Chapter 8 - Lesson 2

Bacteriologic Tests

Introduction

Many veterinary clinics conduct simple bacteriologic tests for diagnostic purposes. For more complex tests, veterinarians submit suitable samples to commercial or state diagnostic laboratories. The veterinary assistant should understand the general principles of conducting the simple tests and of collecting, preparing, and submitting samples to a diagnostic laboratory.

Bacteriologic Tests

There are four general methods of bacteriologic diagnosis procedures:

- Direct microscopic examination
- Culture
- Serologic tests
- Animal inoculation

When diagnosing a disease, one or more of these procedures may be necessary.

Direct Microscopic Examination

Two methods of direct microscopic examination are the inspection of a fresh preparation or unstained slide and the fixing and staining of a slide smear. The veterinary assistant should learn and practice these techniques under the supervision of the veterinarian.

Factors used to identify bacteria using direct microscopic inspection include morphology (form or shape) and the organism’s reaction to stains and dyes. The morphologic classification of bacteria includes:

- Cocci - spherical shape
- Bacilli - cylindrical rod shape
- Spirilla - spiral or curved shape

Gram’s stain is universally used in staining slide smears to allow the easy identification of microorganisms. The reaction of microorganisms to Gram’s stain divides the microorganisms into two groups: Gram-positive and Gram-negative. Gram-positive microorganisms display a blue color when seen under a microscope after staining. Gram-negative microorganisms display a red color.

The staining process includes covering the slide smear with a dye, such as gentian violet. Application of iodine fixes the blue color in the Gram-positive organisms. Flooding the smear with alcohol removes the blue dye from the unstained Gram-negative organisms. Application of a red dye stains the Gram-negative organisms. The Gram-positive organisms will remain blue.
Culture

Culture procedures may be useful if microscopic examinations do not yield a definite identification of the infective organism. To prepare and develop a culture requires an autoclave, incubator, proper growth media, and other culturing equipment. Many veterinarians do not have the necessary equipment to use this analysis method. Therefore, veterinarians send specimens to a commercial or state diagnostic laboratory for analysis.

Serologic Tests

In certain situations, microscopic and culture methods may be inadequate testing methods. Serologic tests may be necessary as another method of diagnosis. Serologic tests determine serum antibody levels. A common example would be the agglutination test. The agglutination test examines the reaction of blood antibodies with a certain antigen. The presence of these antibodies represents present or past infection. The eradication programs for brucellosis commonly use the agglutination test for identification of Brucella-infected animals.

State or federal regulations may govern control or eradication program procedures in diagnosis and/or testing. The veterinarian must be familiar with and follow these government regulations.

Animal Inoculation

The fourth method, animal inoculation, is usually not practical in the office or clinic, but is available at commercial and state diagnostic laboratories. Use animal inoculation when the suspected organism is difficult or impossible to identify and culture, or when specimen contamination is suspected. Rabbits and guinea pigs are commonly used for inoculation tests, but in certain cases other animals may be used.

The inoculation procedure is especially helpful in distinguishing between certain diseases, and in confirming the diagnosis of some virus (viral) diseases.

Reference


Activities

1. Study a microscope used for direct microscopic examination. Draw the microscope and label its parts.
2. Draw the three general forms of bacteria and color the cocci and spirilla Gram-negative and the bacilli Gram-positive.
3. Tour a commercial or state diagnostic laboratory and observe the performance of bacteriologic tests.
4. Observe positive results of the following bacteriologic tests at a diagnostic laboratory or veterinary clinic, or view a picture of positive results in a clinical microbiology book. Make a drawing of your observations.
   a. Fresh bacteria smear slide
   b. Stained bacteria on a smear slide
   c. Bacterial growth on culture plate
   d. Serum agglutination in tube or on plate or card