University of Mosul
Lecture No.:
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Website:

Lecture title: CLINICAL MICROBIOLOGY

Lecturer Affiliation: Department of Internal and preventive medicine

Summary:

1-Collection and submission of diagnostic specimens

General Principles for Sample Collection

1. Specimens should be taken from living or recently dead animals.

Specimens from animals that have been dead for more than 4 hours are usually unsuitable

2. It is useful to collect samples from clinical cases and in-contact animals, particularly ifthere has been an outbreak of disease.

In-contact animals may be at an earlier stage in the infection with a greater chance of shedding microorganisms

3. Samples should be taken from the affected site(s) as early as possible following the onset of clinical signs.

This is particularly important in viral diseases as shedding of virus is usually maximalearly in the infection. This is also true of enteric bacterial pathogens.

4. Samples should be obtained from the edge of lesions and some macroscopically normal tissue included.

Microbial replication will be most active at the lesion's edge.

5. It is important to collect specimens as aseptically as possible, otherwise the relevant pathogen may be overgrown by numerous contaminating bacteria.

6. The laboratory should be informed if treatment has started in order that counteractive measures may be taken to increase the possibility of isolating bacteria or that an alternative method of detection such as polymerase chain reaction (PCR) may be employed. (Specimens should always be collected before the administration of any form of treatment).
7. Sample material sent on swabs is liable to dryness.
8. Samples must be submitted individually in separate water-tight containers
9. If transportation to the laboratory is delayed, most samples should be refrigerated at 4°C and not frozen
10. Precautions must be taken to avoid human infection where a zoonotic condition is suspected.

Specimens for Microbiological tests:

Blood

If collected in a syringe,care must be taken not to cause haemolysis. The needle should be removed prior to expelling the sample carefully into a sterile dry tube.

Abortion cases• Foetal abomasal contents , lung, liver and a sample of any gross lesions should be sent.

• A piece of affected placenta and two or more cotyledons from cattle and sheep.

• Uterine discharge (especially if no placenta is available).

• If leptospiral abortion is a possibility, 20 ml of midstream urine from the dam preserved with 1.5 ml of 10 % formalin should be submitted.

Abscesses :3 ml of pus should be collected, together with scrapings from the wall of the abscess. Pus from recently formed abscesses will yield the best cultural results.

Urine sample:

The preferred methods of collection are by cystocentesis , catheter or mid-stream urine sample.

Samples from skin lesions

If intact pustules or vesicles are present, the surface should be disinfected with 70 % ethyl alcohol, allowed to dry, and material aspirated from the lesion with a sterile syringe and fine needle.

Feces

A faeces sample freshly voided or collected from the rectum is better to a rectal swab which often does not have enough faecal matter for agent detection.

Bacterial pathogens: Microscopy, culture and identification:

MICROSCOPY

Microscopes for microbiology require a higher degree of resolution than those used for haematology or histopathology.

A bright-field microscopy as well as low-power, high-dry and oil-immersion objectives are required. A darkfield condenser is a useful addition necessary for the visualization of unstained preparations such as those of spirochaetes(Leptospira spp.)

Stained Smears from Pathological Specimens

Stained smears made from lesions can yield a considerable amount of information inexpensively and quickly.

Diagnostic uses of stained smears:

1- Gram stain: In pus

Bacteria	Appearance in stained smears
Staphylococcus species	*Gram + cocci, often in clumps*
Streptococcus species	*Gram + cocci, usually in chains*
Streptococcus equi subspecies equi **Strangles**	*Gram + cocci, often in chains*
Corynebacterium pseudotuberculosis *Pasteurella multocida , Pseudomonas aeruginosa*	*Gram + rods*
Fusobacterium necrophorum	*Gram −, long, slender filaments, often staining irregularly*
Actinobacillus lignieresii Bovine actinobacillosis (wooden tongue)	*Gram – rods*
Bovine actinomycosis (lumpy jaw) *Actinomyces bovis*	Gram +, filamentous and branching

2- Dilute carbol fuchsin (simple) stain:

Disease	Samples	Etiology	Appearance in stained smears
Campylobacter infections Infertility in cattle. Abortion in sheep and cattle	Vaginal mucus or foetal stomach contents	*Campylobacter fetus*	Curved rods that can be in chains giving 'seagull' forms.
Foot rot in sheep	Exudate from hoof	*Dichelobacter nodosus* *Fusobacterium necrophorum*	Rods with a knob on one or both ends. Long, slender filaments, staining irregularly.

3- Modified Ziehl–Neelsen (MZN) stain:

Disease	Samples	Etiology	Appearance in stained smears
Brucellosis in cattle, sheep, pigs and dogs	Foetal stomach contents, vaginal discharge, placenta	*Brucella spp.*	Small, red coccobacilli in clumps.
Chlamydial infections Sheep and cattle: abortion. polyarthritis in lambs and calves Cats: feline pneumonitis	Cotyledons, Joint fluid, Conjunctival scrapings.	*Chlamydophila abortus* *Chlamydophila pecorum* *Chlamydophila felis*	small, red coccobacilli in clumps. Similar to *Brucella.*

4-Ziehl–Neelson (acid-fast-) stain:

Disease	Samples	Etiology	Appearance in stained smears
Tuberculosis Cattle and other species	Suspect lesions	*Mycobacterium bovis*	Long, thin, bright red rods, can appear beaded. Usually not numerous in smears
Paratuberculosis (Johne's disease) in cattle and sheep	Faeces, smear from ileocaecal valve area and mesenteric lymph nodes	Mycobacterium avium subspecies paratuberculosis	Fairly short, red, acid-fast rods in clumps.