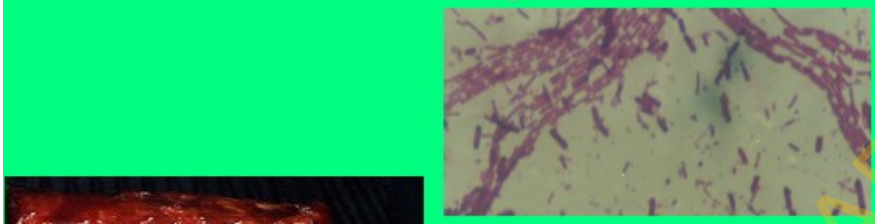
UNIVERSITY OF IBADAN
Faculty of Veterinary Medicine
Department of Veterinary Public Health & Preventive Medicine



Clinic and laboratory manual: Veterinary Public Health- Preventive Medicine- Wildlife and Fish diseases

First edition (2011)

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Preface

This new manual titled" Clinic and laboratory manual: Veterinary Public Health – Preventive Medicine – Fish and Wildlife Diseases" is the first compilation of the laboratory procedures, clinical modules and practical exercises conducted in training undergraduate Veterinary Medical Students of the University of Ibadan. The manual covers in scope the aspects of food and meat hygiene, zoonosis, environmental health, Preventive Medicine, Fish and Wildlife Diseases as well as Veterinary epidemiologic Field investigations. The compilation spans over three decades of teaching Veterinary Public Health and Preventive Medicine to 500 and 600levels students in the Faculty of

Veterinary Medicine, University of Ibadan. The manual will also be useful to 400level students of the Faculty of Veterinary Medicine, University of Ibadan in the current arrangement of industrial training (IT) postings in abattoirs. The compilation is essentially based on epizootiology (Veterinary epidemiology) and population Medicine concepts.

The manual contains mainly the outlines of practical and clinical training requirements in the aspects of Veterinary Public Health and Preventive Medicine curriculum. Hence it is expected to aid teachers and be a learning guide to Veterinary Medical Students especially in the tropical environment.

Dr. I.G. Adeyemi *April, 2008*
Ag. Head

LABORATORY PROCEDURES AND SAFETY MEASURES

DEFINITIONS

Laboratory is derived from the Latin word, '*elaborat*' (to work out, as a problem and with great pains). Laboratory is a place where observations are made, tests are conducted for research work, manufacturing and preparation of chemicals. It is equipped for scientific experimentation or research work. A laboratory may also be a place of specialized work, research, clinical or diagnostic evaluation teaching or learning.

LABORATORY HAZARDS

Hazard is something which may cause injury or damage. The hazards encountered in a laboratory are many and varied hence a large number of available health and safety measures are usually employed in the laboratory.

They can be classified into the following:

(1) Biological – pathogenic, microorganism, biological tissues, animals.
(2) Chemical – corrosives, flammables, toxics
(3) Physical - noise, radiation, and physical falls
(4) Electrical / mechanical – high voltage, apparatus, machinery with moving parts
(5) Psychological – emotional stress

ACCESS TO LABORATORIES

Entry into any laboratory is strictly restricted to personnel authorised to enter the laboratory. The laboratory technologists usually ensure that any person(s) given authority to enter receives appropriate:

- information regarding safety measures required for routine procedures, suitable apparel and so on.
- Supervision
- Children are not permitted in scientific laboratories or workshops

SAFETY MEASURES

A University usually have policies and guidelines on occupational health and safety issues many of which should be relevant to those working in laboratories and readily available for use. Materials Safety Data Sheets (MSDS) are used internationally to provide information required to allow the safe handling of substances at work. It provides the following information;

- The substance; that is – it identifies physical and chemical properties and uses
- Precautions for uses
- Safe handling information

PERSONAL SAFETY

Respiratory and	-	Use fame hoods whenever possible
Body protection	-	Splash proof, safety goggles should be worn at all times in the laboratory.
	-	Laboratory coat/apron should be worn

- Appropriate gloves should be worn as needs arise
- Appropriate closed toes shoes should be worn in the laboratory

Respirators may only be worn after appropriate training.

PERSONAL HYGIENE

Hands should be properly washed before leaving the laboratory
Clothings worn in the laboratory should be laundered separately from other clothings.
Eating, drinking or application of cosmetics is prohibited in a laboratory or areas where chemicals / hazardous agents are stored.
Smoking is prohibited in all areas of laboratories building

WASTE DISPOSAL

- Wastes should be minimized at the source by limiting the quantities of material purchased and used.
- Chemical wastes should be segregated and prepared for disposal in accordance with Health Management Board.
- Wastes should be disposed in designated containers. There are many different types of containers used for collecting waste. (Covered dustbin and incinerator are recommended in biological laboratories).

FIRE PREVENTION

There are many ignition sources in the laboratory such as open flames, heat and electrical equipments, users of lab must take the following precautions:

- Flammable reagents should be purchased and stored in the smallest quantities available
- Flammable liquid that require refrigeration should be stored in explosion – proof refrigerator.
- Flammable liquid should be stored in appropriate safety cabinets or safety cans.
- Incompatible reagents should not be stored together
- Ethers or conjugates should not be stored for extended periods of time as explosive peroxides could form.
- Fire extinguishers must be in good working order at all times.
- Automatic fire sprinklers must remain clear and unblocked to function properly.

. Electrical cords should be in good condition. All electrical outlets should be grounded and all should accommodate a 3 – pronged plug.

- Laboratory personnel should remain out of the area of fire or personal injury unless it is their responsibility to meet the emergency responders.

PUBLIC HEALTH, PREVENTIVE MEDICINE, WILDLIFE AND FISH MANAGEMENT

STAINING TECHNIQUES

SIMPLE METHOD FOR STAINING BACTERIA

1. THE METHOD OF FIXING BACTERIA

The flame fixation of bacterial specimen before staining is unreliable and dangerous. In simple stains and agar cultures only Gram-positive bacteria are fixed. In broth culture, both Gram-positive and Gram-negative bacteria are not fixed. The unfixed bacteria may become infectious during handling. The following reliable and safe method is therefore introduced:

Fixing Solution		**Applicable to:**
0.5% HCL Alcohol		
5% Ethyl alcool	99.5 ml	Non spore forming bacteria
Conc. HCL	0.5 ml	
Mercuric Chloride HCl Alcohol		
95% Ethyl alcohol	99.5 ml	Spore-forming bacteria
Conc. HCl	0.5 ml	
Mercuric Chloride	0.1 gm	

Fixing method:
After drying the bacterial smear under room temperature; add 1-3 drops of the fixing solution (3 drops for Anthrax bacteria).

Wait for several minutes, wash with water (HCI alcohol which causes decolonization) and apply the stain.

Application:
This is a simple stain for inspection of the bacterial morphology.

Remarks:
1. Bacterial surface cover appears in the staining solution .

2. This improved method fixes more bacteria.

II. SPECIAL STAINING FOR MORPHOLOGY

1. FLAGELLA STAIN

Solution 1	
5 % Carbolic acid	10ml
Powdered tannic acid	2gm
Saturated solution of potassium aluminate	10 ml
Solution II	
Saturated alcoholic solution of crystal violet (12%)	

Ten parts of solution I (mordant) and one part of solution II are thoroughly mixed right before staining.

Staining method:

Dry and fix smear from a young culture as usual. Pour the mixed solution on the smear and allow it to act at room temperature for 3-5 minutes, then wash the solution out.

Staining effect:

Cell bodies and flagella stain violet.

Remarks:

1. Three methods of smearing the flagella staining: (1) dispersing the water circle of 1.5 cm in diameter on the slide, then adding the bacteria on it.

(2) 1-2 drops of water on the concave slide, then smear the bacteria on it.

(3) Bacteria suspend in 2% formalin solution, take 1-2 loops, and smear on slide.

2. STAINING OF METACHROMATIC GRANULES

Staining solution:

Saturated aqueous solution of potassium aluminate	50ml
Saturated alcoholic solution of crystal violet	l0ml

Method:

1. Smearing, dry and fix with flame.
2. Stain for 2-3 minutes.
3. Rinse with tap water and blot dry.

Result:

The metachromatic granules stain dark purple, and the cell stain light purple.

Application:

Corynebacterium diphtheria, Corynebacterium renale bovis, Actinobacillus mallei.

Remarks:

Stain solution can be stored for several months.

3. STAINING OF BACTERIAL GRANULES

The ordinary granules of bacteria can not be observed by general staining method. This method makes them easily seen. The micropictures are similar to those of the Dubo's electron microscopy.

Mordants:

Gram negative bacteria 4-5% aqueous solution of carbolic acid

Gram positive bacteria 7 % aqueous solution of carbolic acid

Stain Solution:

Pfeiffer's solution

Ziehl-Neelsen's carbol fuchsin solution 1 part

Distilled water 9 parts

or

Loffier's methylene blue solution

Procedure:

Place a loopful of carbolic acid on a slide.

Mix into the carbolic acid a little of baterial culture smear dry and fix by flame.

(3. Stain with Pfeiffer solution for 10 to 30 seconds.

Rinse with water, blot dry.

If, however, counter stain is wished, the smear is stained first with Loftier methylene blue for 30 seconds and then rinse with water and stain Pfeiffer solution for a few seconds.

Results:

Pfeiffer solution:

The cell stain light red and the granules stain dark red.

Counter-stain:

The cell stain light red and the granules stain dark blue.

4. STAINING OF ECTOPLASMA (CELL WALL)

Ectoplasma, the capsule-like envelope of the bacterial body can not be stained by the common staining method.

The author devised a simple method to demonstrate this ectoplasma clearly as follow:

Procedure:

1. Place 1-2 loops of 0.2-0.3 % agar solution the slide; mix with bacteria and smear to a circle of 1.5....2.0 cm in diameter, then dry it.
2. Fix with 0.5 % HCI alcohol, or flame.
3. After drying, stain the smear with 1 % Gentian violet, crystal violet or methyl violet for 2 to 10 minutes.
4. Rinse with water and blot dry.

Result:

Almost all the species of examined bacteria were found to be provided with the sharply contoured and unstained narrow zone around the deeply stained medullary part.

Remarks:

1. This method is suitable for all kinds of bacteria, but the rough type of bacteria can not be demonstrated.

5. CAPSULE STAIN

This method is designed for the stain of *Streptococcus equi, Klebsilla pneumoniae,* and *Pasteurella spp.*

Procedure:

1. Mix the bacterial culture with 2 % Congo red, and smear on slide to a circle of 2 cm in diameter, dry it.
2. Fix with 2% HCI alcohol.
3. After drying, the bacterial film is then stained with 1 % crystal violet, gentian violet or methyl violet for 1-3 minutes.
4. Rinse with water and blot dry.

Result:

The cell of *Streptococcus equi, Klebsilla pneumoniae, Pneumococcus* stain purple and their capsules stain pale purplish red, the background is greenish purple. The Pasteurella Spp capsule is transparent.

Remarks:

Media for the cultivation of capsule-forming bacteria are suggested as follows:

Blood agar for Strepto. equi.

Egg yolk agar for *Pasteurella multocida*.

6. SPORE STAIN

The method is similar to that of the Acid-fast stain of Mycobacterium described later in this manual.

Procedure:

1. Prepare the smear as usual, dry and heat fix.
2. Cover with Ziehl-Neelsen's carbol fuchsin and boil for 30 seconds to 1 minute.
3. Rinse with water.
4. Counterstain with 2 % malachite green for 1 minute.
5. Rinse with water and blot dry.

Result:

The cell stain green and the spore stain red.

Remarks:

If the time of counterstain isn't enough, the bacteria do not show green color.

7. LEPTOSPIRA AND TREPONEMA STAIN

This method is designed for the simple way to demonstrate the Leptospira and Treponema by staining.

Fixing solution	1 % formalin	
Sensitizing solution	5 % Sodium bicarbonate	
Staining solution	Basic fuchsin powder	0.3 gm
	95% Ethyl alcohol	10 ml
Distilled water	90mI	

Procedure:

1. Prepare a smear and dry.
2. Dip it into 1 % formalin to fix for 1 minute.
3. Take out the slide and drain the formalin solution and place 1 drop of 5% Sod. bicarbonate on the smear.
4. Add immediately *8-10* drops of stain solution and stain for 3-5 minutes at room temperature.
5. Rinse with water and blot dry.

Result:

1. The Leptospira and Treponema stain red.

2. The *Treponema pallidum* in the rabbit testis were visible at the thin layer of area of smear.

Remarks:

1. Old culture of Leptospira is not suitable for staining.
2. Covers the entire smear with 5% $NaHCO_3$, then the treponema will be stained well.
3. It is better to mark a circle to the reverse side of the slide in order to get a clear smear of the culture.

III. SPECIAL STAINING AND DIFFERENTIATION

1. ACID-FAST STAIN

The method is devised to stain the tubercle bacillus from the specimen of the patient.

Method:

1. Smear the patient's sputum on a slide, then dry and fix.
2. Flood the slide with Ziehl-Neelsen's carbol fuchsin, and pass the slide over a fairly strong flame for 1-2 minutes.
3. Wash with water and dry.
4. Stain with 2 % malachite green solution for 1 minute.
5. Rinse with water and dry

Result:

Tubercle bacillus is stained red, other cells, bacteria and sputum are green.

Remarks:

1. If over- stained with carbol fuchsin solution the smeared surface do not appear green.
2. The staining power of brilliant green is too weak, so it cannot be used.

2. GRAM DIFFERENTIATION

The author presented four simple methods by which the Gram positive and negative bacteria could be differentiated without staining. The first method by carbolic acid, the second method by potassium hydroxide, the third method by sulfuric acid, the fourth method by citronella oil. Methods 1-3 are slide-on operating reaction, among them, the second method is more practical at present. The fourth method is a culture method. The principle is to inhibit the growth of the Gram-positive bacteria by the vapor of citronella oil.

KOH method:

1. Place 1-2 loops of 3% KOH solution on a slide.

2. A half loop of young culture (within 24 hours) on solid media is added and mixed.

Result:

Within 30 seconds, the Gram-negative bacteria become markedly viscous (Fig. 15), while the Gram-positive bacteria show no change (Fig. 16).

H_2SO_4 method:

1. A half drop of conc. H_2SO_4 is placed on a slide.

2. A half loop of fresh culture on solid medium is mixed in it.

Result: Immediately, the Gram-negative bacteria become clear and transparent, while the Gram-positive bacteria still showing turbidly.

Citronella oil method:

1. The bacteria under examination is at first inoculate to the test tube which contains nutrient broth and incubated for 24-48 hours.

2. One loop of broth culture is drawn and smeared on agar slant or plate.

3. One loop of citronel1a oil is drawn and streak on the inner wal1 of the test tube or in the cover dish. The tube is then sealed with rubber stopper to prevent the oil from evaporating.

Result:

The culture media are incubated for 24-36 hours, the growth of the Gram-positive bacteria is inhibited, while the Gram-negative bacteria remains unchanged.

Remarks:

1. The second and third methods can only be applied to the colony of bacteria on solid media.

2. The KOH method is better be used as contrast with Gram stain.

3. 3% KOH cause more viscous in Gram-negative bacteria.

4. The citronella oil in the fourth method must be fresh.

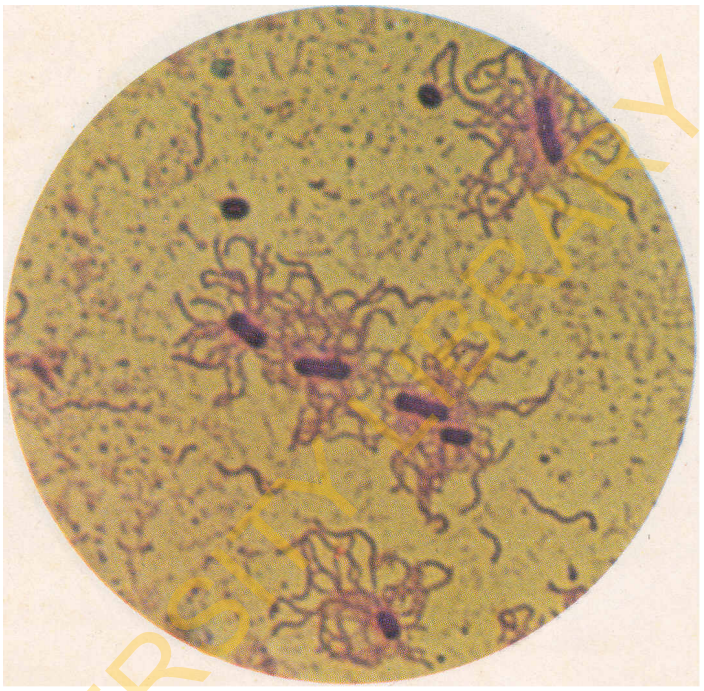

Fig. 1. *Proteus vulgaris*, showing peritrichous flagella, 12 hours culture, 1000 x .

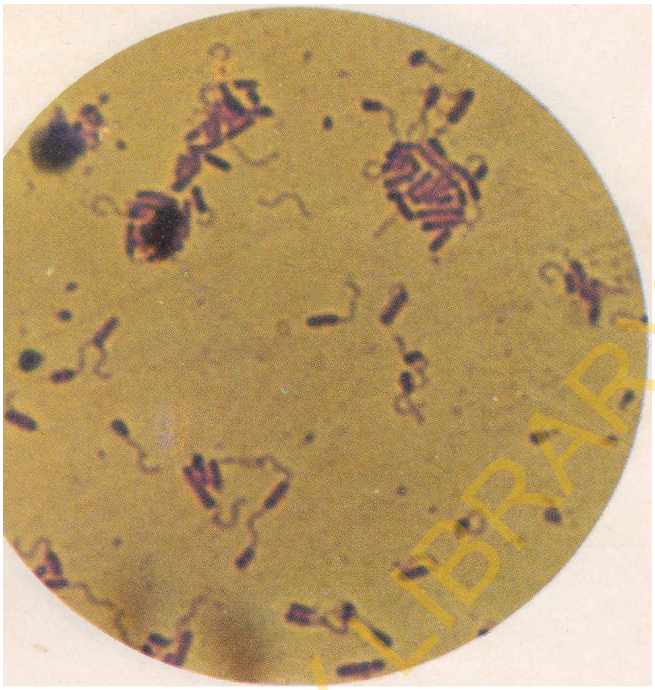

Fig. 2. *Pseudomonas aeruginosa*, showing polar flagella, 12 hours culture, 1000 x

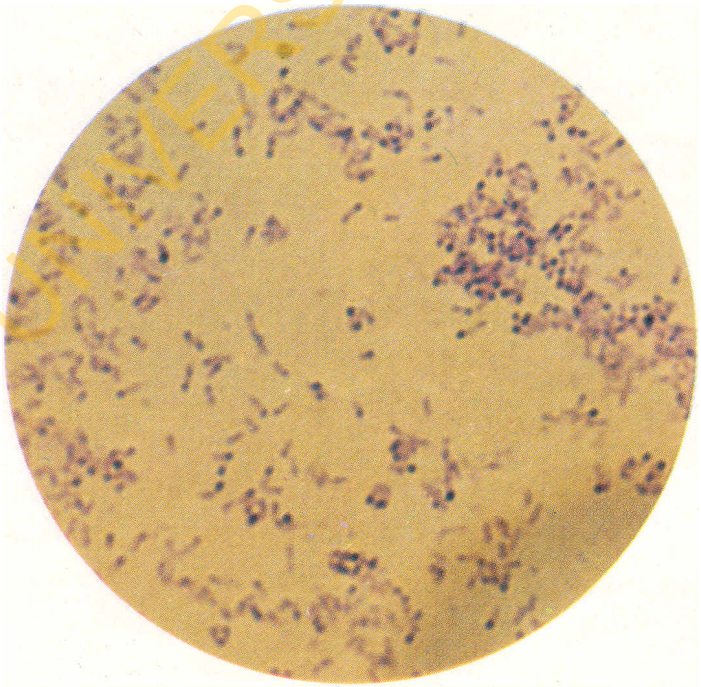

Fig. 3. Metachromatic granules in *Corynebacterium diphtheria*, 24 Hours culture, 1000 x .

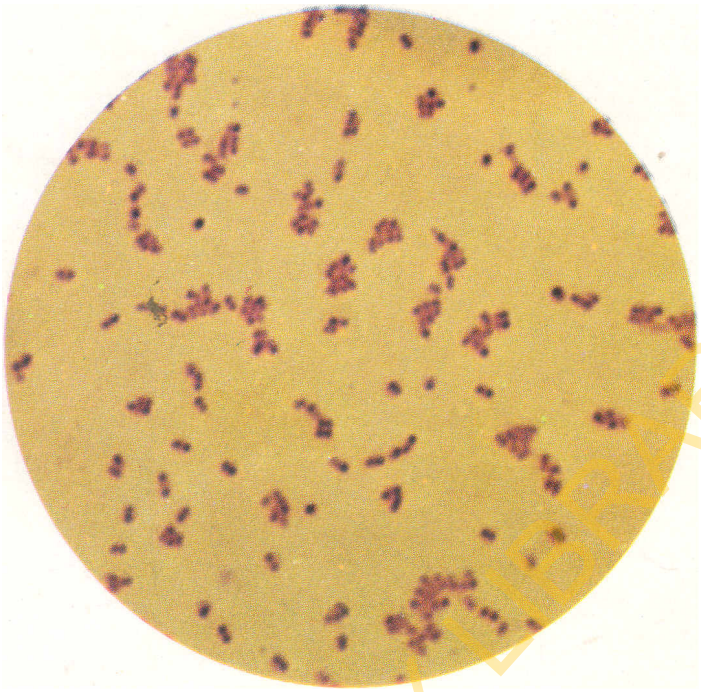

Fig. 4. *Escherichia coli*, showing granules in the cells, 24 hours sculture, 1000 x .

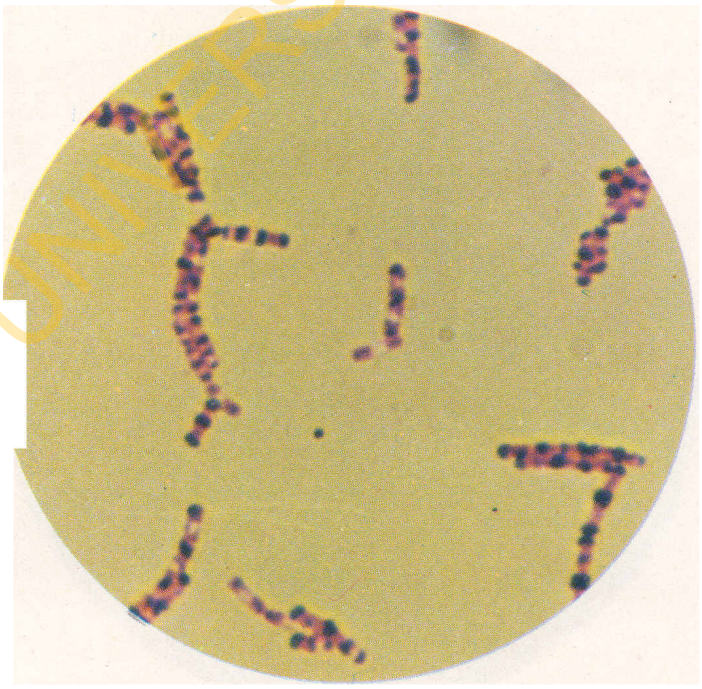

Fig. 5. *Bacillus subtilis*, showing granules in the cells, 24 hours culture, 1000 x .

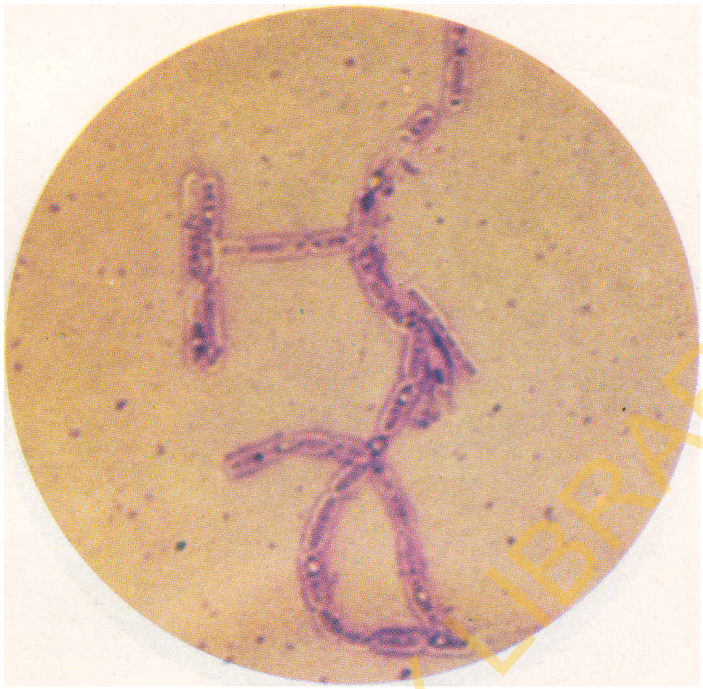

Fig_ 6. *Bacillus subtilis*, showing ectoplasma around the cell bodies, 24 hours culture, 1000x.

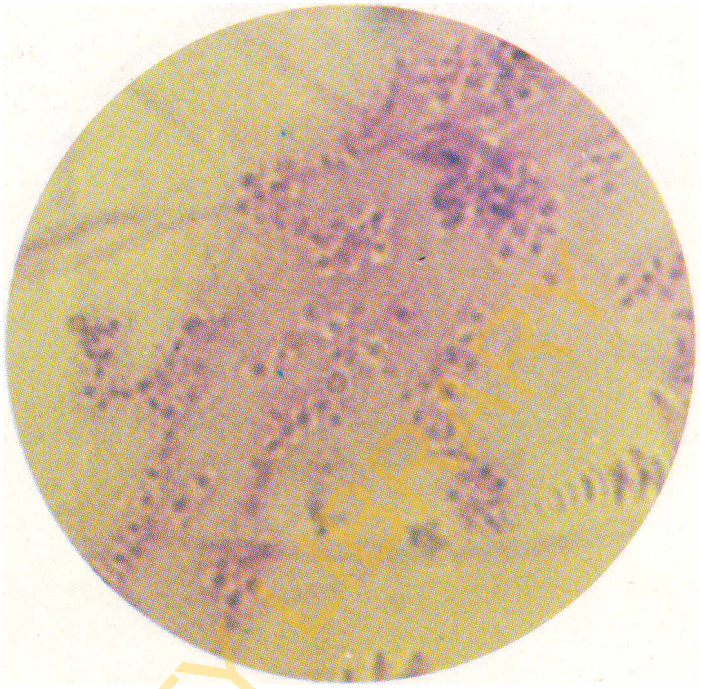

Fig. 7. *Escherichia coli*, showing ectoplasma around. the cell bodies 24 hours culture, 1000 x .

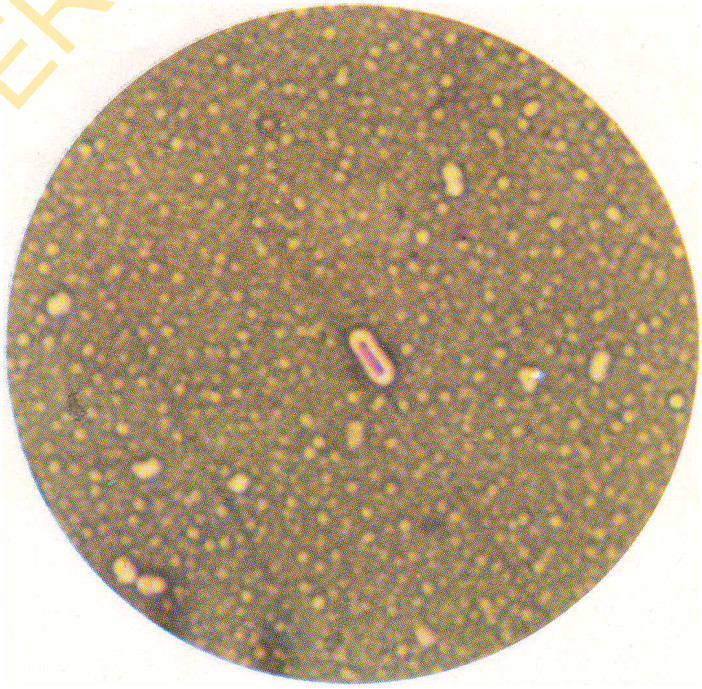

Fig. 8. *Pasteurella multocida* from egg agar, 24 hours culture, showing capsule, 1000 x .

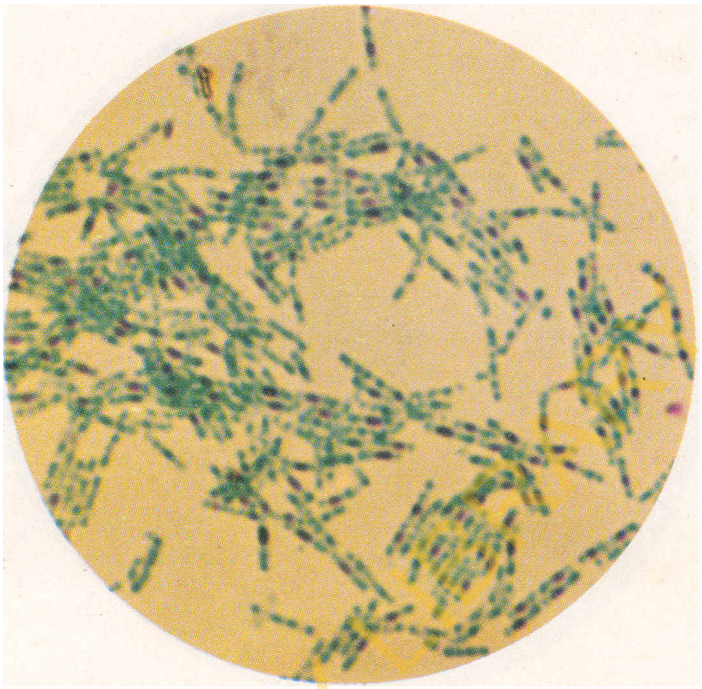

Fig. 9. *Bacillus subtilis*, showing endospores (red) in the central of the cells (green), 72 hours culture, lOOOx.

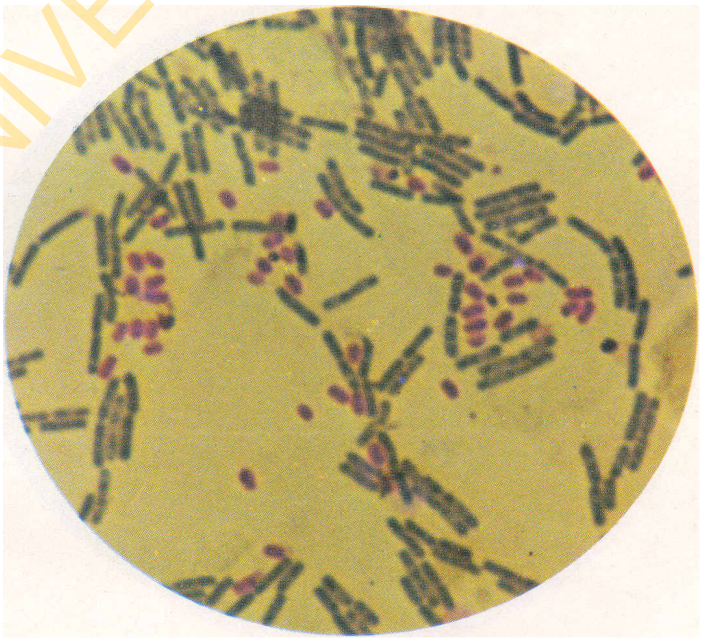

Fig. 10. *Bacillus subtilis*, showing both vegetative cells (green) and spores (red) not *yet* germinated, 6 hours culture, lOOOx.

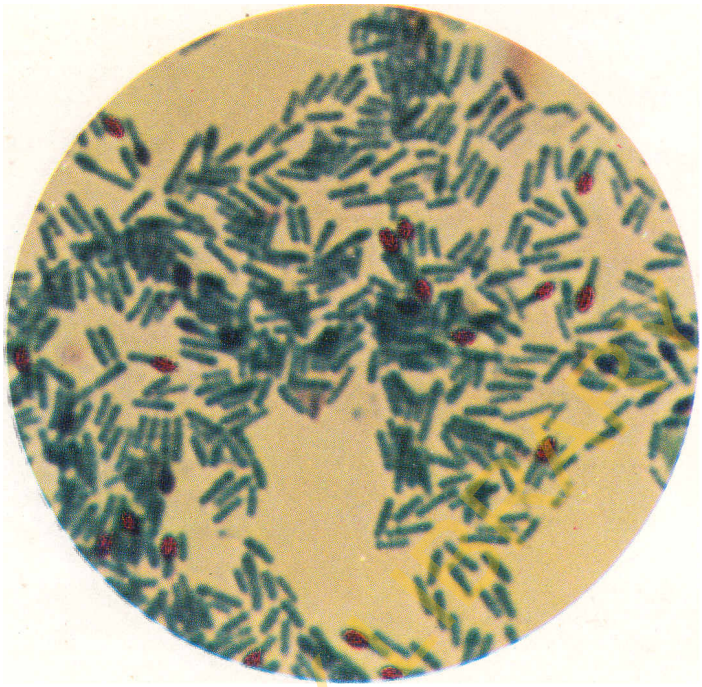

Fig. 11. *Clostridium tetani*, showing terminal spore, 24 hours anaerobic culture, 1000x.

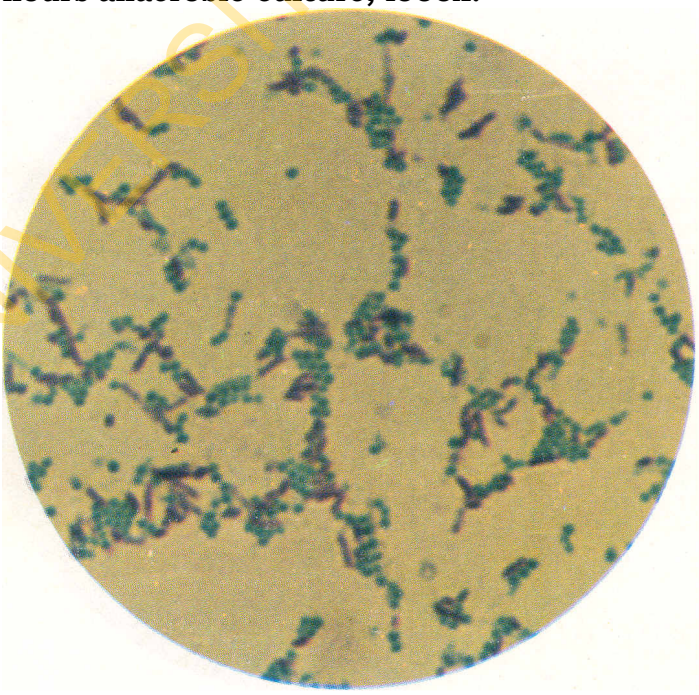

Fig. 12. Acid-fast stain, mix smear with acid-fast Mycobacterium avium (red) and non acid-fast Staphylococcus aureus (green), 1000x.

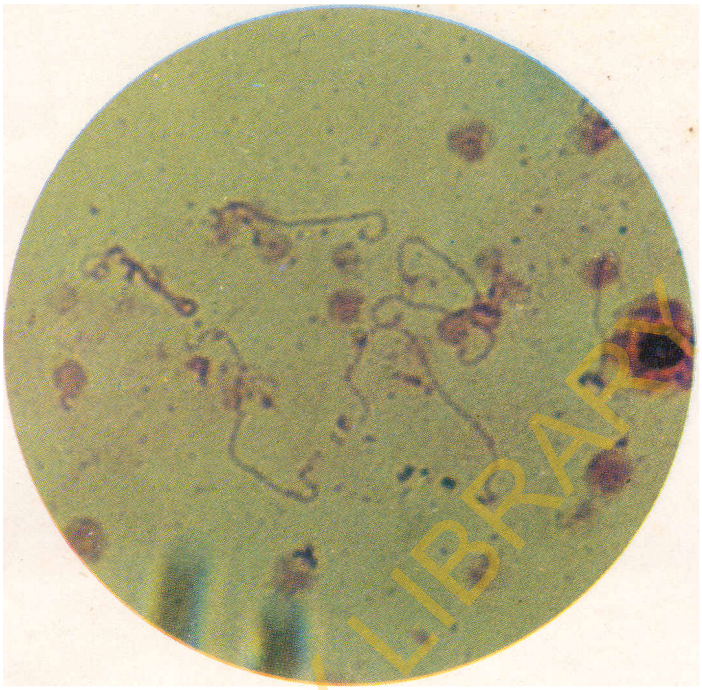

Fig. 13. Leptospira canicola, from Korthof's media, 20 days culture, 1000 x .

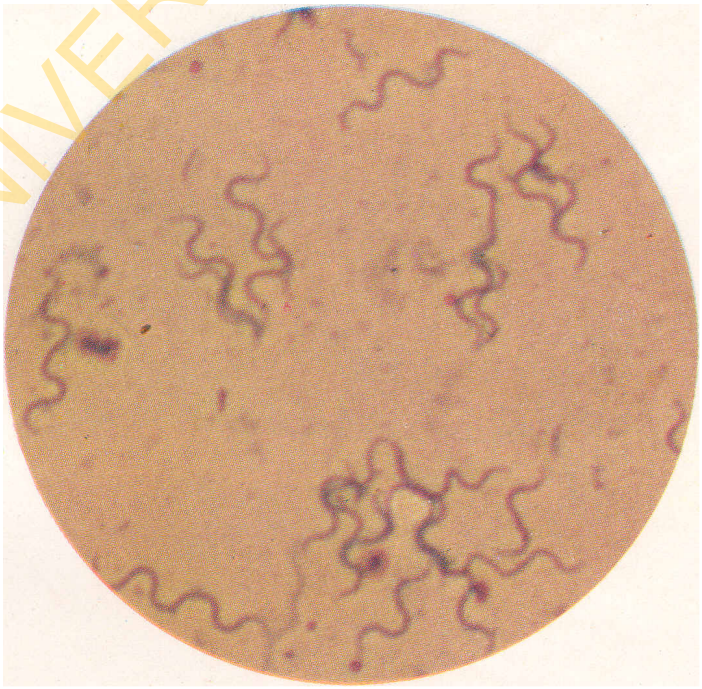

Fig. 14. Treponema hyodysenteriae, smear from rectal swab, 1000 x . .

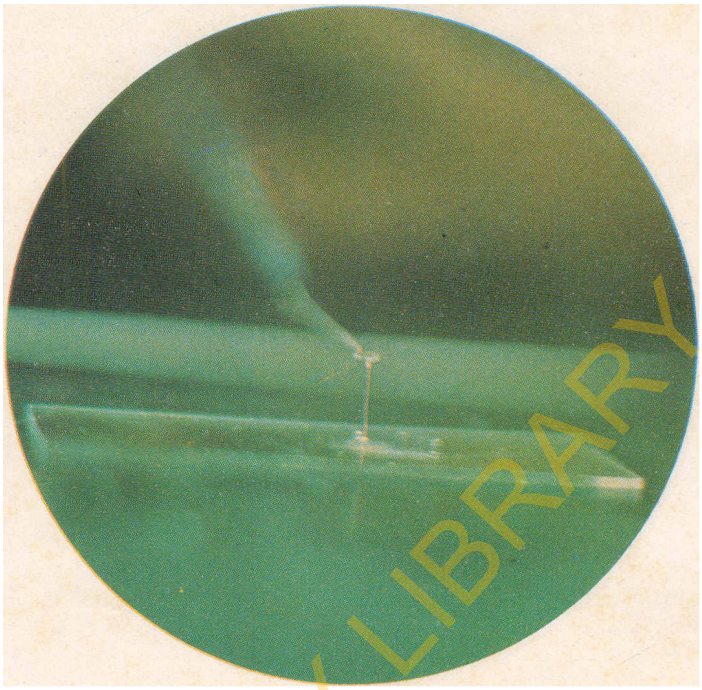

Fig. 15. Differentiation of Gram reaction, KOH method, Gram-negative bacteria showing viscous thread between loop and slide glass.

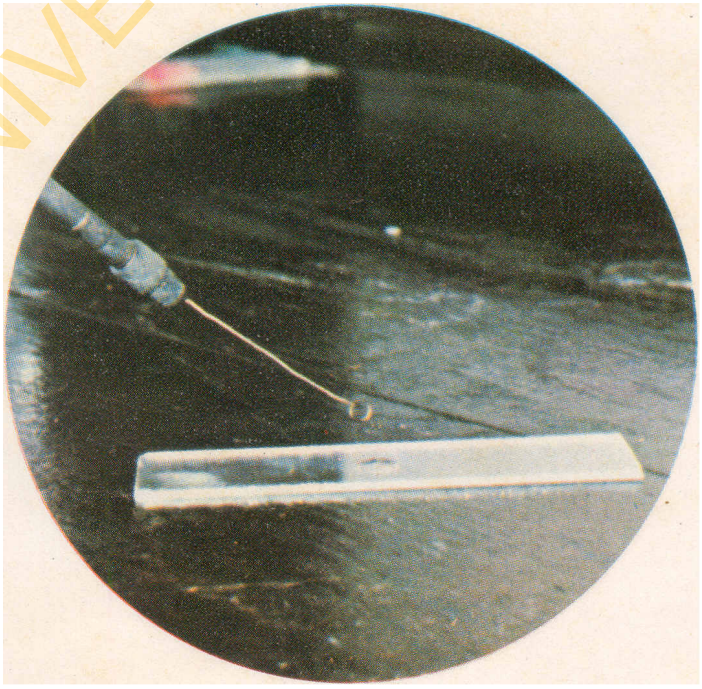

Fig. 16. Differentiation of Gram reaction, KOH method, Gram-positive bacteria showing nothing between loop and slide

Haematological investigation

Changes in haematological indices are usually associated with certain disease conditions. By estimating the levels of appropriate parameters in the laboratory these disease condition can be detected. The parameters commonly evaluated include PCV, Total cell Blood Counts (Red and White) differential white cell count, haemaglobin.
Haematological investigations are often completed as part of a routine physical investigation. It is also included as part of an initial examination of an individual animal presented for routine immunization or surgical procedure. Haematological determinations are of most value in animals with a generalized or systemic disease or in individuals having localized disease process. Through blood examination some blood haemoparasites causing diseases in animals can also be detected.
Major Equipment:- Haematocrite centrifuge, reader, colorimeter for haemoglobin, microscope.
Minor Equipment:- Haemocytometer chamber, diluting tubes, tally counter, glass slide and cover slip.
Reagents: EDTA, white blood cell diluting fluid, RBC diluting fluid, Giemsa stain, Diabskin solution etc.

Total Blood Cell Counts
Total Blood Cell Counts are carried out on unclotted blood using EDTA as anticoagulant. Clotted blood is unsuitable for examination.
EDTA Preparation
2g of EDTA (ethylenediamine tetratacetic acid) is weighed and dissolve in 26.7mls of distilled water. A drop into 1.0ml of blood is sufficient.
Determination of Packed Cell Volume (PCV)
(a) Collect the blood into anti coagulant bottle
(b) Mix the blood by inverting about 20 times
(c) By using a capillary pipette (Pasteur Pipette long type) fill the wintrobe haematocrit, taking care to avoid bubbles.
(d) Sealed up the other end with plastercine
(e) Centrifuge at 3000 r.p.m (revolution per minutes) for 20 minutes.
(f) Remove from the centrifuge and read the height of the red cell column with haematocrite reader i.e. to the bottom of the buffy layer. The tube is divided into 100 division, the height of the column of red cell is read off and is expressed as a fraction of whole blood e.g. if column reading is 42.

$$PCV = \frac{42}{100} = 0.42$$

Determination of Haemoglobin
(a) Collect the blood into an anticoagulant bottle.
(b) Mix the blood by inverting it about 20 times but avoid vigorous agitation
(c) With micro pipette take 20µl of blood, wipe the tip of the pipette.
(d) Wash into 5ml Drabkin's solution. Mix well.
(e) Allow it to stand for 10 minute
(f) At 540nm wave length read in a colorimeter with a tube of Drabkin's solution as blank
(g) Read the standard (std) solution of cyanmethaemoglbin
(h) Calculate as follows:

$$\frac{\text{Reading of test}}{\text{Reading of std}} \times \text{concn of std} \times \text{dilution} = \text{g/dl}$$

Determination of Red Blood Cell (RBC)

(a) Collect the blood into an anticoagulant bottle
(b) Mix the blood by inverting it about 20 times but avoid rigorous agitation.
(c) Take 4ml of RBC diluting fluid into a test tube
(d) By using multichannel pipette take 5µl of the blood and wipe the excess of blood from outside of the tip.
(e) Express the content of the pipette into the tube containing 4ml of diluting fluid. Wash out the blood from the tip by repeatedly drawing up some of the fluid and returning into the bottle.
(f) Mix the content by inversion
(g) Thoroughly clean the haemocytometer and cover slip, ensuring they are both free of oil.
(h) Place the haemocytometer on a flat horizontal surface, and using firm pressure (usually with your both index finger) slide the coverslip into position over the ruled counting areas.
(i) Mix RBC dilution by inverting 10 -12 times
(j) Take up RBC dilution in a capillary tube or a Pasteur pipette.
(k) Fill the counting chamber by holding the capillary at an angle 45 degrees and lightly touch the tip against the edge of the chamber and cover glass (don't allow the channel to be overflow). Let the fluid flow evenly into the chamber.
(l) Mount the counting chamber on the microscope stage.
(m) Using a high dry objective and x10 eye pieces focus the centre square millimeter (ABCD) of the ruled area and count all the cell count and in 80 of the 400 small square i.e. five group of 16 small squares, one at each corner and one at the middle.

RBC calculation = No of cells counted x mag(magnification) x dilution x area e.g. 500 x 10 x 200 x5 = 5,000,000 cell per cubic millimeter or in 51 units 5.0 x 10^{12}/l

Determination of White Blood Cell (WBC)

(a) Place 0.5ml of diluting fluid into a test tube
(b) Mix blood sample by inverting several times avoiding vigorous agitation.
(c) Using multichannel pipette draw blood up to 25µl wipe excess off from die tip and expel it into the diluting fluid.
(d) Mix the tube content by inversion
(e) The dilution of blood contained in the tube is 1:20
(f) Follow the procedure on RBC counting from (g-m) except that the squares counted on the chamber are the four corner 1mm square as

A		**B**
C		**D**

The cells in ABCD are counted as WBC count.
Calculation:
Average no of cell counted x mag x dilution
Calculation directly into 51 units
No of cell counted x 0.05 x 10^{9}/litre

Differential White Blood Cell (leucocyte) Count

(a) Place a drop of blood at one end of a clean grease free slide
(b) Using a beveled piece of glass a little narrower than the slide, allow the drop to spread along its edge.
(c) By holding the spreader at an angle of 45 degree push it along the side drawing the blood behind it until it has all been smeared.
(d) Dry the film rapidly in air by vigorous shaking. Do not heat dry it.
(e) Stain with Giemsa stain or Romanowsky or Leishman stain.
(f) Fix smears in methyl alcohol e.g. methanol for 3 minutes
(g) Allow to dry in the air
(h) Stain it in Giemsa stain for 45 minutes
(i) Remove and wash with clean water and allow it to dry.
(j) Mount on microscope stage and starting from the thin end of the film view under oil immersion.

Derived Erythrocytic Indices

Mean Cell Haemoglobin Concentration (MCHC): This refer to the percentage haemoglobin in one (1) deciliter of packed RBC

e.g. Hb content = 15g/dl blood
PCV = 0.48
MCHC= $\frac{15}{0.48}$ = 31.25%

Mean Cell Haemoglobin (MCH)

This expresses the average Hb content in picograms (pg) of a single RBC e.g. Hb = 14.5 g/dl blood
RBC = 50 ($x10^{12}$/l)
MCH = $\frac{14.5 \times 10}{5.0}$

That is dividing the Hb content in 5.1 units by the RBC count in 51 units and multiply by 10.

Mean Cell Volume (MCV)

This is the average volume of a single cell expressed in femtolitres (fl) or µm. Divide the PCV by the RBC and multiply by 1000

e.g. PC 0.40
50 ($x10^{12}$/l)
$\frac{0.40}{5}$ x 1000 = 80fl

BIOCHEMISTRY

Serum Total Protein By Biuret Method

Principle:- A violet coloured complex is produced by reacting the serum protein and peptides with an alkaline copper sulphate solution (the Biuret). Other non-protein nitrogen compets, creatinne, urea etc do not react.

Reagents:- Biuret reagent, blank reagent containing potassium sodium titrate, cupric sulphate, potassium iodide, sodium hydroxide sodium chloride.

Method

Reagent blank	Std	Sample	Sample blank
		200µl	200µl
5.0ml	5.0ml	5.0ml	5.0ml
5.0ml	5.0ml	5.0ml	-
			5.0ml

Pipette reagent out as above. It is only necessary to prepare a sample blank with aemolysed icteric or turbid sera. Mix all tubes and place in a water bath at 37^0C for 1`5 mins alternatively allow to stard for 30 mins at room temperature. Measure the optical density of the sample against the reagent blank using the EEL colorimeter and filter OGRI, or read between 530 and 565nm if using a spectrophometer.

$$\text{Protein concentration} = \frac{\text{D.D. sample}}{\text{O.D Std}} \times \frac{\text{conc. Of}}{\text{Std}} \times 10$$

When sample blanks are performed it must be read at the same wave length against

$$\frac{\text{O. D Sample} - \text{O. D sample blank}}{\text{O. D Std}} \times \text{Std concn} \times 10 = \text{g/litre}$$

Sodium Sulphite Precipitation Test for Immunoglobin

Principle:- Globidins are relatively insoluble in concentrated salt solutions compared with albumins various salts have been used to separate these protein from ixed solution such as serum, and by using varying concentration of salts fractionation of serum proteins is possible. The immune globulins of serum and colostrums comprise euglobulin and pseudoglobulin which are insoluble in an 18 percent sodium sulphite concentration.

Reagent

18% anhydrous sodium sulphite

Method

To one drop of serum add 19 drops of the reagent.

Interpretation

Newborn calf, pig, sheep etc sera contain no immunoglobulins and therefore give no turbidity. If no colostrums is fed, the fraction does not appear in the serum in appreciable amount for several weeks.

Following feeding of colostrums the immune globulin appears in the serum in a few hours. Peak serum concentration of the acquired protein (30-50g/l) is reached in 18 to 24 hrs and hence there is a decline so that a steady level of about 15 – 20 g/l is reached by about 3 weeks.

Determination of Serum Albumin

Principle

The specific binding of a day (bromocresol green) to albumin results in a changed peak absorbance wavelength. A whole serum of the same species is used as a standard since reconstituted purified crystalline albumin or albumin of a cliff species may show different dye binding properties.

Reagent

(1) Citrate buffer
(2) Sodium hydroxid
(3) 0.6% Bromocresol green* (BCG) solution
(4) working buffered BCG reagent
(5) standard serum

Procedure

(a) To 20µl of the test sample add 5ml working buffered BCG reagent and mix.

(b) Incubate at 20^0C for 10 minutes and measure O. D at 640nm against a working buffered BCG reagent blank. Use std curve to obtain the albumin concentration g/dl in the sample.

Estimation of Gamma – Globulins (δ)

Principle

The Serum immuno-globulin concentration is measured using a selective turbidity produced by zinc sulphate.

Reagents

(1) Stock solution of zinc sulphate
(2) Working solution
(3) Standard Barium chloride
(4) Sulphuric acid

Method

(a) Pipette into two tubes 100µl of serum
(b) Add 6ml of distilled water to the tube acting as blank
(c) To the sample tube add 6ml of the working strength zinc sulphate solution
(d) Mix by inversion and leave both tubes for 1 hr.
(e) Mix again and read in EEL colorimeter against filter 623nm using the blank tube to zero the instrument for each pair.
(f) Express the result as Z.S.T unit by multiplying the EEL reading by 10

Note

The test is very sensitive to temperature

- Above 20^0C and also the time allowed for the precipitation. It is suggested that the temperature of the reagents should be kept at 20^0C and that the readings are taken at 60 minutes.
- Plasma should not be used for this test
- Because Heparin causes a decrease in the turbidity and fibrinogen in varying concentration is believed to affect the turbidity of the samples with the same immunoglobulin level.

COMPETITIVE ELISA

Procedure

- Coat the microplate with required dilution of the antigen in coating buffer using 50 microlitre/well
- Incubate at 37^0C for 1 hr on an orbital shaken
- Wash the plate three times with washing fluid.

- Blot dry on a paper towel
- Add test sera, strong, moderate and negative sera and monoclonal antibody according to test format.
- Incubate at 37^0C for 1 hr on an orbital shaker
- Wash 3 times with washing fluid and blot dry.
- Add conjugate
- Incubate at 37^0C for 1 hr, wash 5 times
- Add substrate/chromogen, incubate at room temperature for 10 minutes.
- Stop colour reaction by adding stopping solution
- Read on the multiscan at 492nm

Major Equipment

(a) Microscopes (including Research types)
(b) Incubators
(c) Water baths
(d) Bench centrifuge, cold centrifuge and Ultra – Centrifuge
(e) Refrigerators and freezers
(f) Autoclaves
(g) Water Distillers
(h) pH Meters
(i) Spectrophotometer/colorimeter
(j) ELISA reader
(k) Hot air oven
(l) Analytical or Top loading balance
(m) Washer
(n) Gas with Bunsen burner
(o) Hand tally
(p) Haematocrit/Reader
(q) Computer systems – with suitable environment
(r) Telephone, modem, fax machine and multimedia facilities

Minor Equipment

(1) Various types of pipettes
(2) Glass slides and over slips
(3) Petri dishes
(4) Reagent bottles
(5) Beakers and flask various size
(6) Slide boxes
(7) Lens tissues
(8) Test tubes
(9) Haemocytometer, haemoglobinometers and thermometers
(10) Filter papers
(11) Screw cap storage bottles
(12) Capillary tubes
(13) Centrifuge tubes (assorted)

Chemical and Biological Reagents

The types of chemical and biological reagents required will vary, depending on the nature of work of the laboratory. Specific requirements are mentioned along with the relevant techniques.

HELMINTHOLOGICAL INVESTIGATION

Laboratory investigation of diseases caused by worm infection of the alimentary tract is routinely carried out by faecal examination for the presence of worm eggs or larvae.

Collection of Faeces

Faecal samples required for the examinations should preferably be collected from the rectum and examined fresh. If it is difficult to take rectal samples, then fresh faeces can be collected from the ground or floor (in the case of the abattoir). A plastic glove is suitable for the collection, the glove being turned inside out to act as the receptacle. For small pets a thermometer or glass rod may be used ideally, about 5g of faeces should be collected since the amount is required for some of the concentration refrigerator unless the examination is carried out within a day of the specimen collection. For samples sent through the post, the addition of twice the faecal volume of 10% formalin to the faeces will minimize development and hatching.

Methods of examination of faeces

Several methods are available for preparing faeces for microscopic examination to detect the presence of worm eggs or larvae. However, whatever method of preparation is used, direct faecal smears on the slides should first be examined under low power (10x) objective lens of the microscope as most eggs can be detected at this magnification. Higher magnifications are employed for more detailed examination.

The Direct Smear Method

(1) Mix a few drops of water with an equivalent amount of faeces on a microscope slide.
(2) Tilt the slide to allow the lighter eggs flow away from the heavier debris
(3) Place a cover slip on the fluid
(4) Examine under the microscope, using x 10 (and later the 40/45 objective) lens

Note:
It is possible to detect most eggs or larvae by this method, but due to the small amount of faeces usually used, it may only detect relatively heavy infestation.

Floatation Methods

The basis for any floatation method is that when worm eggs are suspended in a liquid with a specific gravity higher than that of the eggs, the latter will float up to the surface. *Nematode* and *cestode* eggs float in a liquid with a specific gravity of between 1.10 and 1.20; *trematode* eggs, which are much heavier, require a specific gravity of 1.30 – 1.35. The floatation solutions used for *nematode* and *cestode* ova are mainly based on *sodium chloride* (NaCl) of sometimes *magnesium sulphate* ($MgS0_4$). A saturated solution of each of these is prepared and stored for a few days before usage. In some laboratories a sugar solution is preferred.

For trematode eggs, saturated solution of zinc chloride ($ZnC1_2$) or *zinc sulphate* ($ZnS0_4$) are widely used.

Direct Method

(1) Add 2g of faeces of 10ml of the floatation solution
(2) Mix thoroughly and pour the suspension into a test tube adding more solution to fill the tube to the top.
(3) Place cover glass on top of the liquid surface.
(4) Allow the tube and cover slip to stand for 10 – 15 minutes
(5) Remove the cover slip vertically, place on a clean glass slide and examine under the microscope.

McMaster Method

This is a quantitative technique and is used where it is desirable to count the number of eggs or larvae per gram of faeces.

(1) Weigh 3.0g of faeces or if faeces are diarrheic, 3 teaspoonfuls
(2) Break up thoroughly in 42ml of water in a plastic container. This can be done using a homogenizer (if available) or in a stoppered bottle containing glass beads.
(3) Pour through a fine mesh sieve.
(4) Collect filtrate and fill a test tube, preferably using a 15ml and flat bottomed tube
(5) Centrifuge at 2,000 r.p.m for 2 minutes – in a standard centrifuge
(6) Pour off supernatant, agitate sediment and fill tube to previous level with flotation solution
(7) Invert tube 6 times and remove fluid with pipette to fill both chambers of the *McMaster's* slide. Leave no fluid in the pipette or else pipette rapidly, since the eggs will rise quickly in the floatation fluid.
(8) Examine one chamber and multiply number of eggs or larvae under one etched area by 100, or two chambers and multiply by 50 to arrive at the number of eggs per gram of faeces (epg)

If 3g of faeces are dissolved in - 42ml
Total volume is - 45ml
Therefore 1g - 15ml
The volume under etched area is - 0.15ml
Therefore the number of eggs is multiplied by 100
If two chambers are examined, multiply by 50

Sedimentation Method (for trematode eggs)

(1) Homogenise 3g of faeces with water and pass the suspension through a coarse-mesh sieve
(2) Wash thoroughly the material retained on the screen using a fine water jet and discard the debris
(3) Transfer the filtrate to a conical flask and allow to stand for 2 minutes
(4) Remove the supernatant and transfer the remainder (approx 12 – 15ml) to a flat-bottomed tube.
(5) After sedimentation for a further 2 minutes the supernatant is again drawn off

(6) Add a few drops of 5% methylene blue
(7) Examine the sediment using a low power stereomicroscope

Any *trematode* eggs are readily visible against the pale blue background

BACTERIOLOGICAL (MICROBIOLOGICAL) INVESTIGATION

Bacteriological investigation is concerned with the etiological diagnosis of infectious diseases by means of isolation and identification of infectious agents, the demonstration of immunologic responses in the patient and with rational selection of antibacterial agents to be utilized in treatment. The results of bacteriological diagnostic tests in the study of infectious disease are principally a function of the nature of the specimen, the care with which it is collected, the timing of this collection and the technical proficiency of the individual performing the tests. A veterinarian should be competent to perform some of the simple techniques that will enable him to render an accurate diagnosis of an infectious disease process. He must know how and when to take a specimen, what examinations can be completed in his laboratory, how to interpret the results of his own work and how and when specimens should be submitted to a laboratory for confirmatory tests.

Collection of specimens

Since results of many diagnostic tests in infectious diseases depend largely on the timing and method of collection, the veterinarian should be aware of certain basic rules for the collection and handling of specimens. Specimens must be obtained from the site most likely to yield the infectious agent at a particular stage of illness and must be handled in a manner that will favour survival and growth of the infecting agent.

Direct Smears

The direct smear is a simple technique and should be used routinely as a part of any microbiologic determination. The proper preparations of direct smears, their staining and examination will often reveal significant information regarding the type of infection present and may enable the Veterinary Officer to institute proper therapy. The direct smear technique for the detection of micro-organisms is applicable in the examination of exudates, urine, cerebrospinal fluid, milk and tissues. A direct smear can be readily prepared by rolling a cotton applicator that has been used for collection of the sample over the surface of a clean glass slide. In preparing tissue impressions the tissue should be blotted on a piece of paper towel of other absorbent surface. Several tissue impressions may be made by gently touching the tissue to the surface of a clean glass slide following removal of excess blood by blotting. Sample fluids can be centrifuged and the sediment used for the smear for most microbiological work.

Examination of direct smears (for some specific bacterial diseases)

Anthrax

Direct smear prepared from blood and spleen impressions may provide evidence suggestive of the existence of anthrax. Bacillus anthracis in blood and tissue occurs as a relatively large square ended Gram-positive rod, appearing in chains or sometimes occurring singly and in pairs.

Procedure

(1) Make smears from blood or tissue impression
(2) Heat-fix by placing over Bunsen flame.
(3) Stain for 6 minutes with 1% aqueous methylene blue, or with gram stains
(4) Examine under oil immersion microscope

Note: With the use of methylene blue stain, a faint outline of a capsule may be noted. Correct interpretation of a direct smear is difficult as the carcass is often contaminated with other Gram-positive rods having an appearance similar to *Bacillus anthracis*. The 'purple detritus' effect should be recalled.

Brucellosis

A quick method for the direct examination of the stomach contents of an aborted foetus is by the use of a modified acid – fast stain.

Procedure

(1) Prepare a thing smear of stomach contents and lightly heat fix
(2) Stain 10 minutes with Ziehl-Nielsen, Carbol fuchsin diluted water
(3) Wash in tap water
(4) Decolorize for 20 seconds with 1% acetic acid
(5) Counter stain for 30 seconds with 1% methylene blue
(6) Blot and examine

Note

Brucella sp. appears as extracellular red coccobacillary rods usually occurring as in clumps.

Agglutination Test

Direct bacterial agglutination can be conveniently performed using either reference bacterial suspensions to detect anti-bodies in a test serum or using reference anti-serum to identify an unknown bacterium. Suspension of bacterial species such as *Brucella*, *E. coli*, Salmonella, Mycoplasma, Streptococci, Staphylococci, etc are available commercially. Stained bacterial suspensions are also available which often make interpretation of results easier. An example of this is the Rose Bengal Plate Test for Brucellosis. The test can be performed quantitatively by just mixing a drop from Pasteur pipette of each of the antigen suspension and test serum sample on flat surface such as slide. After a few minutes, the mixture is examined for agglutination of cells indicating positive reaction. Qualitative Agglutination are usually used as diagnostic screening test. Animal blood group can also be determined by this method.

Quantitative tests involve the serial dilution of the test samples in test tube and then adding specified quantity of diluted antigen suspension. The reaction is incubated in water bath or incubator at 37 degree Centigrade and left for specified period of time. The level of antibody response can be quantified by this method.

PUBLIC HEALTH LABORATORY WORK/CLINICS

FOOD HYGIENE LABORATORY PROCEDURES

500level posting

Determination of Decomposition of Meat

Test 1a: Andryjewzki's Filtrate Test

Equipment & Reagent

Stomacher Machine
Stomacher Bags
Water Distiller
Bunsen Burner
5% Acetic Acid

Procedure

- Mince 10g spoilt meat in 100 ml distilled water for 15 minutes
- Filter 10 ml into a test tube
- Add 1-2 drops 5% Acetic Acid
- Heat to 80 ^{o}C while shaking

Result

Apply heated reagent to the Meat

Fresh Meat = Rose Red

Decomposed Meat = Cloudy

Determination of Decomposition of Meat

Test 1b: Reder's Test

Equipment & Reagents

Stomacher Machine

Stomacher Bags

Water Distiller

Loeffler's Methylene Blue

Carbol Fuchin

Procedure

Cover minced meat with the mixture of Loeffler's methylene blue and carbol fuchsin.

Result

Fresh Meat = No colour Change (light green).

Decomposed Meat = Brownish green

General Bacteriological Classification of Drinking Water

To classify drinking water as to its suitability for drinking, Incubate 100 ml water in:

- A coliform Selective Medium for 24 hrs and
- An *E. coli* Selective Medium and Interprete the result as follows:

Coliform/100 ml	*E.coli* / 100 ml	Category
0	0	Excellent
1-3	0	Satisfactory
4- 10	0	Suspicious
Greater than 10	0	Unsatisfactory
Any number	1 or more	Unsatisfactory

YELLOWNESS OF MEAT DUE TO ICTERUS OR DIET

Remington & Fowrie Test:

The Test differentiates between Yellowness due to Icterus and that of Diet (nutritional) Yellowness

Equipment & Reagents

Bunsen Burner; Distilled Water; 5% NaOH; Ether

Procedure

- Cut 2gm yellow fat; Add 5 ml 5% NaOH; Boil for 1 minute;

- Allow to cool under tap water; Add 5 ml Ether, mix well.

Result

The mixture separates into two parts.

If due to:

Icterus = the lower layer is yellow

Carotene = the upper layer is yellow

TOTAL BACTERIAL COUNT

Equipment and Reagents

Petri Dishes;

Pipettes

Plate Count Agar;

Incubator set at 37 ^{o}C

Water or Meat Sample

Procedure

Pipette 1 ml sample water into 2 sterile empty Petri dishes

In the case of meat sample, blend 10 gm meat sample in 100 ml sterile normal saline or peptone water

Pour approximately 10 ml sterile melted Plate Count Agar (PCA) into each Petri dish.

Incubate the Petri dishes in an inverted position at 37 ^{o}C for 18-24 hours.

Count the total colonies of bacteria at 10^{-4} to 10^{-6} ;

Calculate the Total Plate Count.

A total of 10 per ml count for water or 100,000 per ml meat is normal.

DETERMINATION OF TOTAL VOLATILE BASES (TVB)

Principle: The sample is distilled from magnesium oxide under standard conditions and volatile bases are titrated with boric acid.

Apparatus:

Kjeldahl macro-distillation unit

Reagents:

1. Magnesium oxide, solid

2. Antifoam, silicon preparation or ethyl alcohol.
3. Boric acid, 2% aqueous solution
4. Methyl Red indicator. Dissolve 0.016g methyl red and 0.083g bromo-cresol green in 100 ml neutral denatured ethanol.
5. Sulphuric acid, 0.1 N

Procedure:

Add 1O0ml of water to 109 of the minced sample in a food blender .and homogenize for one minute. Wash into the distillation flask with a further 200ml of water. Add 2g magnesium oxide and a drop or two of anti foam solution. Bring to the boil in exactly 10 minutes and distil for exactly 25 minutes, using the same rate of heating, into 25ml of 2 percent boric acid solution with added indicator in a 500ml conical flask. The tube on the end of the condenser should dip below the boric acid solution. Disconnect the splash-head, stop heating and wash the condenser down with distilled water and titrate the contents of the conical flask with 0.1 N H_2SO_4. Carry out a blank determination.

CALCULA TION

Total volatile bases (as mg N per 100 g flesh) = 14 (titre - blank)

INTERPRETATION

The method is valid only for fresh or frozen meats and not for bacon and other cured meats. M at should give a value of less than 20mg percent calculated on a fat- free basis, values over 30mg percent are considered to correspond to staleness and appreciable production of trimethylamine. However, it is important to compare with values obtained from satisfactory samples of the same meat.

DETERMINATION OF FREE FATTY ACIDS AND PEROXIDE VALUE

PRINCIPLE

Fat spoilage can be assessed by estimating the free fatty acids (FFA) and peroxide value on a common chloroform extract.

The FFA in ‘the sample extract is distilled with alcohol and neutralized by titrated with sodium hydroxide. The FFA is expressed as % oleic acid on the extracted fat. This

method may be used for determining FFA in lard (edible pig fat) and dripping (edible beef and mutton fat), as well as tallow and fats extracted from fresh meats.

The analysis for peroxide value depends on the reaction of potassium iodide in acid solution with the peroxide oxygen followed by titration of the liberated iodine with sodium thiosulphate. The amount of iodine liberated is expressed as milli-equivalents of peroxide oxygen per kg extracted fat. This method determines all substances which oxidizes potassium iodide under the conditions of the test. These substances are generally assumed to be peroxides or other similar products of' fat oxidation. The test may be used to determine the peroxide value of fats extracted from fresh meat.

APPARATUS

1. Erlenmeyer flasks, 250m!;
2. Filter paper, Whatman No.1 or equivalent.
3. Water-bath with temperature regulator.
4. Drying oven maintained at 100 ^{o}C $\pm$ 20 C.
5. Glass desiccator, charged with any efficient desiccant.

REAGENTS

1. Chloroform.
2. Anhydrous sodium sulphate.
3. Sodium hydroxide standard solution (0.002N).
4. Glacial acetic acid.
5. Freshly prepared Potassium iodide, saturated solution.
6. Sodium thiosulphate standard solution (O.OIN).
7. Phenolphthalein indicator, 1.0% solution in 95% ethanol.
8. Starch solution as indicator, 1% (m/v) freshly prepared.
9. Ethanol, 95% neutralized with 0.1 N sodium hydroxide using 1% phenolphthalein solution as indicator.

PROCEDURE

Trim as much fat as possible from the sample. Macerate50g of the fat mechanically with 200m I chloroform, filter. Re-filter through a paper containing anhydrous sodium sulphate and

keep the filtrate in a stoppered flask.

Pipette 20ml of the filtrate into a tarred evaporating dish. Evaporate off the chloroform on a water-bath and then dry in an air oven at *1O0* °C for 3 hours. Cool the dish in desiccator before weighing. (This is to determine the fat content).

Determine the FFA as follows: Pipette 20ml of filtrate into a 250ml conical flask. Add 20ml of neutralized ethanol. Titrate with 0.02N sodium hydroxide solution using phenolphthalein as indicator. Shake vigorously during the titration.

Next, determine the peroxide value as follows: Pipette 20m I of filtrate into a 250m I glass-stoppered Erlenmeyer flask. Add 15ml glacial acetic acid and 0.5ml of saturated potassium iodide. Allow the solution to stand with occasional shaking for exactly I minute and then add 30ml of distilled water. Titrate with 0.01 N sodium thiosulphate adding it gradually and with constant 'and vigorous shaking. Continue the titration until the yellow colour has almost disappeared. Add about 0.5ml of starch indicator solution. Continue the titration, shaking the flask vigorously near the end point to liberate all the iodine from the chloroform layer. Add the thiosulphate dropwise until the blue colour has just disappeared.

Conduct a blank determination of the reagents daily. The blank titration must not exceed a.5ml of the O.OIN sodium thiosulphate solution.

CALCULATION

Fat content in 20ml of chloroform extract:

M = MI - M2

where: M 1 = mass in grams of dish and contents

M 2 = mass in grams of empty dish

FFA (as oleic acid on extracted fat), % (M/M):

$$\% \text{ F.F.A} = \frac{(V\ 1 \times N \times 28.2)}{M}$$

Where: V 1 = volume of mililitres of Sodium hydroxide

N = normality of sodium hydroxide

28.2 = milliequivalent weight of oleic acid (include factor of 100 for %)

(Note: *FFA* are frequently expressed in terms of acid value instead of % oleic acid. The acid value is defined as the number of mg of KOH necessary to neutralize 1 gram of extracted fat.

To convert % oleic acid to acid value, multiply the former by 1.99).

Peroxide value, milliequivalent of peroxide oxygen per kg of extracted fat:

$$\frac{(V2 \times N2 \times 1000)}{M}$$

Where: V2 = volume in millilitres of sodium thiosulphate solution used.

N2 = normality of sodium thiosulphate solution used.

INTERPRETATION

Animal fats largely consist of glycerides which are esters of the trihydric alcohol glycerol with fatty acids of various types. These acids are of the long - chain variety, having 14 to 18 carbon atoms in each chain. They may be fully saturated, such as stearic and palmitic acid, or unsaturated, such as oleic acid and linoleic acid where one or more reactive double bonds occur in the side chain.

Unsaturated fatty acids are much more unstable chemically than are saturated acids as oxidation can readily occur at the site of the double bonds causing the fatty acid chain to break down into fragments. This causes the development of rancid off-odours and off-flavours. Such oxidation is caused by atmospheric oxygen and increases with increasing temperature. The extent to which rancidity has developed in a fat is measured by its peroxide value. Most fresh beef samples give a peroxide value of 0 - 1. A value of 5 could be considered a maximum acceptable level.

Animal tissues contain enzymes called lipases which have the ability to hydrolyse fats, splitting fatty acid molecules from the glycerol molecule. The extent to which this occurs car be determined by measuring the free fatty acid content. In a good quality product, the free fatty acid content should not exceed 1.2%, expressed as oleic acid in the extracted fat.

DETERMINATION OF THE MEAT CONTENT OF A MEAT PRODUCT

Routine Analysis

The determination of the meat content of a meat product is made on the assumption that a specific lean defatted meat has average nitrogen content, referred to as the nitrogen factor. For example, lean defatted raw beef is taken to have an average nitrogen content of 3.55 percent. If a sample is found to contain 1.75 percent of nitrogen, the lean defatted

beef content is taken to be 50 percent. Addition of the amount of fat in the sample gives its total meat content.

Meat products may have to be examined for preservatives, especially S02, nitrite and nitrate. Other constituents that may have to be determined or looked for are salt, added colour, starch, cereal and other fillers, textured vegetable protein, dried milk, soya bean meal, added phosphates and ascorbic acid. Canned products should be examined for lead, tin and arsenic. The lead content of meat products such as corned beef in tins sealed by spot 50ldering may be high near the seal only. Tests that are routine for canned foods such as vacuum, water capacity of the can, headspace and drained weight (where appropriate) may also be made on canned meat products.

Bacterial spoilage due to *Clostridium* species, *Streptococcus faecalis,* etc. may be due to a mixture of poor hygiene and inadequate pickling. Analysis for salt, pH, nitrate and nitrite may be helpful.

DETERMINATION OF MEAT CONTENT

The moisture, fat, protein and ash are determined and the carbohydrate or cereal filler obtained by difference. The amount of nitrogen derived from the filler (assumed to be present herein to the extent of 2 percent, or 12.5 percent as protein) is subtracted from the total nitrogen and the rest assumed to be derived from the meat. The total meat content is then calculated from the meat-derived nitrogen and fat contents, as follows:

APPARATUS

1. Tecator Digestion System or equivalent.
2. Kjeldahl Steam Distillation Apparatus.
3. Burettes; Pipettes.

REAGENTS

1. Catalyst - 1 part sulphate plus 10 parts $NaSO_4$. Grind to powder in a mortar.

2, Concentrated Sulphuric acid,

3, Sodium hydroxide standard solution (40 %): Dissolve 40 g NaOH in water and make to 100ml.

4, 0,1 N Sulphuric acid solution.

5, 0.02 N NaOH solution,

6, Screened methyl red/methylene blue indicator:

Mix equal parts of aqueous 0.2% methyl red and 0.1% methylene blue.

PROCEDURE

Accurately weigh 2 - 3 g of muscle into the digester. Add 15 g of catalyst and 35 ml of conc. H_2SO_4 Digest until solution is clear (i.e no more visible particles of sample).

Allow digest to cool. Transfer the clear digest solution into a 100 ml volumetric flask with several rinsings of distilled water and make up to mark when solution has cooled. Pipette 5 ml and transfer to steamed-out Distillation apparatus.

Stopper funnel, add 5 ml of 40% NaOH to the funnel. Attach a 100 ml distillation receiving flask containing 5 ml of 0.1 N acid with about 6-8 drops of screened mixed indicator added. When steam reaches the condenser, slowly run down the 40% NaOH solution until the digest turns brown. Distil for 5 minutes.

Lower the distillation flask and rinse down the condenser with distilled water. Titrate the distillate with 0.02N NaOH solution to a permanent green end-point. Carry out a blank titration by titrating 5 ml of 0.1 N acid with 0.02N base.

CALCULATION

$$\text{Total Nitrogen \%} \quad \frac{(B - S) \times 1.4007 \times N}{\text{Sample Weight gm}} \times 20$$

Where: B = ml of NaOH solution used for "lank

S = ml of NaOH solution used for sample

N = Normality of NaOH

1.4007 = meq. wt. of nitrogen

20 = Dilution factor (5 ml of digest used out of 100 ml)

Crude Protein % = total nitrogen % x protein conversion factor (see table below). The nitrogen factor is the nitrogen content of lean defatted meat of the species present in the sample under analysis.

Protein Conversion Factor

Meat	Nitrogen Factor	Meat	Nitrogen Factor
Beef	3.55	Chicken, breast dark meat	3.9 3.6

		whole	3.7
Veal	3.35	Turkey, breast dark meat whole	3.9 3.5 3.65
Pork	3.45	Blood	3.2
Tongue (Ox and Pig)	3.0	Pig Liver	3.65
Ox Liver	3.45	Kidney	2.7

Total meat = lean defatted meat plus fat.

Alternatively, it may be considered that lean meat contains 10 percent of fat (interstitial). Therefore, lean meat = 1.1 x lean defatted meat and the remaining fat is regarded as free fat. The amount of interstitial fat in lean meat varies considerably but 10 percent is taken as an average for the purposes of the calculation.

The nitrogen factors apply to the fresh meat with the normal amount of accompanying water. Pressed and cured meats such as canned beef, cured pates and salami ingredients have had water removed during processing and therefore contain a higher proportion of protein. For example, a factor of 4.7 has been suggested for traditional corned beef.

Carbohydrate © = 100 - (moisture + fat + protein + ash)

Nitrogen attributable to filler = 0.02 x C

Lean defatted meat:

$$\frac{N - 0.02C \times 100}{\text{Nitrogen Factor}}$$

Meat analysis for the presence of adulteration by other protein sources (e.g. soy... flour. textured vegetable protein (TVP) which give an elevated apparent meat content has been an area of great analytical interest in recent years. Soya can be detected and quantified microscopically, using histological staining techniques. A more recent method, the ELISA technique (Enzyme-Linked Immuno-Assay) is becoming more widely accepted as a routine quantitative test for Soya Protein.

The addition of offal to meat products can be detected histologically and this method can provide a semi-quantitative assessment. Gel electrophoresis methods can be used to identify offal. Foreign meats (i.e. other meat types present in meat products) has for many years relied upon Unlenluths test which is a quantitative method for uncooked

products and is non-specific in many cases. Electrophoresis methods have become more widely used for identifying meat types.

DETERMINATION OF THE NUMBER OF BACTERIA IN MEAT

The number of bacteria in a food is determined to establish the quality of the product. The shelf-life is more or less depending on the number of bacteria, and so arc the health hazards. There are 3 principles of establishing the number of bacteria in foods:

INDIRECT METHODS By these methods we do not count the cells, but measure the result of the action of bacteria. (The amount of microbial nitrogen, utilization of oxygen, lowering of ox - red potential etc.) Since each cell has a given quantity of N_2, the total N_2 in a culture could be related to the number of cells. Likewise the growth of bacteria will lower the oxidation reduction potential in the sample. If we then add an ox-red indicator (methylene blue, resazurin) to the sample, the time until colour changes may be used as an indicator of the number of bacteria in the sample.

THE RESAZURIN REDUCTION TEST

Principle

Resazurin - - - --+ Bacteria - - - _ Resorufin - - - _ Colorless

(Blue) reduction (pink) breakdown products

Procedure

1. A Resazurin strip, moistened in clean water, is placed on the surface of a – large piece of meat (good contact needed).
2. Strip transferred back to small plastic bag, the, air pressed out, and the bag placed in darkness at 22- 23°C.
3. The colour of the strips controlled after 10, 30 and 60 minutes. (blue - violet - red violet - pink - decolouring).

Result

If time necessary for change to pink or colourless is:

• < 10 minutes:	Very poor quality
• 10.. 30 minutes:	Poor quality
• 30-60 minutes:	Acceptable quality
. > 60 minutes:	Very good quality

CULTURAL METHODS: PLATE COUNTING

The principle is that we inoculate a known amount of the sample onto PCA. We have to realize that when inoculating directly from the sample, the plates most of the times will be overgrown after incubation, making it impossible to count the colonies. We therefore have to dilute the samples. By this dilution we shall have in mind the importance of a well homogenized sample to start with, and that we by each dilution get a representative part over to the next one. Two other important things to remember are not to contaminate the sample or dilutions with additional bacteria and to keep track of the dilutions made, e.g. with which f actor the number of colonies counted on the plate in to be multiplied to find the actual number of bacteria in the sample. As we do not know the number of bacteria to start with, it is difficult to predict the appropriate dilution to obtain a suitable number for counting on the plate. We therefore make inoculation from several dilutions to get at least one which can give in the range of 30-300 colonies on the plate, which will be suitable for counting.

ASSESSMENT OF CLEANLINESS OF SURFACES

Agar Sausage Technique

This method is an impression method of assessing microbial contamination of possible meat contact surfaces in the abattoir. Sterile agar media contained in a synthetic casing is pressed against the equipment to be sampled. The agar surface picks up bacteria, which after incubation at a suitable ten1perature develop into visible colonies and appear as small spots on the media. The number of spot give a measure of the number of bacteria on the equipment sampled and this can be related to the thoroughness of cleaning. The smaller the number of bacteria the better the cleaning.

The agar sausage picks up only a proportion of the bacteria on the surface being sampled depending on the type of surface and the manner in which the sample is taken. It is not suitable for sampling dirty, wet or rough surfaces.

Equipment

Agar Sausage, Knife, Sterile petri dishes, Alcohol and Burner.

Procedure

1. Take the agar sausage in the left hand.
2. Sterilize the knife blade by dipping it in alcohol and flaming.
3. Slice the tip off the agar sausage with the sterilized knife.
4. Squeeze the agar out of the sausage to a distance of about 2cm. Be careful not to squash the agar.
5. Press the exposed end of the agar against the surface to be sampled, and hold it there with a firm pressure for
6. 5 seconds.
7. Re-sterilize the knife blade.
8. Remove the agar sausage from the surface and with the sterile knife cut off a thin slice of agar about 1/8 inch
   thick.
9. Transfer the slice, on the blade of the knife, and lay it in the sterile petri dish exposed side uppermost.
10. Incubate the petri dishes in an enclosed spot in a warm room until the petri dishes have grown enough to form visible
    colonies.
11. Count or estimate the number of colonies on each slice. A magnifier is a useful aid in counting.

Results:

++++ 4 slices greater than 100 colonies

+++ 3 slice greater than 100 colonies

++ 2 slices greater than 100. colonies

\+ 1 slice greater than 100 colonies

All slices less than 100 colonies

0 Not examined.

Cleaning has been satisfactory when all slices have 100 or fewer colonies

Remember - sterile conditions must be adhered to throughout the technique, otherwise the results will be meaningless and misleading.

DIAGNOSIS OF *BACILLUS ANTHRACIS*

Bacillus anthracis is a spore-forming, aerobic bacillus, Gram + rods in chains. When exposed to air they form equatorial oval spores, but do not form spores in vivo (blood smear). They form capsules in the organism or If grown on 50 % serum agar in 50 % CO_2 atmosphere. It is the only species in genus Bacillus that is non-motile.

In most inspection an anthrax case may be seen as:

(a) Dead animals on transport arrival

(b) Dead animals in the lairages

(c) Pharyngeal abscesses in swine.

The diagnosis is established by:

i) Direct microscopy of blood smear or organ samples (always done when suspects are found),

ii) Cultivation - characteristic appearance,

iii) inoculation of material intraperitoneally into mice. Mice will die within 3 days. Sub-culturing is done.

iv) Serology (Ascolis thermoprecipitation test).

Great Care Should Be Taken When Suspect Material Is Handled!!

When an anthrax case is diagnosed, the carcass and other material should be burned or deep buried with lime on top. If material is to be transported care should be taken not to contaminate during transport.

DETERMINATION OF ANTIBIOTIC RESIDUES IN MEAT

In meat inspection we might sometimes suspect that an animal has been treated with antibiotics or sulphonamides prior to slaughter. In meat inspection we might sometimes suspect that an animal has been treated with antibiotics or sulphonamides prior to slaughter. The residues (i.e. the parent drugs and their metabolites) of veterinary drugs and pesticides in meat have public health consequences thereby require monitoring in order to prevent the entrance to food chain the level above the international maximum residue limits (MRLs) standards which are set by joint FAO/WHO codex alimentarius committee. Several tests classified into screening and confirmatory methods are employed to detect residues in meat, milk, egg and honey, screening methods involve fast and less expensive equipment and procedures. Microbiological assays are commonly

used for screening of antibiotics working on the principle of inhibition of growth of bacteria seeded in test agar. A more rapid screening microbial assay test developed by DSM Foods, Netherlands called Premi®Test a commercially available kit containing agar pre-seeded with *Bacillus stearothermophilus,* which sensitive to most commonly used antibiotics providing a simple yes or no response to presence of antibiotics shall be demonstrated.

(A) Premi®Test Materials and Methods

Meat samples, Petri dish and DSM Premi®test kit containing meat scissors, meat presser, micro-pipette, pipette tips, ampoules of agar pre-seeded with *Bacillus steareothemophilus var calidolactis*, incubation blocks, thermometer timer and distilled water.

Steps

1. Cut the required number of ampoules with a pair scissors: be careful not damage not to damage the foil of the remaining ampoules.
2. Take approximately $2cm^3$ of lean meat and use a meat press to extract 250ul of meat juice.
3. Pippete 100ul of meat juice slowly unto the agar in the ampoule.
4. Allow the ampoule to stand at room temperature for 20minutes for prediffusion.
5. Flush the meat juice away by washing twice with distilled water.
6. Close the ampoule with a foil, insert into a preheated incubator block maintained at 64^oC.
7. Withdraw the ampoule from the block heater after 3 hours of incubation and observe for colour change

NB: Colour change (yellow) indicates negative for antibiotic residues
No colour change (pink) indicates positive for antibiotic residues

(B)The principle of the test is that .if some of these substances are present in the tissue for example kidney, it will prevent growth of bacteria when placed onto an agar surface in which a test organism is inoculated.

Several media .may be used for this purpose: Plate count agar; Blood agar; Muller Hinton agar.

The last one is especially fitted when sulphonamides are suspected due to low content of para-aminobenzoic acid and folic acid as these substance acts antagonistically towards sulfonamides.

PROCEDURE

The most used test organism is *Micrococcus luteus* (Sarcina lutae).

1. From a fresh culture of the test organism, 2-3 typical, pure, colonies are transferred to a tube with about 10 ml. sterile physiological saline and mixed by shaking.
2. To 100ml of melted Muller Hinton agar cooled to 45 ^{0}C is added 0.5 ml of the test organism suspension and mixed well.
3. From kidneys to be tested a piece (1.5 x 1.5cm) is cut both from cortex and medulla. The pieces are placed tight on the agar surface with a space of at least 3cm between each piece and l.5cm, from the edge of the plate.
4. The plates are stored in refrigerator for 2 hours to allow diffusion of growth inhibiting substance into the medium before incubation at 37°C for 18-24 hours.

Result:

If inhibiting substance is present in the kidney piece, there will be a zone free of growth of at least 1.5mm around the piece of kidney. Sensitivity of the test is 0.01 LU. Penicillin when *Micrococcus luteus* is used.

ACID-FAST BACILLI TEST FOR TUBERCULOSIS IN MEAT

Certain organisms, particularly the Mycobacterium (M. tuberculosis, M. bovis, M. avium. lepra,. M. para-tuberculosis and others) are able to resist the anilin dyes that are commonly used for staining of bacteria because these bacteria contain large (74%) amounts of lipid material that hinder the absorption of these dyes. In order to stain these bacteria, the bacteria have to be exposed to the stain for long periods (hours) or heating could be used.

When they then have accepted the stain, they are not easily de-stained, and even strong mineral acids will not decolorize the bacteria, giving the description “acid-fast bacilli”.

For smears the following solutions are to be used:

i) Carbol fuchsin ii) Basic Fuchsin 1 g

ii) Absolute alcohol 10 g.)

iii) Phenol Solution 5% 100g. (Dissolve in absolute alcohol at 40°C for

iv) 24 hours before addition of phenol-solution.

v) Sulphuric Acid (20%)

vi) Solution of malachite green. 0.5% in water (or of methylene blue 0.5%).

PROCEDURE

1. Fix smear by heat.
2. Flood the slide with carbol fuchsin using a piece of filter paper to hinder evaporation, heat over Bunsen flame, reheat at intervals and stain for 10 minutes. Do not let the slide dry add more stain If necessary.
3. Wash in running tap water.
4. Pour 20% Sulphuric acid on the preparation and leave for 15-30 seconds.
5. Repeat till the preparation has got a reddish colour—10 minutes.
6. Wash in running tap water.
7. Counter-stain with malachite green for 1minute.
8. Wash in water.
9. Blot dry and examine in microscopy using oil immersion.

EXAMINATION FOR LARVAE OF *TRICHINELLA SPIRALIS*

Adult Trichinella are found in the small intestines, usually the ileum of mammals, which have recently eaten trichinosed flesh. The larvae are liberated from their cysts by the action of the gastric juice, develop into sexually mature adults in the intestine, and copulate within 2 days.

The females have duration of life of some 5-6 weeks, and penetrate the mucous Inen1brane of the intestine where they give birth to about 1,500 larvae, which at this stage are 0.1 mm in length.

The larva is spread from the thoracic duct through the arterial system and undergoes further development in the skeletal muscles, especially muscles poor in glycogen. Here they penetrate the walls of capillaries and enter the muscle sarcolemma 7-8 days after the infected meat is ingested. They are actively motile, grow to 4 to 8 times their original size, and curl up within the muscle fibre in a typical spiral coil. In about 3 weeks after infection the embryo are about 1 mm long and the growth ceases.

PROCEDURE

1. One piece of muscle of about 30 gm shall be taken from the thick fleshy part of the diaphragm.

2. Using scissors cut 12 grain-sized pieces longitudinally to the muscle fibers as near as possible to the tendon and placed in a compression glass for examination under the microscope.
3. In case Trichinella is found, six new samples should be taken from different parts of the pig and examined in the same way.
4. In case salted or smoked samples shall be examined, they shall first be put in boiling hot water for 20 minutes and shall be treated with a 2-5 % solution of sodium hydroxide immediately before examination.

VIABILITY OF PARASITES IN MEAT

Certain treatments (temperature, salting - etc.) are in many countries allowed or enforced by law to render moderately parasites infested *meat* safe *for* consumption.

Best known is the low temperature (freezing at – 10^0C *for* 10 days) to treat meats with *Cysticercus bovis* and *Trichinella spiralis.* In Kenya no freezing of Trichinella infested pork, as this will be condemned, which also is the case with *Cysticercus cellulosae* infested pork. To make sure that the required treatment is performed satisfactorily the viability of parasites present should be controlled.

1. **Viability of Cysticerci** (C. *bovis* - T. *saginata*; C. *cellulosae*- T. *solium*)

(a) Carefully remove cysts from meat to petri dish.

(b) Add a saline solution of 30% ox bile, or a saline solution of 5% sodium taurocholate.

© Incubate at 37°C for 2 hours, cysts are considered dead if there is no evagination of the scolex within 2 hours.

2. **Viability of Trichinae**

a. Place 100 grams of ground pork in a glass container.
b. Add a mixture of 1,000ml water, 10 ml HCl and 1.4 grams Pepsin.
c. Incubate *for* 24 hours at 37°C.
d. Fitter through linen filter to remove undigested parts.

e. Centrifugation of filtrate. Microscopy of sediment to find live, free larvae of *Trichinella spiralis*.

BRUCELLOSIS AND TUBERCULOSIS DIAGNOSIS LABORATORY PROCEDURES

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Brucellosis diagnosis

a. Rose bengal test (rbt)

A drop of the test serum is taken using a clean Pasteur pipette and placed onto the test plate beside an equal drop of RBPT antigen added using another clean Pasteur pipette. This is then mixed well using a sterile applicator stick. The mixture is now rocked manually for 4 minutes before examination. The presence of distinct pink granules (agglutination) is recorded as positive result while samples that appeared clear without agglutination granules are recorded as negative.

b. Milk ring test

Milk samples stored at 4°C for at least 2 hours are removed from the refrigerator and left at room temperature for 1 hour before setting up the tests.

The milk in the sample tube is gently mixed to ensure even distribution of the cream. 1.0 ml of the milk is the placed in a plastic "agglutination" tube to give a column of milk about 2 cm high. One drop (O.03ml) of the MRT antigen is added to tl1e milk holding the dropping pipette vertically. Then, the contents are gently mixed within one minute of adding the antigen and incubated at 37°C for 1 hour.

Readings are recorded as follows:

Colour of Cream Ring	**Colour of Milk Column**	**MRT Reading**
Definitely Blue	White	+++
Definitely Blue	Slight Blue	++
Definitely Blue	Definitely Blue	+
Slightly Bluer than or same as the milk column	Definitely Blue	+/-

White	Definitely Blue	--

Tuberculosis diagnosis

specimen processing for tuberculosis diagnosis

Using a sterile, 50 ml centrifuge tube with a screw cap, equal amounts of specimen (e.g. sputum or homogenized tissue) and activated NALC (N-acetyl-L-cysteine)-NaOH of about 5 ml each are added. The centrifuge tube is capped and mixed on a vortex-type mixer until the specimen was liquefied. The mixture is allowed to stand at room temperature for 15 min with aoccasional gentle shaking. Prepared phosphate buffer is then added to the 15 ml mark on the centrifuge tube and mixed, followed by centrifugation for 15 to 20 min at 3,000 x g. The supernatant is carefully decanted, and 2 ml of phosphate buffer of pH 6.8 is added to re-suspend the sediment.

Microscopic examination: The sediments are smeared on glass slides (a sample each per slide) and stained using the Ziehl Neelsen staining technique for the detection of acid fast bacilli after decontamination and digestion as described above.

LABORATORY TEST TO DETERMINE THE QUALITY OF MILK
500level posting
Standard Plate Count Method

This test can be used to determine the quality of milk and other liquid food items.
Equipment and Supplies

(a) Very clean dust-free rigid support well lighted and well ventilated work area.
(b) Storage space – cabinets, drawers or shelves free of dust and insects
(c) Glass wares apparatus, measuring pipettes, dilution bottles, plates, test tubes, petri dishes
(d) Thermometers, Incubator
(e) Water bath, sterilizer, and hot-air-oven
(f) Colony counter and Tally.
(g) Agar and Culture media

Procedure

Milk samples to be examined are first serially diluted before plating on culture media to ease counting of colonies, which other wise may be too many to count (TMTC)

Serial dilution is done by using several test tubes each containing 9ml of sterile physiological saline solution (PSS). One milliliter of the liquid milk sample is added to the first tube and mix thoroughly for this mixture.

Pipette 1ml and transfer to the next tube of 9ml of saline and mix, 1ml of this in second tube is again transferred into the next tube and exercise continues to the maximum dilution desired. These are then inoculated on the culture media thus.

1ml (1) (2) (3) (4) (5) (6) (7)

(0)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
	.1	.01	.001	.0001	.00001	.000001	.0000001

The culture media used could be any of the following MacConkey agar; Brilliant green agar, Nutrient agar, Sabourand dextrose agar (SDA)
There are two methods of inoculation of sample onto culture media

Pure plating Technique

Procedure
In the pour plating techniques, first prepare the media (according to manufacturers prescription), e.g. MacConkey, by weighing 26gm of MacConkey powder, add 500ml of distilled water and boil completely until dissolved. Autoclave the media at 121^{o}C for 15 mins and keep in refrigerator until the need arises. At the point of use keep the media in a hot water bath at 55^{0}C. One milliliter or 0.1ml of the serially diluted milk samples are first measured into sterile Petri-dishes followed by the addition of molten media, mix both the milk and media gently allow to cool with the cover placed to avoid contamination. Then incubate at 37^{0}C for 24 hours.
Advantages and Disadvantages
In the pour plating technique, there is an increased chance of detecting bacterial organism due to higher volume of sample (1ml) used especially if the bacterial load is low. Also both aerobic and anaerobic bacterial would grow by this method. The disadvantages are that fragile organism may be destroyed by the hot media. Colonies may be difficult to count in the solid media and the morphologies of colonies may be distorted especially those at the underneath of media.

Surface Plating Technique
In this technique, the media is first poured and allowed to solidify. Then the serially diluted milk samples (0.1 – 1ml) are poured, evenly spread out by rotating the culture plate or dish and then incubated at 37^{0}C for 24 hours.
Advantages and Disadvantages
In the surface plating techniques, the fragile organisms can grow. Typical colonies appear well expressed. The disadvantages include the chances of high degree of contamination, which may occur during cooling of media, and contamination would prevent easy detection of the bacterial colonies.

Colony Counts and Interpretation
Growths on culture media are enumerated with colony counter and tally and the counts expressed in colony forming units per ml (CFU/ml) of the diluted sample under consideration.
For example, if in pour method, 1ml of the 10^{-4} dilution yielded (8) eight colony forming units after incubation, then the number of bacterial organisms in that sample is 8 x 10^{4} CFU/ml. Similarly, if by the surface plate method using 0.1 ml of the dilution 10^{4} then the dilution on the plate is 10^{5}. If colony count is 8, the sample therefore contains 8 x 10^{5}CFU/ml.

California Mastitis Test (CMT)

This text is designed primarily to detect cows with mastitis and it is usually performed at the side of the animal. However, CMT can also be applied to ordinary milk supplied for sale at the collection centre.

Requirements:

CMT paddle, reagent and milk sample. The reagent consists of a detergent (Alkylaryl sulphate) and a pH indicator (bromocresol purple). The detergent reacts with the DNA of the somatic cells in milk to from a gel while the pH indicator detects whether milk is acidic (yellowish) or alkaline (deep purple).

***Procedure*:**

Equal volume of milk and reagent are added to each of the four compartments of the paddle from each quarter. The paddle is then rotated and swirled for proper mixing. The mixture is observed for colour change, viscosity, and gel formation.

Methylene *Blue Reductase Test*

The methylene blue reducates test (MBRT) can be conducted to detect the presence of reductase enzyme, which affects the keeping quality of milk.

The higher the bacteria load in milk, the more the enzyme is produced Milk kept overnight for example could be detected by this test, as more enzymes would have been produced.

The test can also be used to grade milk especially at the receiving station, condensing plants and cheese factory. MBRT is a very simple test that requires small amount of equipment and materials. It also permits simultaneous testing of several hundred samples since it combines speed and adaptability. The indicator dye is a solution of methylene blue thiocyanate (MBT) which is even available commercially in tablet forms.

Procedure: Three test tubes are set up as follows:

(a) Positive Control – Add 10ml of known positive milk plus 1ml of MBT dye.
(b) Negative Control – Add 10ml of distilled water plus 1ml of MBT dye.
(c) Test Sample – Add 10ml of test milk sample plus 1ml of MBT dye.

Incubate the tubes in water bath at 37^{o}C for 1 hour during which the colour changes. Observe the changes at regular intervals.

Results and Interpretation

Complete reduction in15minutes – Very bad milk
Complete reduction in 30-60 mins - Bad milk
Complete reduction in 12-2 hrs - Poor milk
Complete reduction in 3-4 hrs - Doubtful milk
Complete reduction in 42-6 hrs - Good milk
Complete reduction after 6 hrs - Very good milk

Production of bacteriocin like substance by Lactic acid bacteria (LAB) isolated from milk

Preparation of culture

Lactic acid producing organisms will be sourced from milk samples. Samples will be inoculated on man de Rogosa Sharpe (MRS) agar and modified (MRS) M17 agar for isolation of Lactic acid bacteria. Biochemical tests (catalase, oxidase, coagulase, urease, citrate utilization, indole, starch hydrolysis and growth in 2% NaCl) and microscopy will be used to confirm the species. Identified isolates will then be stored in glycerol at -20^{o}C and subcultured thrice before use for this test.

Test for inhibitory quality of the *Lactobacillus* and *Lactococcus spp*

The test for inhibitory quality will be done against pure cultures of *E. coli L. monocytogenes (Pal et al*, 2005).

Antimicrobial effect of LAB

Mueller Hinton II Agar base for antimicrobial disk diffusion susceptibility testing (Becton, Dickinson and Company Sparks, MD 21152 USA) will be prepared according to manufacturers' prescription (30g/liter of purified water).

Preparation of test culture

A 24hr fresh culture of the test strains (*E. coli* and *L. monocytogenes*) will be prepared in tryptose soy broth by inoculating 24hr culture of an appropriate agar plates into tryptose soy broth and incubated at 37^{o}C for 24hrs. Each Mueller II Agar plate will then be seeded with 0.1 ml (10^{6} - 10^{8}cfu / ml) of culture evenly spread. The inoculum will be standardized by plating 1ml diluted and undiluted cultures on appropriate media to obtain the desired inoculum level. The seeded plates will be left to dry. Twelve wells will be made on each plate specifying a positive control (acetic –nitric acid (150ml 80% acetic acid and 15ml conc. Nitric acid) and a negative control (sterile water) and test samples of 10 strains of the LAB. The 12 wells will then be inoculated appropriately with 2-3 colonies of LAB by using a wire loop. Ten microliter of acetic-nitric acid and sterile water will be inoculated for positive and negative controls. The plates will be left to dry slightly and then incubated at 37^{o}C for 24hrs. LAB positive strains showing inhibition zone of at least 8mm will be regarded as positive. These positive strains will be used for further bacteriocin production study.

Test for bacteriocin production

A 24hr fresh culture of the LAB positive strains grown on M17 agar will be inoculated into tryptose soy broth. This will be incubated at 37^{o}C for 24hrs. The 24hr broth culture will then be centrifuged at 3000rmp for 5minutes. The supernatant will be decanted into sterile test tubes. This supernatant will be adjusted to 6.5 - 7pH with NaOH (40G/1000ml) to remove the organic acid effect. Effect of H_2O_2 was removed by addition of catalase from bovine liver at 200µ/ml. The mixture of the supernatant, NaOH and catalase will then be filtered for sterilization with a 0.45αm millipore filter membrane. Inhibitory effect of free bacteriocin on test bacteria will then be determined

by agar well diffusion method using the filtrate from the mixture and the supernatant alone.

Mueller Hinton II Agar plates will be welled with sterile clubs into 8mm diameter. The bottom of each well will be leveled with viscous agar which will be allowed to solidify to prevent spreading of the mixture and supernatant through the base. The wells will then be filled with 20µl of the mixture and filtrate of supernatant alone and refrigerated for 2hrs to allow diffusion of test substrate. Incubation will be for 24hrs at 37^{o}C. The diameter of inhibition will then be measured with ruler.

ZOONOSIS CLINICS
500level posting

INTRODUCTION
The following zoonoses are systematically considered for clinical discussion because of their prevalence and socio-economic importance to both animal and human population.
VIRAL
Rabies
Avian Influenza

BACTERIAL
Tuberculosis
Brucellosis
Leptospirosis

PARASITIC
Taeniasis
Cysticercosis

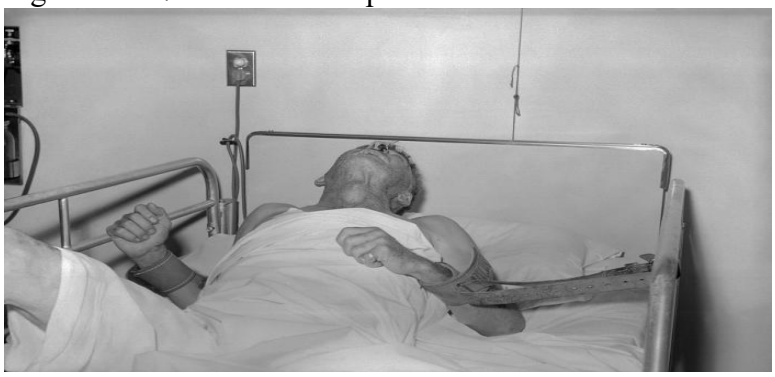
FOOD AND WATER BORNE ZOONOSES

Salmonellosis
Escherichia coli (O157)
Toxoplasmosis

APPROACHES
Hypothetical cases are presented for clinical discussion with a view to bringing to bear the Host-Vector-Environmental triad; the reservoirs; route and mode of transmission; diagnosis; treatment; Prevention and Control. Relevant pictures of typical cases if available are also shown.

RABIES

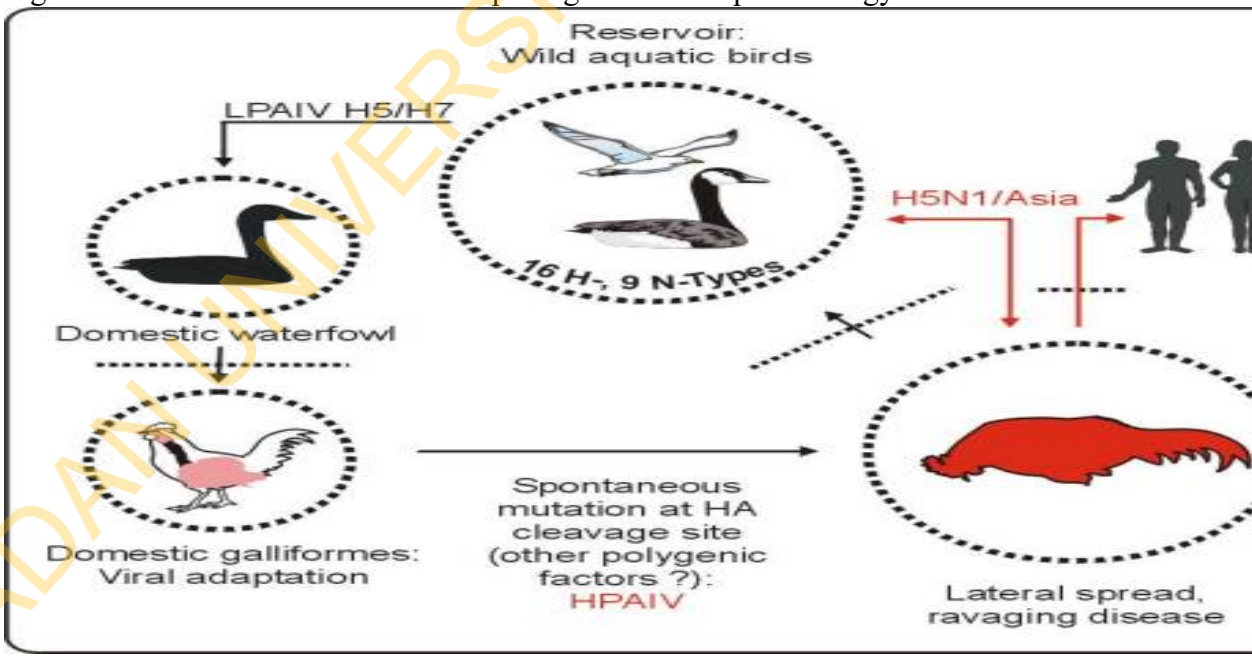
Figure 17: A hospitalized human rabies victim in restraints.



Description: Rabies in humans is almost always fatal. Symptoms may be headache, fatigue, fever and pain at the site of the bite can be present. Behavioural changes like apprehension, anxiety, agitation, irritability, insomnia and depression may also appear.
Provider: CDC

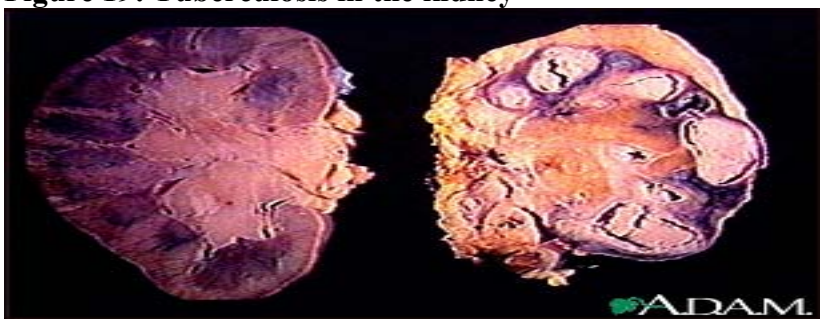
AVIAN INFLUENZA

Figure 18: Scheme of avian influenza pathogenesis and epidemiology



TUBERCULOSIS

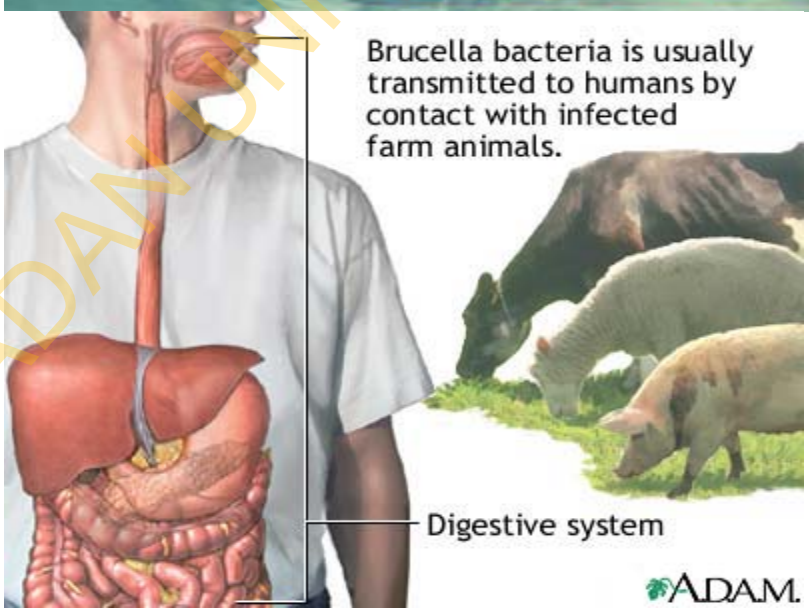
Figure 19: Tuberculosis in the kidney



Kidneys can be damaged by tuberculosis. Tuberculosis generally affects the lungs, but may cause infection in many other organs in the body.
(Image courtesy of the Centres for Disease Control and Prevention)

BRUCELLOSIS

Figure20: aborted foetus from an infected animal



LEPTOSPIROSIS

A loving lick to the face from the family dog may be a welcome feeling, but it could carry a number of pathogens that are dangerous to humans. The bacterium, *Leptospira*, can cause the infectious – and potentially deadly – disease leptospirosis. *Leptospira* lives in its host's kidneys, and once infected, dogs show a variety of symptoms that can range from lethargy and depression to vomiting, kidney failure or even death.

Figure 21:A traditional show of loyalty and affection between this man and his best friend could actually be harmful. Researchers are finding an innocent lick to the face could unwittingly spread leptospirosis, a re-emerging disease that can cause kidney failure or even death in dogs, and flu-like symptoms in humans.

Photo: David Fieldhouse

TAENIASIS

Figure 22: **Schematic diagram of the mode of infection of taenia solium in man**

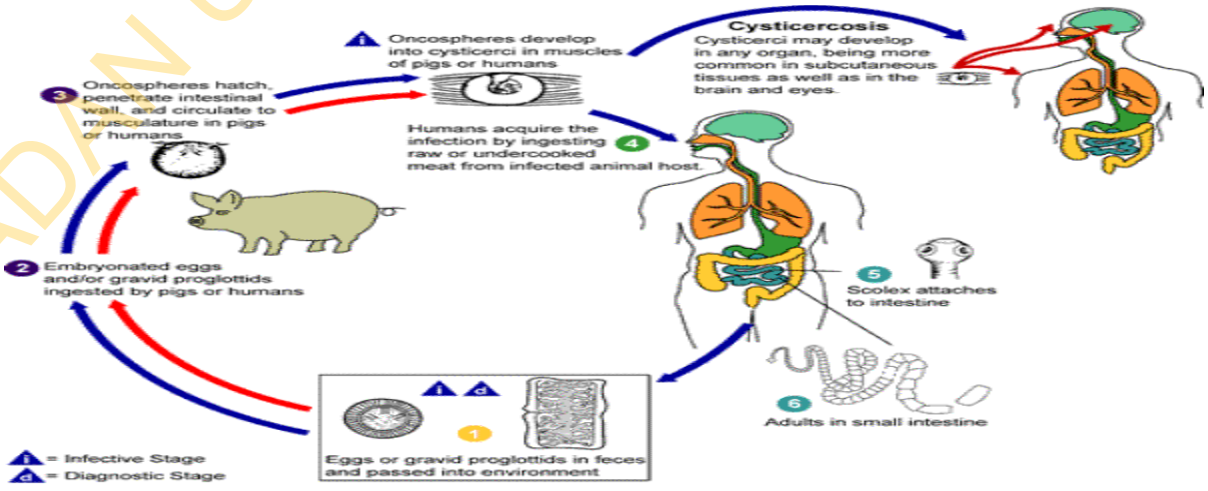
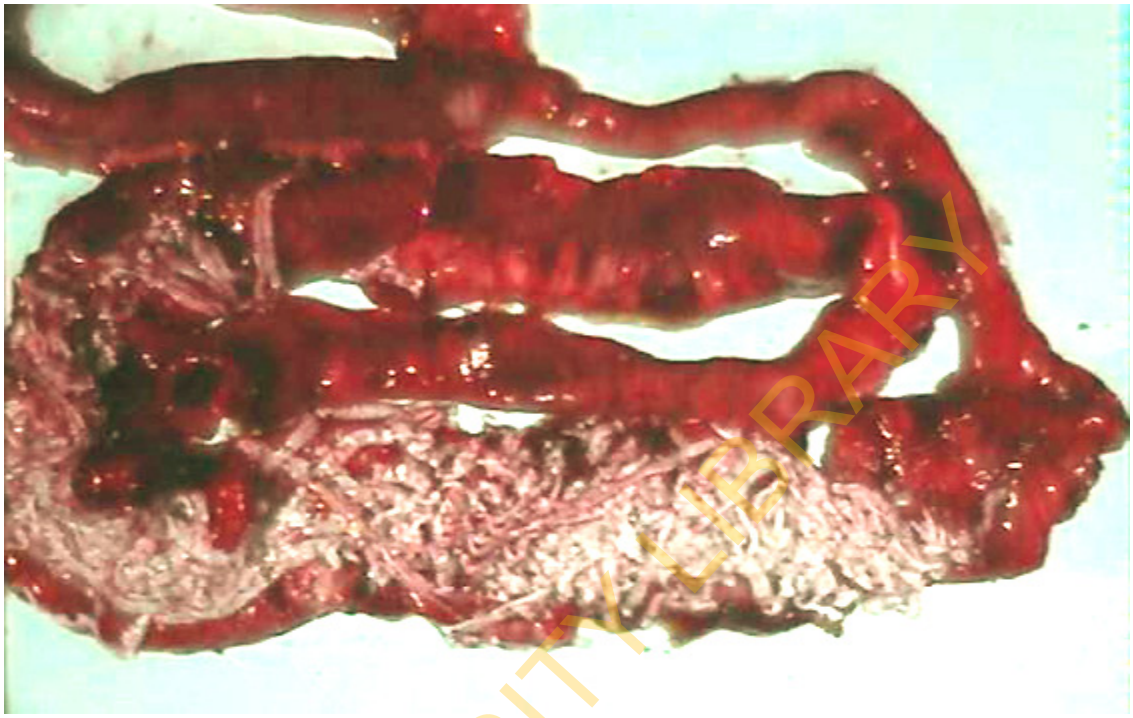


Figure 23: Schematic diagram showing the mode of infection of taenia solium



Gross picture of an intestine laden with adult taenia worms

CYSTICERCOSIS

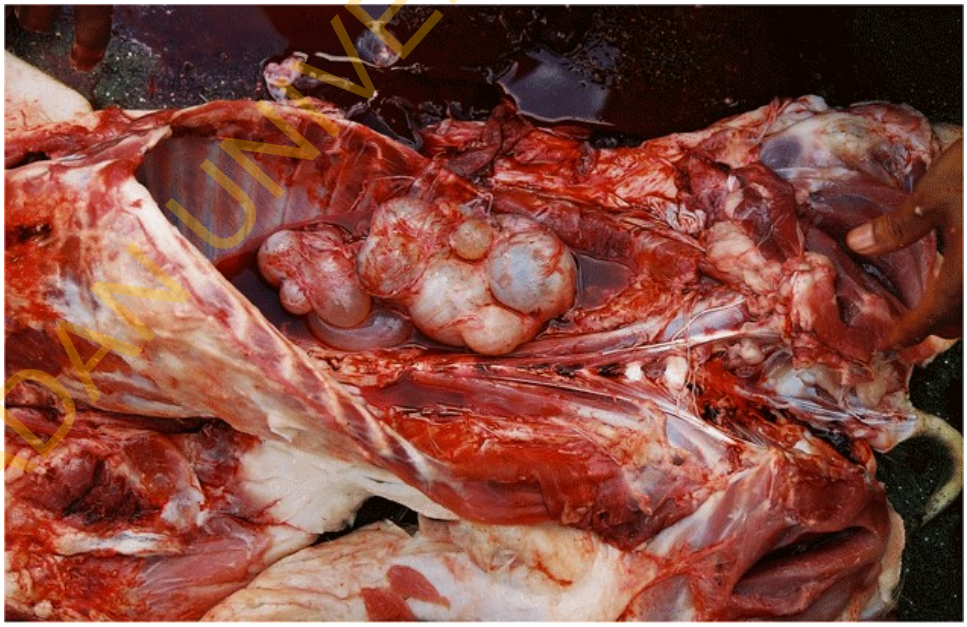


Figure 24: Gross picture of a carcass showing taenia cyst

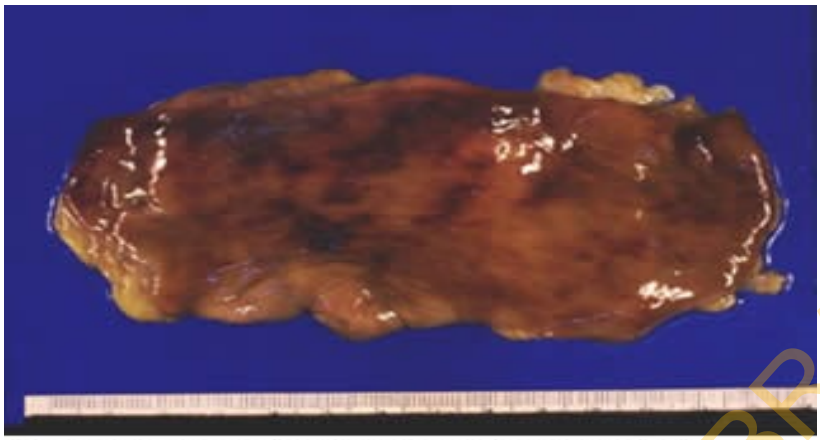
SALMONELLOSIS

Figure 25*:* Salmonellosis in poultry

E. coli 0157

Figure 26: Intestinal wall showing bleeding caused by verotoxin from *E. coli*

A



Piece of intestine wall (pathologic specimen) showing bleeding caused by verotoxin from E. coli. Source:

B

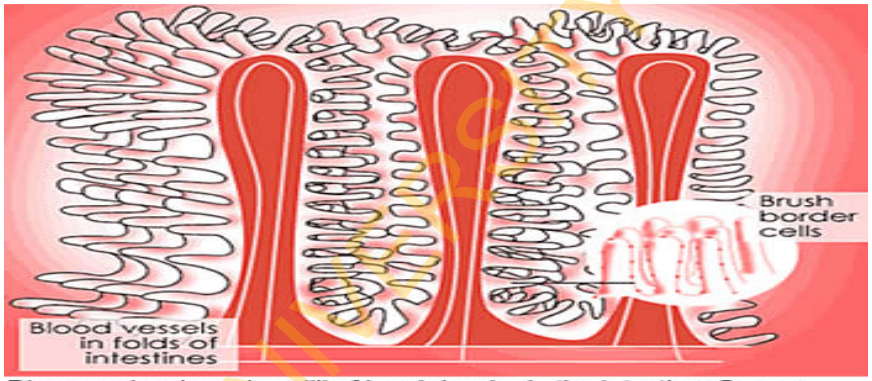


Diagram showing microvilli of brush border in the intestine. Source: http://img.sparknotes.com/figures/9/92fefe6f9160757a6207a120b3637c38/figure4.gif; accessed October 1, 2006.

C

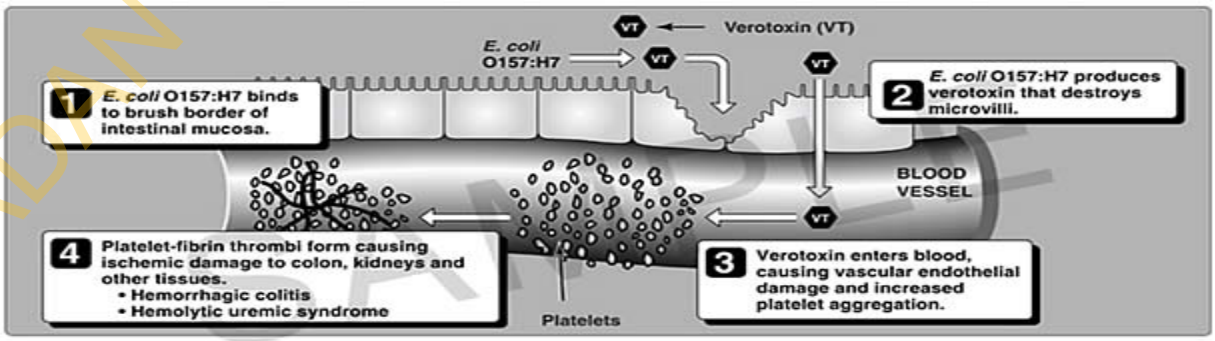


Figure 15.4
Pathogenesis of *E. coli* O157:H7 infection.

Pathogenesis of poisoning by VTEC. Source: http://connection.lww.com/Products/strohl/documents/SampleIB/jpg/F0001-15-04.jpg; accessed October 1, 2006.

TOXOPLASMOSIS

Figure 27: Sources of infection of the mother

Figure 28: Girl with hydrocephalus due to congenital toxoplasmosis. (From Dubey JP, and Beattie CP. Toxoplasmosis of animals and Man. CRC Press, Baca Raton, Florida, 52, 1988)

Environmental Health Laboratory Work
600level posting

Progress towards bringing about a cleaner environment has relied on a philosophy of pollution control. This has involved sometimes costly measures and controversial political decisions. As a result, developing countries, poor communities and financially constrained enterprises have often argued that the environment is an expensive luxury that diverts resources from more productive uses. This perspective is giving way to a new paradigm stating that neglecting the environment can impose high economic and even financial costs, while many environmental benefits can in fact be achieved at low cost (World Bank, 1998).

In this lab-work we attend to four issues namely:

1. Determination of the quality of wastewater on animal farm and abattoir in view of their management by recirculation method
2. ENVIRONMENTAL HEALTH CLINICS
3. Determination and analysis of selected risk factor on farm animal health in view of their spatial distribution and appropriate biosecurity profile to control them
4. Control of pests of farm and companion animals by fumigation: Assessment of their efficacy and environmental residual effects
5. Basic techniques in serological diagnosis and mapping of infectious animal disease: A case study of Rabies

Practical Exercise 1
Determination of the quality of wastewater on animal farm and abattoir in view of their management by recirculation method

Background

Water is an essential component of farm and abattoir operations. It is important to plan for water quality and quantity to ensure health of animals, wholesomeness of meat and cleanliness of the environment. In the process, used water needs to be disposed and eventually re-circulated. Used water is here regarded as wastewater. Wastewater from the farm is farmyard wastewater. Wastewater from the abattoir is abattoir wastewater.

Skills to develop

In this exercise the physico-chemical and patho-bacteriological qualities will be assessed. Students would exercise their sense of smell, sight, and touch to describe odour, colour and texture of samples provided respectively. Students will carry out filtration to determine suspended solids and various chemical tests of emitted gases and solids formed.

Source of Sample

The University of Ibadan Teaching and Research Farm and Bodija Municipal Abattoir are two locations from which materials for this laboratory work may be collected.
Equipment

1. Universal bottle - 30
2. Collection bucket - 5

3. Hand gloves - 50
4. Funnel - 5
5. Nose/Mouth cover - 50
6. Conical flask - 5
7. Beaker - 5
8. Filter paper - 100
9. Desiccator - 5
10. Weighing balance - 1
11. Glass slide - 100
12. Bunsen burner - 5
13. Inoculation hood - 1
14. Microscope - 5
15. Autoclave - 1

Chemical reagents

1. Lead acetate paper - 50
2. Concentrated HCl - 10ml
3. Detergent
4. Disinfectant
5. Giemsa stain
6. Methanol

Biological reagents

1. Nutrient agar
2. Nutrient broth
3. Sheep blood

Collection of Samples

Collect ten samples daily at hourly specifications with universal bottles. Each sample should be 20mls in volume. Of these, two samples should be collected before use, and three samples collected during slaughter. Two samples should be collected 3 hours after slaughter. Three samples should be collected 9 hours after slaughter. Daily samples should be collected in this way on 5 non-consecutive days. Thus a total of 50 samples of wastewater should be collected for laboratory assessment.

Assessment of physico-chemical properties

On each of these samples, immediate physico-chemical parameters assessment (smell, colour, texture, filtration for suspended solids) were conducted. The samples were then kept on the shelves for 2 weeks after the date of collection. Physical changes at the end of 2 weeks on shelf were determined in the same way as on the fresh samples.

Assessment of patho-bacteriological content

Prepare nutrient broth and inoculate with 0.2ml of each sample collected. Incubate at 37^0C for 24 hours. Prepare a total of 100 plates of Nutrient Agar. Inoculate the prepared broth and nutrient agar with pre-use water and wastewater samples collected. Thirty (30) plates prepared a day. Ten samples should be inoculated (into 20 Nutrient and blood agar plates). Place the inoculated plates into the incubator for four days. Colonies of bacteria that grow on the plates should then be observed visually and their physical characteristics described as they appear on the plates. A total of 150 glass slides smears should be made

from the colonies. The slides should be stained with Giemsa stain and examined under microscope.

Records of findings

Record your findings in the table below. Results obtained may be used to explain the state of environmental pollution in Bodija-Agbowo stream, Ibadan.

Table 1: Physico-chemical properties of abattoir wastewater

Specimen	Colour	Dissolved solid (mg/ml)	Odour	Colour after 2 weeks on shelf	Odour after 2 weeks on shelf	Chemical Inference	Pathogenic bacteria

Further studies

Visit a treatment facility for wastewater adjacent to the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan and discuss its mode of operation. How does this procedure remove solids and gases that you identified in the lab?

Note for Further Discussion

Water pollution has continued to generate unpleasant implications for health and economic development in Nigeria and the third world. Despite policy focus of some public and international agencies on this problem, the situation in Nigeria seems degenerating and therefore demands increased attention. Water Engineering and Development Center in Edinburgh, United Kingdom published papers on *People and Systems for Water, Sanitation and Health* in 2001 and the case of wastewater quality in Bodija, Ibadan was provided by (Coker *et al*, 2001). In 2005, Adelegan designed a protocol for converting cattle waste into bio-gas at Bodija, Ibadan called *Cow to Kilowatt*. His design won a grant from the United Nations Development Project to implement the same, worth USD500,000:00. This has not been actualized yet and liquid and solid wastes in Bodija abattoir continue to attract local and international attention.

Practical Exercise 2

Determination and analysis of African swine fever risk factors on pigs in view of spatial distribution and appropriate biosecurity profile

Background

Nigeria was free of African swine fever (ASF) until 1997. At present ASF is an endemic disease in some parts of Nigeria. The continuous presence of recovered pigs in the population enables virus spread through trade and breeding. It is important to describe the distribution of pigs that have recovered from ASF, since these have been shown to serve as carriers of the virus and to investigate the extent of contribution to other risk factors such as ticks, rodents, birds and personnel (farmer/worker, visitors and stock-buyers).

Skills to develop

In this exercise students will visit pig farms to collect ticks and pig whole blood samples. They will administer questionnaires on farm biosecurity and use the GPS to collect geographic coordinates of locations visited. Students would be put through the art of preparing tissue culture for virus isolation.

Source of Sample

Pig farms in Ibadan that are owned by members of Pig Farmers' Association of Nigeria.

Equipment

1.GPS
2. Medium with Hearle's solution
3. Needle and syringe
4. Bijou bottles
5. Tissue culture flasks
6. Cotton wool
7. Restraint rope
8. CO_2 Incubator
9.Centrifuge tube
10.Refrigerator
11.Light Microscope
12.Cryovals
13.Centrifuge
14.EDTA bottle
15.Liquid Nitrogen Refrigerator

Biological reagents

1. Minimum Essential
2. Methylated spirit
3. Phosphate Buffered Solution (PBS)
4.Normal swine serum
5. Normal Bovine serum
6. Amphotericin B, Fungisone, Penicillin, Streptomycin and Neomycine

Experimental animals

1. ASF virus-free pigs

Collection of blood

Collect 4-5ml of blood from each pig through the anterior vena-cava into an EDTA bottle. Use immediately for gradient centrifuge to extract swine leukocyte in Ficoll Paque.

Tissue culture procedure

Constitute a mixture of Minimum Essential Medium (MEM) with Hearle's salt and Normal swine serum as specified by instructor. Inoculate leukocytes that were harvested from Ficoll Paque centrifugation above into the MEM and incubate in CO_2 Incubator at

38^0C. Examine the culture daily for growth performance. Harvest and store the Swine leukocyte culture into cryovals placed in Liquid Nitrogen Refrigerator.

Farm visit and geographic coordinates recording

On the farm, the use of a GPS (Magellan 360) will be demonstrated. Students will practice how to discussion effectively with farmers and collect potential ASF risk agents, including ticks and rodents. A group of students will administer a questionnaire on farm biosecurity practice on the farm.

QUESTIONNAIRE ON FARM BIOSECURITY

Serial no. _ _ _ _ _ _ _

Please, we shall be delighted if you could willingly fill in this questionnaire. All information and identity given shall be treated with utmost respect and confidentiality. Thank you.

Name of Farm:_____Address:_____
State, Town and Local Govt. Area: _____Tel_____
Name of Owner: _____Age: _____ Sex: _____
What is your highest Educational level? No formal education [ ] Functional literacy [ ]
Primary School [ ] Secondary School [ ] Tertiary education [ ]
Others (specify) _
Primary occupation: Civil servant [ ] Trading [ ] Crop farming [ ] Livestock keeping [ ] Artisan [ ] Others (specify) _
How many pigs are on your farm now (2008)? _
How old is your farm? _

Section A

1. Do you use an isolation facility for incoming breeding stock? ____Yes ____No

a. If you answered no to 1., are all replacements produced and grown within your farm? ____Yes ____No

i. If you answered yes to 1.a, disregard the remainder of this section and go to **Section B**.

ii. If you answered no to both 1. and 1.a, your isolation procedures are unacceptable and you are at very high risk for introduction of a new pathogen into your herd. Please use the rest of this questionnaire as a guide to develop an effective isolation facility to protect your herd.

b. If you answered yes to 1., continue with the remainder of this questionnaire.

2. Is the isolation facility located…

a. Less than 300 meters from any other pig farm or free range pigs? ____Yes ____No
b. Greater than 300 meters from any other pig farm or free range pigs? ____Yes ____No
c. Greater than 3 km from any other pig farm or free range pigs? ____Yes ____No

3. Is the isolation facility…

a. Completely outdoors/open? ____Yes ____No
b. Indoor/Outdoor? ____Yes ____No
c. Totally enclosed (100% confinement)? ____Yes ____No

4. Blood testing animals in isolation for known disease agents of concern:

a. No animals are tested in isolation facility ____Yes ____No
b. A few animals are tested in isolation facility ____Yes ____No
c. A statistical sample of all animals are tested in isolation facility ____Yes ____No
d. All animals are tested in isolation facility ____Yes ____No

5. Animals are blood tested in isolation facility:

a. Only on arrival ____Yes ____No
b. Once around 14 days post-arrival ____Yes ____No
c. Once just prior to entry into the breeding herd following a minimum of 30 day isolation ____Yes ____No
d. 14 days post-arrival and again just prior to entry into the breeding herd following a minimum of 30 days isolation ____Yes ____No

Section B

6. Location

a. Considering the proximity of your pig farm to the nearest unrelated pig farm:
i. Less than 300 meters ____Yes ____No
ii. 300 meters to less than 3 kilometers ____Yes ____No
iii. Three kilometers or greater ____Yes ____No

b. Considering the proximity of your pig farm to a public road:
i. Less than 200 meters ____Yes ____No
ii. 200 to 500 meters ____Yes ____No
iii. Greater than 500 meters ____Yes ____No

7. Access deterrents

a. No biosecurity or information signs at entrance ____Yes ____No
b. No surrounding fence or gated driveway ____Yes ____No
c. No surrounding fence; driveway is gated and not locked ____Yes ____No
d. No surrounding fence; driveway is gated and locked ____Yes ____No
e. Buildings are secured with locks ____Yes ____No
f. An occupied dwelling exists on the site ____Yes ____No
g. Surrounding fence exists and driveway is gated and locked ____Yes ____No

8. Pest / Wildlife control programs

a. No pest control program ____Yes ____No
b. Pest control program maintained by farmer ____Yes ____No
c. Professional biosecure pest control program ____Yes ____No
d. Excessive debris and vegetation inside perimeter ____Yes ____No
e. Birds have access to pigs or feed in the pig houses ____Yes ____No
f. Dogs, cats, or wildlife have access to pigs and feed in pig house ____Yes ____No
g. Feed spills are cleaned up immediately ____Yes ____No

9. Feed

a. Feed or feed ingredients are produced and delivered from a mill servicing other pig farms: ____Yes ____No

i. Feed is delivered to your farm on the same load as other farm deliveries ____Yes ____No
ii. Feed truck is dirty on arrival (either inside cab or externally) and enters your farm ____Yes ____No
iii. Driver wears coveralls and clean boots to each delivery ____Yes ____No
iv. Driver enters your farm and pig house during deliveries ____Yes ____No
v. Feed truck remains outside of surrounding fence and driver does not enter farm ____Yes ____No

b. Feed is produced internally and delivered with a dedicated truck ____Yes ____No
c. Source of ingredients (corn, meat and bone meal, fish meal) is known____Yes ____No
d. Feed mill follows adequate biosecurity and quality control procedures ____Yes ____No

10. Carcass removal

a. Carcasses are disposed of in a timely manner according to state regulations ____Yes ____No
b. Carcasses are kept in an enclosure that prevents access by dogs, cats, or wildlife ____Yes ____No
c. Dead stock transporter observes all pickup cleansing protocols ____Yes ____No
d. If burning to ashes is used, the driver picks up carcasses on sit ____Yes ____No
e. If burning to ashes is used, the truck picks up carcasses at the entrance gate

OFFICIAL USE ONLY

(i) Location of Farm ……. State …… LGA …….
(ii) Coordinates (GPS readings) ……
(iii) Names of Adjacent Roads ……
…… Coordinates ……
(iv) Names and proximity of streams/Rivers ……
……. Coordinates ……...
Name of Investigator: BOO/…… Date of Farm Visit ……...

Post-field lab-work

Collected ticks and blood samples should be processed based on the specifications of instructor. The questionnaire should be summarized and all data inputted into a laptop or desktop computer that has ArcGis 9.0 software for spatial autocorrelation.

Practical Exercise 3

Control of pests of farm and companion animals by fumigation: Assessment of their efficacy and environmental residual effects

Background

Several chemical compounds are available commercially for the control of ectoparasites of dog, cat and farm animals. There are various reports of failure to control and results that are questionable. The purpose of this exercise is to practice the use of graded concentrations of formaldehyde and evaluate their potency for tick control in dog kennel.

Skills to develop

Measurement and dilution of formaldehyde for fumigation of kennel.

Equipment

1. Universal bottle
2. Bucket
3. Hand gloves
4. Funnel
5. Nose/Mouth cover
6. Measuring cylinder
7. Knapsack sprayer

Chemical reagents

1. Water
2. Formaldehyde (40%)
3. Hypochloric acid (Jik)

Source of Sample

From the cases presented to the Veterinary Teaching Hospital for fumigation of kennel, select three and give them the same date for treatment. Students should be taken to each of the kennels at least a day before the fumigation exercise is done for tick load assessment.

Procedure

Under the guidance of the clinical supervisor, select graded dilutions between 1.0 - 3.0% solution for formaldehyde in water. Also use same dilution gradient (1.0-3.0%) of hypochlorate in the same water to which formaldehyde is added. Divide the group into three and each group should mix, deliver and evaluate the outcome of application of each dilution, namely 1.0%, 2.0% and 3.0% each for fumigation exercise.

Evaluation of results

Count the number of surviving ticks that could be picked on the kennels after three days of fumigation and compare the outcome of the three dilutions. If there are need to repeat based on poor performance, discuss and repeat the exercise where necessary. Decide on an appropriate dosage that was economically feasible and effective in this exercise.

Practical Exercise 4

Basic techniques in serological diagnosis and mapping of infectious animal disease: A case study of Rabies

Background

Veterinary public health laboratory serves as a community eyepiece in the diagnostic intelligence it provides about zoonotic diseases. In this practical exercise, serological diagnosis of positive response to rabies vaccine in dogs and the level of exposure risk in terms of non-protection will be determined.

Skills to learn

Collection of blood from the dog and use of Rapid Test Kit for laboratory confirmation.

Source of samples

Dogs presented to the Veterinary Teaching Hospital for vaccination and other treatments will be bled via the cephalic vein and tested.

Equipment

1. Needle and syringe
2. Bijou bottles
3. Rabies virus Rapid Test Kit
4. Cotton wool
5. Methylated spirit
6. Mouth guard

Procedure

Allow the blood collected to clot and collect the serum. This procedure takes less than three hours to complete. The serum is dropped into the receiving vent of the test kit. Each serum sample is tested with a stick of the test kit. A positive result is qualitatively indicated by a band on the kit result area. This exercise should be discussed with the supervision clinician in view of the other viral zoonoses that such rapid test kits are available for.

EPIZOOTIOLOGY AND PREVENTIVE MEDICINE CLINICS

Preventive Vet Medicine Clinics Module
500level posting

(1) Introduction to Preventive Vet Medicine Clinics
-Differences between PVM & other Clinics
-PVM & Epizootiology
-PVM & Public Health.

(2) Preventive Veterinary Medicine Approaches:Biosecurity, Prevention, Eradication and Prophylaxis ……..definition & measures employed

(3) **Principles of Vaccine and Vaccination**

a Introduction of Vaccines and Vaccination Technique in Animals.

-Definitions, Basic immunology, immunological Basis for vaccination and Herd immunity.
-Reasons for vaccinations
-Types and Presentations of vaccines
-vaccine failure & vaccine break
-Adverse Reactions

(b) **Handling and administration of vaccines.**
-Legislations and Regulations on vaccination in Nigeria
- Production and supply of vaccines
- Storage, Transport and Disposal of vaccines
-Biosafety in relation to vaccine
-Vaccines Quality Assessment
-Materials required for vaccinations
-Considerations on routes of vaccinations
-Reconstitution of vaccines
-Personnel responsibilities and supervision

(c) **Pre-vaccination Assessment of Animal Patient & Vaccination procedures**
- Assessment of patient's health status (clinical &immunological)
- Environmental, nutrition &management assessment
- Cold-chain assessment
- Patient's preparation, restraint @ individual and flock/herd levels
- Biosecurity and biosafety procedures

(d) **Vaccination Schedules in different animal species**
-Review of vaccines preventable diseases of socioeconomic importance
-Vaccination protocols in different species of animal (age, timing, routes, and dosage) including cattle, sheep, goat, pigs, dogs, cats, poultry, equine, primates, wildlife and fishes.

(e) **Post Vaccination Cares**
- Record keeping and Certification
- seromonitoring

- Stress and Environmental Control
- Productivity and health assessment.
- Disposal Methods
- Handlers personal hygiene and bio-safety

PREVENTIVE VETERINARY CLINICS

600level posting

PLANNING and Implementation of Preventive Veterinary Programmes

Epizootiological Investigations of Diseases in U.I. Teaching and Research Farm and designated farms, ranches, abattoirs and livestock markets

- survey and surveillance.

Practice of preventive medicine

- mass actions/campaign
- animal husbandry systems and implications against disease development and prevention.
- Biosecurity practices at different animal units
- Chemoprophylactic schedules for large animals , small animals and poultry
- Animal health systems; components, responsibilities and organization
- Records and data management in preventive veterinary medicine.
- Production, supply, procurement, handling and administration of veterinary vaccines and biologicals at individual, flock/herd or mass levels in VTH, UIT&R farm and designated farms; routine vaccinations @ VTH pvm clinics, teaching and research farm and ambulatory clinics, practical exposures and demonstrations to vaccination exercises at faculty poultry project and selected farms.
- Veterinary economics; livestock/veterinary project planning, implementation and appraisal, veterinary professional & operative charges, book keeping and accounting in veterinary practice.
- Practice of veterinary extension at animal producers, processors, marketers, consumers service-men and policy makers levels.

- Review , applications and implementations of animal disease prevention and control regulations and programmes; vet surgeon acts, animal disease control decree meat law/edict JP24, PACE, NADIS, TADS EMPRESS OIE Int. Codes
- **Pest and vector control field and laboratory works**

  Pest and Vector survey

  -Trapping procedures

  - Pesticides and insecticides application and toxicity evaluation
  - Bloodmeal procedures and analysis
  - Biosafety and security in pesticide and insecticide applications
- **Veterinary professional/ occupational Hazards;**
  - Types, risks factors, prevention and control
  - Fumigation and pest control.
  - biosafety approaches at farm, clinics, abattoir and laboratories
  - First aid practice

EPIZOOTIOOGY AND SPECIFIC DISEASE PREVENTION CLINICS TOPICS

The basic principle of Epizootiology Clinics is the demonstration and instruction on diseases and herd health of animal populations, which are of financial, socioeconomic, public health (including food safety) and zoonotic importance. This is by the use of practical clinical, video-recorded and/or field cases. It so involves studies of the interrelationship of animals, disease agents and the environment, and the contribution of the veterinarian to the overall public health.

Specific topics to be of Epizootiology clinical importance are:

a. Epizootiological studies of common animal diseases in food and production animals.

b. Relevant Field Sample collection (hematological, parasitological, pathological) for laboratory examination/confirmation studies

c. Analysis of data to determine epizootiological patterns (Incidence, prevalence, epizootic, enzootic, sporadic outbreaks) in animal populations

d. Epizootiological studies in effect of Environmental factors on disease situation.

e. Epizootiological studies in Transboundary Diseases including Rinderpest and other epidemic animal diseases (Contagious Bovine Pleuropneumonia, Foot-And-Mouth Disease, Contagious Caprine Pleuropneumonia, Peste De Petit Ruminants,, Newcastle Disease, Avian Influenza And African Swine Fever)

f. The EMPRES (Emergency Prevention System for transboundary Animal Pests and Diseases)

g. The Foreign Animal Diseases concept

h. Ambulatory field trips for off- campus exposure to livestock production farm (poultry, piggery, fisheries), and nomadic/ pastoralists farms.

i. Ambulatory field trips for off- campus vaccination, de-worming and extension education exercises

j. Field exercises in practical Participatory Epizootiology approaches to disease investigation and data gathering

k. Practical exercises in the power of observation in diagnosis of animal host, agent and environment diagnosis

l. Field exercises in Computer Epizootiology/ Veterinary Geo-Informatics (Geographical Information System and Global Positioning Systems usage

m. Practical exercises in Data analysis and Mathematical Epizootiology

n. Analysis of socio-economic analysis of herd health and disease data

o. Participation and Discussion of past and ongoing Epizootiological research

- **Economic analysis**

- cost/benefits analysis of diseases events and interventions
- funding of epizootiolgical practice.

Principles, operations and application of serum banking

Serology, materials for collection of blood, procedures of collection and processing of serum for different serological works, maintainance of serum bank, uses of serum and precautions in serum banking.

Disease reporting, monitoring and surveillance clinic

1. Basic concepts: principles and practice of Survey, Notification, Reporting, Monitoring and Surveillance of animal diseases.
2. National and International animal codes and agencies: regulations, agencies, classification and management of animal diseases, reporting channels and format
3. Principles and applications of bioclimatology in veterinary practice: ecological concept and measurements, physical geography i.e. topography and vegetation, map reading, application of geographical information system (GIS) in disease reporting and surveillance.
4. Surveillance exercises on TADs and major zoonoses: field, farm, abattoir data collection, laboratory data analysis, interpretation and actions; prevention, control and eradication.

Materials, equipment and facilities

- Farms-contacts some corporate farms/Ranches
- Animal Stock
- Abattoirs/slaughter slabs
- Livestock markets
- Automatic Syringes
- Needles as Syringes , wing web jabbing needles
- Samples and specimen bottles
- Protective clothing
- Cooler/Cool boxes
- Ice packs
- Refrigerators
- Different vaccines
- Deep Freezer
- Antivenom, Antitetanus
- Restraining Materials, Ropes, tape muzzle, Mouth guard
- GPS Readers
- Ambulatory bus/van
- Universal bottle
- Microscopes
- Slides/Cover Ships

- Jabbing needle
- Cotton wool
- Marker
- Knap Sack
- Disinfectants
- First aid Boxes
- Fridge and Freezer, cool boxes
- Hot and cold water ruing
- Uv culture hood
- Bacterial Culture incubator
- Standard centrifuge
- Cold Centrifuge
- Ultracentrifuge
- Microheaemotocrit centrifuge
- Microcentrifuge
- Thermocycler
- Spectrophotometers
- Standard Lab. balance, (chemical balance)
- Light Microscope
- Water Distiller
- C02 and 02 enriched tissue culture incubators
- Glass drying oven
- Water bath
- Centrifuge tubes (assorted)
- Sample tubes/bottles
- Microsocope Slides
- Capillary tubes
- Venapencture hypodermic needle
- Eppendorf tubes

FISH AND WILDLIFE

LABORATORY WORK/CLINICS

FISH AND WILDLIFE CLINICS/LABORATORY WORK

600 level posting

INTRODUCTION TO AQUATIC ANIMAL HEALTH MANAGEMENT

Structural Assessment of Fishponds

Field trips to fish farms to assess different types of culture ponds.

Identification of the enemies of fish

Including; Insects, frogs (tadpoles, fishes, birds, reptiles, mammals and man)

Pond Water Quality Assessment

The single, most important factor affecting fish health and influencing disease in fish ponds and tanks is water quality. Raised levels of ammonia or nitrite, sub-optimum pH and water hardness levels or a high level of organic pollution will be stressful to fish; predisposing them to disease.

Procedure: Samples of pond and river water will be obtained and assessed for their quality and suitability for aquatic life.

Requirements:

1. Sample bottles
2. Hach's water quality test kit.
3. Thermometer
4. DO Meter
5. BOD Meter
6. pH meter

Quick Diagnosis for culture water quality

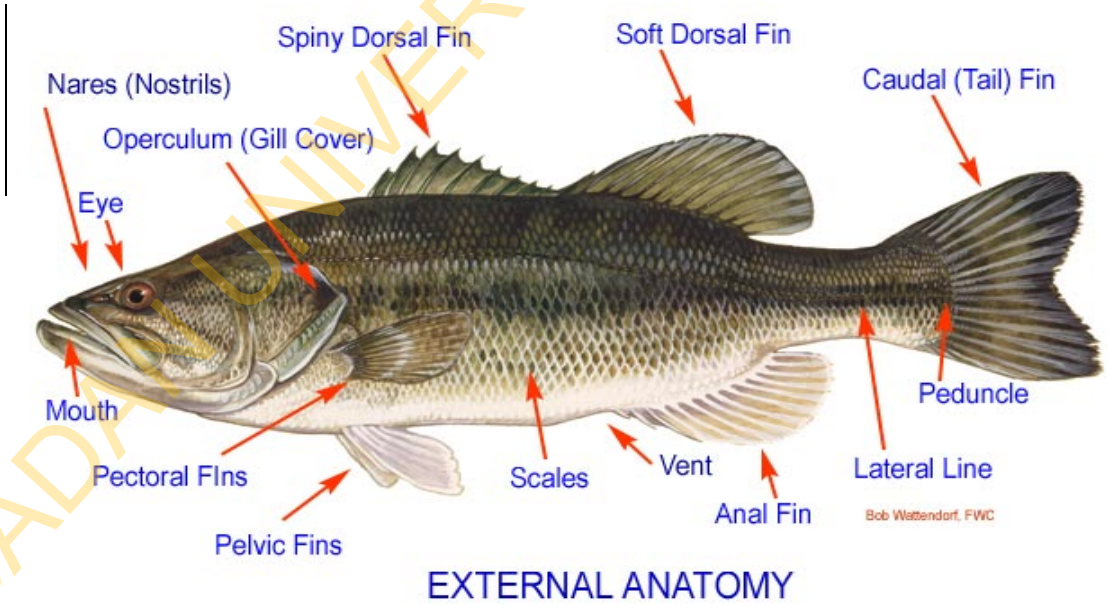
Symptom	What It Is	Treatment
Green Water / Pea Soup	Algae Bloom	Algicides
Small Floating Debris	Filter Needs Cleaning / Insufficient Mechanical Filtration	Use Coagulator/Mechanical Filtration
Brown Cloudy Water	Often Rain Run-off / Dirt in Water /	Use Coagulator
pH Tests High (over 8.0)	Pond Too Alkaline	Agricultural gypsum
pH Tests Low (under 7.0)	Pond Too Acidic	Agricultural Calcium carbonate over a few days
Ammonia Tests Positive	Inadequate Biological Filtration / Nitrosomas Bacteria	Introduce bio-filters
Nitrite Tests Positive	Inadequate Biological Filtration/ Nitrobacter Bacteria	Introduce bio-filters

Collection of some cultured fish specimens
Specimens of commonly cultured fish species will be purchased for class demonstration. Examples include:

1. Catfish
2. Tilapia
3. Carp

Gross External Examinations
Locate major external anatomical parts:
Dorsal Fin
Posterior Dorsal
Pectoral Fins
Pelvic Fins
Anal Fins
Caudal Fin
Gill Covers (operculum)
Lateral line - sensory organ of fish
Remove several scales
Prepare a dry-mount of a scale.
Observe it under a microscope
What function do you think scales serve?

Figure 29: External anatomy of fish



Blood sampling in fish
Blood samples from larger fish can be collected from the caudal vessel, by insertion of a fine needle from the ventral midline behind the anal fin until the spine can be felt, and withdrawing the needle slightly before collection. Blood can also be collected from

the heart. For blood enzymology in sacrificed fish, blood is best collected from the exposed heart, immediately after the fish has been killed by anaesthetic overdose. The major vessels supplying the gills are another suitable site that is readily visible during life in large fish. In very small fish, tail truncation will provide sufficient blood for smears

Fish necropsy
Remove operculum with scissors
Observe gill anatomy: Including
Rakers - white, comb-like arches

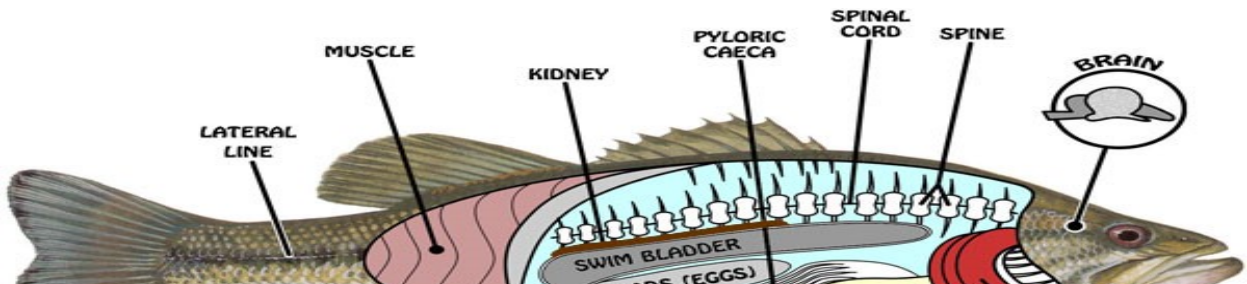
Filaments - Red fingerlike projections

Figure 30: Gill

Dissection
The main incision will be made by opening the abdomen (below the gill) carefully with a scalpel. The incision is widened with a scissors and an oval-shaped piece of skin (only skin) running from underneath the gills, to the anus, up to the lateral line, along the lateral line, to the gill, down to where you started the incision is removed.

Figure 31: Identification of internal organs in Fish

Different organs will be identified.



Requirements

1. Fish specimens for demonstration of fish species, blood sampling, gross anatomy and necropsy.
2. Dissecting sets
3. Benzocaine
4. Tricaine Methane Sulfonate (MS-222)
5. Needles and syringes
6. Sample bottles
7. Nets
8. Buckets
9. microscopes
10. Microscope slides
11. Coverslips

Determining if fish are sick

Farm visits will be made to assess fish insitu. Fish will be assessed for signs of sickness. Signs and symptoms to look out for include:

- Presence of dead or dying animals.
- Anorexia
- Fish that are observed hanging listlessly in shallow water,
- gasping at the surface, or
- rubbing against objects
- presence of sores (ulcers of hemorrhages),
- ragged fins or abnormal body conformation (i.e., a distended abdomen or "dropsy", and exopthalmia or "popeye".

Sampling Procedures for Fish Health Assessment

This module will discuss methods of obtaining the best available samples for fish disease diagnosis under a variety of circumstances, especially where specialised fish diagnostic services are not routinely accessed. Laboratory submissions for fish diagnosis should include suitable specimens of fish, water and any other material that is suspected to be involved, and a full history. Fish show a relatively small range of clinical and gross signs, so quality of samples and history are paramount. Poor quality specimens will probably result in no diagnosis being established. The specific methods for collecting, preparing and submitting the samples varies with the analysis to be conducted.

The following is a list of samples to be submitted for general diagnosis of fish diseases, in decreasing order of preference.

a) Preferred samples

(i) Live affected fish, plus
(ii) Suitable water samples (see below).
(iii) Any suspected contaminant material or suspected feed.
(iv) Blood or tissue samples, if tests are required for chemicals which will change during transport (see below)
(v) Smears or fixed tissue of gills and skin, especially where gross lesions are detected.
(vi) History or

b) If fish cannot be maintained live, submit
(i) Freshly killed fish on ice.
(ii) Fixed tissues, especially gills, gut (see below).
(iii) Blood samples if required.
(iv) Frozen fish or tissues for labile chemistry if required (see below).
(v) Suitable water samples (see below).
(vi) Any suspected contaminant material or suspected feed.
(vii) Smears of external or gill lesions.
(viii) History ; or

c) If transport is delayed, submit samples as for (b) plus specific samples for virology, bacteriology and fixed parasites, as appropriate; or

d) if no live fish are available, submit
(i) Dead fish (largely unsuitable), on ice and frozen
(ii) Fixed tissues if autolysis is slight.
(iii) Water samples (sampling possibly too late).

Sample Selection for Pathology

Fixatives

For general finfish pathology, 10% isotonic formalin fixative preferably buffered or at least balanced to neutral pH, is usually suitable. As finfish tissues are of the same isotonicity as higher vertebrates, standard vertebrate fixatives are suitable.
Exceptions are where samples contain small parasites that wash off epithelial surfaces during processing; where rapid penetration is required; and with tissues that tend to separate during processing. Under these circumstances, both Bouin's and Davidson's fixatives are useful.Both are acidic fixatives with slight decalcifying action. Use of these fixatives results in reduced tearing of scaled skin sections during sectioning and reduced need for further decalcification.

Bouin's Fixative

Bouin's fixative is suitable for small specimens, especially very small fish that can be fixed and sectioned whole. It is also useful for skin and eyes.

Requirements

Picric acid [$(O_2N)_{3C6H2O8}$] sat. Aq. sol. 75 ml
Formalin (40% w/v commercial soln) 25 ml
Glacial acetic acid (CH_3CO_2H) 5 ml

Wet Smear Examination for Microscopic External Parasites

Wet smears are conducted immediately as many parasites leave the host rapidly after death, or are loosely adhered and are not retained in sections. Wet smears are prepared from a scalpel blade scrape of mucus from skin and gill, diluted on the slide by a drop or two of water to give a thin film, and examined immediately. The same source of water for dilution as that of the fish is used, or at least of roughly the same salinity and temperature to preserve parasite integrity

Common Fixatives for Parasites

Schaudinn's fixative

Requirements

Mercuric chloride ($HgC1_2$) sat. aq. sol. 200 mL

Ethanol 95% (C_2H_5OH) 100 mL

Glacial acetic acid (CH_3CO_2H) 15 mL

(add just before use)

AFA fixative

Requirements

Ethanol 9.5% (C_2H_5OH) 50 mL

Formalin (commercial soln. 40% w /v) 10 mL

Glacial acetic acid (CH_3C0_2H) 5 mL

Distilled water 45 mL

Record Keeping On Fish Farms

These records should include:

The date fish were stocked,
Size of fish at stocking,
Source of fish,
Feeding rate,
Growth rate,
Daily mortality and
Water quality.

Good records, a description of behavioral and physical signs exhibited by sick fish, and results of water quality tests provide a complete case history for the diagnostician working on a case. Water quality parameters should be monitored once a week and also anytime treatments has been carried out or any changes made on a fish farm. Ideally, daily records should also be available for immediate reference when a fish disease outbreak occurs.

Requirements: A prototype record keeping register suitable for use on a fish farm.

INTRODUCTION TO WILD ANIMAL HEALTH MANAGEMENT

Field trips to University of Ibadan and Agodi Zoological gardens

Students will familiarize themselves with the animals currently in captivity at the zoological gardens.

Procedure:

- Common diseases associated with the different species of wild animals will be reviewed by assessing the case notes at the zoological gardens.
- Routine vaccinations and other preventive measures will be observed by the students.
- Husbandry and management of animals in captivity
- Introduction to wildlife Veterinary Medicine; including observations on restraint techniques, drug administration, and record keeping.

APPENDIX

Evaluation of student workbook-attendance-5
-appearance-5
-participation-10
-Report- 10

NATIONAL VETERINARY RESEARCH INSTITUTE, VOM, PLATEAU STATE ESTABLISHED 1924

A GUIDE TO THE USE OF NVRI VIRAL VACCINES

VACCINE	STORAGE AT (^{0}C)	DOSAGE	SITE AND ROUTE OF ADMINISTRATION	MINI MUM	DURATION OF IMMUNITY

				AGE AT VACCINATION	
Newcastle Disease Vaccine Intraocular (NDV-i/o)	12 Months	200 dose vial to be reconstituted in 10mls of sterile distilled water/cold sterile normal saline. Inject 0.05ml/bird	Instill one drop (0.05ml) into each open eye. Vaccine can also be given by aerosol spray.	17 days	3 months. After 3 weeks, vaccin
Newcastle Disease Vaccine Lasota (NDV-L) (200 Dose)	12 Months	200 dose vial to be reconstituted in 2 litres of chlorine – free drinking water (10mls of water/bird). Or dissolve 200 dose vial in 10mls sterile water or normal saline and instill one drop (0.05mls) into each open eye of the bird.	Administer the vaccine in cool chlorine –free drinking water or by intra-ocular administration.	3 weeks	4-6 months. Deprive birds of wa vaccination.
Newcastle Disease Vaccine Lasota (NDV-L) (500 Dose)	12 Months	(a) 500 dose vial to be reconstituted in 5 litres of drinking water. (b) Or dissolve 500 dose vial in 25ml of sterile water (instill one drop into each eye).	(a) Administer the vaccine in cool chlorine-free drinking water (b) By intra-ocular administration	3 weeks	4-6 months. Deprive birds of wa vaccination.
Newcastle Disease Vaccine Kamarov (NDV-L) (200 Dose)	12 Months	200 dose vial to be reconstituted in 40mls of sterile water or normal saline. Inject 0.2ml per bird	Intramuscular injection	6 weeks	About 1 year. Previous vaccinat compulsory
Newcastle Disease Vaccine Kamarov (NDV-L) (500 Dose)	12 Months	500 dose vial to be reconstituted in 100mls of sterile water or normal. Inject 0.2ml per bird	Intramuscular injection	6 weeks	About 1 year. Previous vaccinat compulsory
Newcastle Disease Vaccine (Strain I_2)	12 Months	200 dose vial to be reconstituted in 2 litres of chlorine – free drinking water (10mls of water/bird). Or in 10mls of normal saline for intraocular use (0.05ml/bird).	Administer vaccine in cool chlorine-free drinking water and keep away from sun. vaccine can also be given by eye drop (one drop into each open eye of the bird)	3 weeks	4-6 months. It can be used for th turkey, guinea fowls, ducks and
Fowl Pox Vaccine (FPV)	12 Months	200 dose vial to be reconstituted in 4mls of sterile water or normal saline.	Two stab punctures in the wing web.	6 weeks	About 1 year
Gumboro Vaccine (IBDV)	12 Months	200 dose vial to be reconstituted in 2 litres of drinking water or in 10mls of water for eye drop or in 10mls of sterile water for 0.2mls i.m/bird.	Administer vaccine either in drinking water, or by eye drop or by intramuscular injection	10-21 days	About 1 year
Peste des Petit Ruminants Virus Vaccine (PPR Vaccine)	12 Months	50 dose vial to be reconstituted in 50ml of distilled water and give 1ml per animal.	Subcutaneous injection	4 Months	About 3 years, but vaccination c
Rabies (LEP) Flurry Vaccine (ARVD)	12 Months	One dose vial to be reconstituted in 2.5mls or sterile water or normal saline	Deep intramuscular injection	3 months	About 3 years. Annual revaccina in recommended

Note: All Reconstituted vaccine must be used within 1 hour , Balance (if any) should be discarded

A GUIDE TO THE USE OF NVRI BACTERIAL VACCINE

VACCINE	STORAGE AT (^{0}C)	DOSAGE	SITE AND ROUTE OF ADMINISTRATION	MINIMUM AGE AT VACCINATION	DURATION OF IMMUNITY
Anthrax Spore Vaccine (ASV) (400 Dose Bottle)	12 Months	0.5mls Ready for use	Subcutaneous	6 Months	12 Months
Blackquater Vaccine (BQV) (500 Dose bottle)	12 Months	2mls or 5mls per head. Read to use	Subcutaneous	4 Months	12 Months
Brucella S. 19 Vaccine (100 Dose vial)	3 Years	5mls	Subcutaneous	6-9 Months	Life, Revaccination may not be necessary
Contagious Bovine Pleuropneumonia Vaccine (CBPPV). (100 Dose vial.	12 Months	0.5mls	Subcutaneous	6 Months	12 Months
Haemorrhagic Septicaemia Vaccine (HSV)) (40 Dose bottle)	3 Months	5mls Ready for use	Subcutaneous	4 Months	6 Months
Hantavac (40 Dose bottle)	18 Months	2mls or small ruminants, 5mls for cattle. Ready for use	Subcutaneous	4 Months	12 Months
Dried Fowl Typhoid Vaccine (DFTV) (100/1000 Dose vial)	12 Months	0.5mls	Subcutaneous	6 Weeks	12 Months
Fowl Cholera Vaccine (WFCV) (100 dose bottle)	3 Months	1ml	Subcutaneous	6 Weeks	12 Months

Note: Vaccine once opened or reconstituted, must be used within 1 hour
Discarded the remaining unused vaccines safely
Ref: NVRI vaccine Guide, Fed. Min. of Agric and Rural Development

AGE	VACCINATION	VACCINES TYPE	ROUTE OF VACCINATION	REMARKS

1 Day	Infectious Bronchitis	IBH 120	Spray or Intraocular	Spray:- 1 vial of 1000 doses in 500ml distilled water for 2000 birds. Intra-occular:- 1 vial of 1000 in 30ml normal saline. By means of a dropper, apply a drop into the eye
5 Days	Coccidiosis	Immucox or Livacox	Drinking water	Follow the manufacturers instruction on application
10 Days	Newcastle Disease	ND Hitchner B1	Spray or intraocular	Spray:- 1 vial of 1000 doses in 500ml distilled water for 2000 birds intra-ocular:- 1 vial of 1000 in 30ml normal saline By means of a dropper, apply a drop into the eye
16-18 Days	Infectious Bursal Disease	Gumboro (Intermediate plus)	Drinking Water	Dissolve 1 vial of 1000 dose in 10 It of water
23-5 Days	Infectious Bursal Disease	Gumboro (Intermediate plus)	Drinking Water	Dissolve 1 vial of 1000 dose in 10 It of water
4 Weeks	Mycoplasmosis	MG (Optional)	Subcutaneous	Inject recommended dose into back of the neck Vaccination Recommended only in endemic area
5 Weeks	Newcastle Disease	ND Lasota	Drinking Water	Dissolve 1 vial of 1000 dose in 20 It of water
7 Weeks	Colibacilosis	(A). E.coli I (Optional)	-Intra-muscular	Inject recommended dose into the thigh or breast muscle
	Salmonellosis	(B). Salmonella vacc. I	_Subcutaneous	Inject recommended dose into back of the neck Note:- Do not give any antibiotics a week before and after Salmonella vaccine
	Fowl pox	Fowl pox	Nebulisation	-Stab by the wing- web with Jabbing needle supplied with vaccines.
8 Weeks	Infectious Bronchitis	IBH 120	-Drinking Water	1 vials of 1000 doses in 20 It of water for 1000 birds
10 Weeks	Newcastle disease	(A) ND Clone	-Drinking Water	Dissolve 1 vial of 1000 doses in 20 IT of water
14 Weeks	Colibaccilosis	(A). E. Coli II (Optional)	-Intra-muscular	Inject recommended dose into the thigh or breast muscle
	Salmonellosis	(B). Salmonella Vacc. II	-Subcutaneous	Inject recommended dose into back of the neck Note:- Do not give any antibiotics a week before and after Salmonella vaccine
	Infectious Bursal Disease	©. Gumboro oil	-Intra-muscular	Inject recommended dose into the thigh or breast muscle
16 Weeks	Newcastle Disease Infectious Bronchitis Egg Drop Syndrome	(A). ND + IB + EDS	-Intra-muscular	Inject recommended dose into the thigh or breast muscle
18 Weeks	Gumboro Mycoplasma	Gumboro oil MG (Optional)	Intra-muscular Subcutaneous	Inject recommended dose into the thigh or breast muscle Inject recommended dose into back of the neck

POULTRY VACCINATION PROGRAMME OF IMPORTED VACCINES

Vaccine strains and method of application may differ based on type and manufacturer. Follow the vaccine manufacturer's instruction on route and time of vaccination.

HINTS AND PRECAUTION ON VACCINATION

- Ensure that the vaccination equipments (sprayer, drinkers, droppers) are clean and free from any sediment, corrosion and traces of disinfectant prior to use.
- Vaccinate healthy birds only
- Always give Vitamin in water starting from a day prior to any vaccination for the next 3 days
- Do not expose the vaccines to direct sunlight
- Keep record of the vaccines type, batch number and expiry date.
- Destroy any unused reconstituted vaccines, vaccine's containers and needles by burning
- Adhere strictly to all other biosecurity measures.

Drinking water route

- Deprive birds of water for about 2-4 hours prior to vaccination
- Do not use chlorinated water. If doubtful, add skimmed milk at rate of 2g per It of water
- All materials for mixing and application of the vaccines should be cool, clean and free from disinfectant, detergent, chemicals sediments and corrosion.
- The vial should be opened under cool, clean non-chlorinated water free
- Dilute vaccine with quantity of water recommended based on age
- Reconstituted should be consumed within 1 – 2 hours at temperature not higher than 25 C.
- Provide enough drinkers and adequate drinking spaces so that all birds can have access to drinking as quickly as possible

Intra-ocular instillation

- Dilute vaccine in normal physiological as recommended (1000 doses in 30ml)
- One drop should be applied from a height of few centimeter onto one eye and allowed to spread evenly across the surface of the eye
- Do not release bird until swallowing motion is noticed.

Spray Method

- This should be carried out by experienced trained operatives as it could increase the risk of respiratory infection if not well done.
- Ensure that the vaccination equipment (sprayer) is clean and free from any sediment corrosion and traces of disinfectant prior to use
- Dilute vaccine with distilled water as recommended based on age
- Administer by course spray at a distance of 30-40 cm preferably when birds are sitting together in dim light.

Parenteral route (Intra-muscular and Subcutaneous)

- Ensure that all vaccination equipments (needle, injectors) are clean and sterile prior to use
- Allow the vaccines to gradually reach room temperature (20-25C) prior use
- Shake well before use and at regular intervals during vaccination (killed vaccines only)
- Opened bottle should be used within 24 hours
- Do not mix vaccine with other vaccines

RECOMMENDED MAXIMUM RESIDUE LIMITS (MRLS) FOR SOME ANTIHELMINTHICS AND ANTIBIOTICS

DRUG	SPECIES	MUSCLE (µg/kg)	LIVER (µg/kg)	KIDNEY (µg/kg)	FAT (µg/kg)	MILK (µg/l)	Eggs (µg/kg)
Febantel, fenbendazole, oxfendazole	Cattle						
	Horses	100	500	100	100	100	
	Pigs	100	500	100	100		
	Goats	100	500	100	100		
	Sheep	100	500	100	100	100	
Procaine benzylpenicillin	Cattle	50	50	50		4	
	Pigs	50	50	50			
	chicken	50	50	50			
Chlortetracycline, oxytetracycline, tetracycline	Pigs	200					
	Goats						
	Sheep	200	600	1200		100	
	Cattle	200	600	1200		100	
	Poultry	200	600	1200			400
	Fish [2,3]	200	600	1200			
	Giant prawn[2]	200					

[1.] singly or in combination. [2.] applies only to oxytetracycline. [3.] temporary pending evaluation of use pattern of oxytetracycline in aquaculture (FAO/WHO, 1998)

Chlortetracycline, oxytetracycline, and tetracycline

Acceptable daily intake: 0-30 µg/kg bw (group ADI for oxytetracycline, chlortetracycline, and tetracycline)

Residue definition: Parent drug

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)	Eggs (µg/kg)
Cattle	200	600	1200		100	
Pigs	200	600	1200			
Sheep	200	600	1200		100	
Poultry Fish2,3	200 200	600	1200			400
Giant prawn2 (*Penaeus monodon*)	200					

Diclazuril
Acceptable daily intake: 0-30 µg/kg bw
Residue definition: Diclazuril
Recommended maximum residue limits (MRLs)

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/litre)
Sheep Poultry Rabbits	500 500 500	3000 3000 3000	2000 2000 2000	1000 1000[1]	1000

Dexamethasone
Acceptable daily intake: 0.015 µg/kg bw (established at the forty-second meeting of the Committee (WHO TRS 851, 1995))
Maximum residue limits: The forty-second and forty-third meetings of the Committee recommended
temporary MRLs of 0.5 µg/kg in muscle, 0.5 µg/kg in kidney and 2.5 µg/kg in liver of cattle, horses and pigs and 0.3 µg/L in cattle milk based on an ADI of 0-0.015 µg/kg body weight.

Flumequine
Acceptable The Committee re-established an ADI of 0–
daily intake: 30mg/kg bw.
Residue Flumequine
definition:

Species	Fat (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	(m
Cattle	1000	3000	500	500
Black tiger shrimp (P. monodon)	—	—	—	500[a]
Chickens	1000	3000	500	500
Pigs	1000	3000	500	500
Sheep	1000	3000	500	500
Trout	—	—	—	500[b]

Cypermethrin and a**-cypermethrin**
Acceptable The Committee established a group ADI of
daily intake: 0–20mg/kg bw for cypermethrin and a-cypermethrin
Residue Total of cypermethrin residues (resulting from the
definition: use of cypermethrin or a-cypermethrin as veterinary drugs)
Recommended maximum residue limits (MRLs)

Species	Fat (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	Milk (mg/kg)	Muscle (mg/kg)
Cattle	1000	50	50	100	50
Sheep	1000	50	50	100	50

(FAO/WHO. 1998)

NORMAL RANGES AND MEANS FOR BLOOD VALUES IN DOMESTIC ANIMAL AND MAN

Differential Leukocyte Count in Percent

Animal	RBC (10^6/mm^3)	Hb (g %)	PCV (%)	MCV (μ^3)	MCHC (g/dl)	WBC 10^3/ mm^3	Band Neut.	Seg. Neut.	Lymph	Mono	Eos.	Bas
Dog	5.5 -8.5 (6.8)	12-16 (15)	37-55 (45)	60-77 (70)	31-34 (33)	6-18 (11)	0-3 (0.8)	60-77 (70)	12-30 (20)	3-10 (5.2)	2-10 (4)	Rare (0)
Cat	5.5 -10 (7.5)	08-14 (12)	24-45 (37)	40-55 (45)	31-35 (33)	9-25 (17)	0-3 (0.5)	35-75 (59)	20-55 (32)	1-4 (3)	2-12 (5.5)	Rare (0)
Cow	5.0-10 (7.0)	8-11 (11)	24-48 (35)	40-60 (51)	26-34 (20)	4-12 (8)	0-2 (0.5)	15-45 (28)	45-75 (58)	2-7 (4)	2-20 (9)	0-2 (0.5)
Sheep	8.0 – 16 (12)	8-16 (12)	24-50 (38)	23-48 (32)	29-35 (32)	4-12 (9)	0-2 (0.5)	10-50 (30)	40-75 (62)	1-6 (2.5)	1-10 (4.5)	0-3 (0.5)
Goat	12.0-20 (15)	8-14 (11)	24-48 (35)	18-24 (20)	30-35 (32)	6-16 (12)	0-2 (0.5)	30-48 (36.5)	50-70 (55)	1-4 (2.5)	3-8 (5)	0-2 (0.5)
Horse (cold)	5.5 -9.5 (7.5)	8-14 (11.5)	24-44 (35)	39-52 (44)	31-35 (33)	6-12 (8.5)	0-2 (0.5)	35-75 (54)	15-50 (35)	2-10 (5)	2-12 (5)	0-3 (0.5)
Horse (hot)	7.0-13.0 (9.8)	10-18 (13.5)	32-55 (42)	37-50 (42)	31-35 (33)	7-14 (10)	0-2 (0.5)	30-65 (48)	25-70 (44)	0.5-7 (2)	0.5 - 11 (4)	0-3 (0.5)
Pig	5.0 -8.0 (6.5)	10-16 (13)	32-50 (42)	50-68 (63)	30-34 (32)	11-22 (16)	0-4 (1)	28-47 (37)	39-62 (53)	2-10 (5)	0.5-11 (3.5)	0.2 (0.5)
Chicken	2.9	9-12	-	-	-	21	-	23	64	9	2	2
Human	4.8-5.5	13-16	42-47 (45)	82-92 (87)	32-36 (34)	4-11	-	50-70	20-40	2-8	1-4	0-1

NORMAL RANGE FOR CLINICAL PARAMETERS IN DOMESTIC ANIMALS

Animal	Rectal temp. (^oC)	Critical Temp. (^oC)	Pulse rate (/min)	Resp. rate (/min)
Dog	38.3 – 38.9		60 – 130	15 – 30
Cat	38.0 – 38.5		110 – 130	20 – 30
Horse	38.0	39.0	30 – 40 (foal: 70 – 80)	8 – 10
Cattle	38.5	39.5	60 – 80 (calf 100 – 120)	10 – 30
Pig	39	40.0		10 – 30
Sheep	39	40.0	70 – 90	10 – 30
Goat	39.5	40.5	70 – 90	25 – 35

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