Bacteriology

in

Veterinary Practice

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IN THE PAST undergraduates in veterinary medicine were not able to envisage any practical use for their laboratory training in bacteriology. When a practitioner required a cultural examination it was necessary to submit the specimen to an established laboratory and three or more days would elapse before a reply was received. There has been a marked change in this approach to bacteriology within the last few years and it is the purpose of this presentation to explain how bacteriological methods can be used in a clinical practice.

The need for an early bacteriological diagnosis has increased with the broad scope of chemotherapeutic agents available for specific action against causative organisms. It is futile to treat a colon infection with penicillin. Broad spectrum antibiotics while offering some advantage with their coverage, also fail against certain bacteria where another would be useful. A more urgent need for sensitivity testing has arisen in recent years as the resistance of many strains of bacteria to antibiotics has become evident. The value of diagnosing a case of staphylococcal mastitis is reduced if it is among the ten per cent of resistant mastitis strains. Consequently the antibiotic sensitivity testing of bacteria has become a necessary part of bacteriological diagnosis.

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Equipment Required for a Clinical Laboratory

(a) *Culture Plates*: The production of sterile disposable petri-plate solved one drawback for the busy practitioner. These are available from commercial firms at a moderate cost and eliminate the need for the washing and sterilization of plates. A few may wish to prepare their own, but most prefer to secure the plates containing a suitable medium. In routine veterinary work the five per cent blood agar plate will support the growth of most pathogens. These plates have a shelf life of a few months when kept in the refrigerator. A number of firms supply a variety of sterile culture plates.

(b) *Sensitivity Disks*: The use of the antibiotic sensitivity disk has become the accepted method of determining the sensitivity pattern of an organism. They are available as single disks or incorporated in a wheel. The latter are more expensive and some antibiotics are included which are not practical in certain diseases. The level of antibiotics used in each disk should be noted and should correspond to

a level that can be secured by a therapeutic dose of the antibiotic.

(c) *Incubator*: A small commercial incubator is suitable or one can be constructed using a small insulated box with electric light bulbs as the source of heat. A chicken brooder thermostat will control the temperature at 37° C.

(d) *Gas Burners*: A propane gas cylinder with a torch nozzle makes an appropriate burner for the sterilization of bacteriological loops.

(e) *Other Equipment* includes microscope, stains, inoculating loops, and sterile swabs (available in sterile plastic tubes.)

Method for Culture

In our experience, the sterile swab is usually used to collect material for culture from a discharging sinus and body orifices. Urine and milk samples are collected in sterile vials. In all cases cultures should be made immediately. If one wishes for individual colonies, the swab is streaked across one quarter of the plate; then a sterile loop is used to continue streaking across the remainder of the plate. In the case of fluids, a loopful is used for streaking.

Tissues from necropsy can be cultured by searing the surface with a spatula, then inserting the loop deep into the tissue. Sufficient material will adhere to the loop for a satisfactory culture.

When a direct sensitivity test is required the plate must be inoculated to ensure an even growth of the organism over the entire plate. This is accomplished by streaking the swab over the entire surface in one direction then repeating at right angles. When using the loop it is advisable to use three loopfuls and streak in three directions. The sensitivity disks are placed on the plate using sterile forceps. If individual disks are used, they should be placed equal distance from one another. The plates are incubated for at least 18 hours.

Following the plating, the specimen should be smeared on a sterile glass slide. (A slide can be adequately sterilized by dipping it in alcohol and burning in the flame). The smear is then stained with Gram's stain.

Examination of Plates

After 18-24 hours of incubation, the plates are examined. The type of organism involved can usually be determined from the size, consistency, and color of the colony together with its activity on the blood agar plate and its staining characteristics. If further identification of the organism is required, the plate can be sent to a diagnostic laboratory. When the organism is sensitive to the antibiotic, there is a distinct zone of inhibition surrounding the disk. One cannot compare the relative effectiveness of different antibiotics by the width of the zone as there is a variation in their ability to diffuse. The organism is resistant when there is no inhibition of growth. It is wise to consider that factors such as diffusion of antibiotics through tissues or their absorption from the intestinal tract, as well as their antimicrobial activity are concerned in antibiotic therapy.

Common Pathogens Isolated From Various Conditions of Animals

DOG: Skin Conditions: — The staphylococci cause more skin disorders than any other bacteria. Fifteen to fifty colonies will be found on the blood agar exhibiting a distinct zone of hemolysis. They exhibit the varied pattern of antibotic sensitivity peculiar to this organism.

Otitis Externa: The persistant cases are frequently caused by gram negative organisms such as *Pseudomonas, Proteus,* and *Escherichia coli.* As a group they are resistant to most antibiotics. Hibitane and furacin sensitivity disks usually produce a zone of inhibition. Species of Candida (Yeast) grow on blood agar in 24 hours producing a small colony. The yeast is resistant to all antibiotics except Nystatin.

Discharging sinuses — A wide variety of organisms are isolated from these which occur at various sites.

EQUINE: Nasal Swabs: — The streptococci which cause strangles are characterized by a wide zone of beta hemolysis and are frequently encountered.

Cervical Swabs: A cervical examination is frequently made before breeding to determine freedom from bacterial infection. It is important that the material be streaked on the plate soon after collection.

A cotton swab is satisfactory although some prefer a bacteriological loop which can be sterilized in the flame and used for direct inoculation. The plates should be examined in 18-24 hours when most pathogens encountered will have grown. They include hemolytic Streptococci, *Klebsiella* and hemolytic *E. coli.*

PORCINE: Rectal Swabs: — Certain serological types of *Escherichia coli* are responsible for intestinal tract infections of young pigs and enterotoxemia. Many of these strains are hemolytic and are the predominate aerobic organism on blood or MacConkey's agar. The direct sensitivity test on the rectal swab will indicate the chemotherapeutic agent most likely to be successful in controlling the infection. *Vibrio coli* will not grow under the conditions described, but can be observed in large numbers as a curved gram negative organism on a gram stained smear of feces

BOVINE: Milk Samples — In the diagnosis and treatment of bovine mastitis, a cultural examination has marked value as most pathogens grow in 24-48 hours. These include hemolytic *Staphyloccus aureus, Streptococcus agalactiae,* other streptococci species exhibiting varying degrees and types of hemolysis, *E. coli, Klebsiella pneumonia, Corynebacterium pyogenes* and yeast. The colonies are sufficiently distinctive for recognition although a 24 hour growth of a yeast will resemble a non-hemolytic streptococcus. The sensitivity pattern and a stained smear help to identify the organism, i.e. a yeast will be resistant to all antibiotics while the streptococcus is sensitive to most. The number of colonies will vary but the average loopful will give 10 colonies or more on the plate. There will be no growth in approximately 25 percent of the cases. These prove to be a disappointment to the clinician when he begins culturing milk from acute cases. One reason, for this is the fact that during the acute phase the number of bacteria is drastically reduced due to leucocytic activity.

Summary

A brief outline of a method has been presented whereby a clinician may conduct simple and useful procedures for diagnosis of bacterial infections. *End*

Bovine Teat Surgery

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vade the teat sinus and in which milk is leaking from the teat are more difficult to treat. Lacerations with little swelling are gently cleansed and a rubber band or a compression forcep is placed across the base of the teat to prevent the flow of blood and milk over the operative area. The mucosal layer and the tough connective tissue layer just beneath it are sutured with a simple continuous stitch. Mattress tension stitches of synthetic material are then used to bring the edges of the wound in apposition. The skin is closed with simple interrupted stitches of synthetic material. The teat is infused with antibiotic and a Larson type plastic teat tube is inserted with the cap off. The wound is covered with a plastic spray. The teat tube is removed in three to four days, the synthetic sutures in about eight days. If the papillary duct is opened by the laceration, Mutrux places the teat tube in the duct first and closes the wound over it.

Contusions and infected lacerations are debrided to remove the infected tissue. Then if enough tissue remains, the wall is closed by using a subcuticular stitch and closing the skin if possible. Bandaging the greatly swollen teats is sometimes of value.

In conclusion it can be said that more successful operations could be performed on the bovine teat if careful attention were given to the use of sterile instruments, the preparation of the operative field, restraint, technique of the operation, and to post operative care.

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End

Superior meat and wool have resulted from U. S. Department of Agriculture crossbreeding of Hampshire, Shropshire and Southdown sheep.