolate grew heavily after 24 h on pyrixodal and pyridoxamine vaginalis agar but it grew lightly on supplemented blood agar after 24 and 48 h.

This strain grew lightly to moderately well satelliting around streaks of

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