A STUDY TO DETERMINE THE MOST EFFICIENT METHODS OF ROUTINE LABORATORY DIAGNOSIS OF THE DISEASES OF ANIMALS

Minor Thesis in Animal Pathology prepared

by

Charles R. Davis

June 1930

Approved

Dean

Approved

Acting Head of
Department of Zoology
and Animal Pathology

CONTENTS

A STUDY TO DETERMINE THE MOST EFFICIENT METHODS OF ROUTINE LABORATORY DIAGNOSIS OF THE DISEASES OF ANIMALS

INTRODUCTION

Diseases and parasites among domesticated birds and animals are fast becoming more prevalent. Frequent interchange of birds and animals between farms spreads disease. Permanent pastures and close confinement under unsanitary conditions increase parasites. As a consequence the number of specimens sent to this laboratory each year is increasing.

The poultryman and live stock owner each year are realizing the necessity for knowing the exact cause of the losses of their poultry and live stock from disease. These owners are often at a loss to know what the disease may be. The local veterinarian may not have the necessary equipment to make a laboratory diagnosis. This department is well equipped to carry out this work. Therefore, it is the purpose of this laboratory to assist the poultry and live stock owner and the veterinarians, in diagnosing disease, when special technic is needed which cannot be used in the field.

OBJECT

The main object of this problem is to determine which of our present

methods of laboratory diagnosis are most satisfactory and to improve them where possible.

ROUTINE TECHNIC EMPLOYED IN DIAGNOSING

Following is a description of the methods that gave the most satisfactory results this year:

Acariasis: Acariasis was diagnosed by microscopic examinations of deep scrapings from the diseased part of the skin. A small amount of the deep scrapings was placed in approximately two drops of water on a glass slide, and a cover glass applied. In Sarcoptic Acariasis the mite is oval shaped. The Dermodectic Acariasis mite is club shaped, having a long body and four pairs of legs (5).

Acariasis in chickens was diagnosed by microscopic examination of the serum found under the scales on the leg.

Actinomycosis: A small amount of the suspected pus was placed on a glass slide and gently pressed under a cover glass. The colonies of Actinomycosis bovis when present, could easily be seen under a microscope, using 10 x eye piece and mm objective. These micro-organisms are radiating hyaline club shaped bodies. Smear preparations stained with Gram's stain show branching filaments (1).

Ascariasis: Ascariasis was easily diagnosed in some cases by the presence of the parasites themselves. However, many fecal specimens showed no parasites so a microscopical examination was necessary. The feces were mixed with enough water to make them in suspension. The suspension was then centrifuged slowly for approximately fifteen minutes. Then the supernatant fluid was poured off and a sugar solution added to the sediment in the bottom of the tube, and this thoroughly mixed and centrifuged for approximately fifteen minutes. Two drops of water was put on a glass slide. A glass rod that had been flattened on the end so that its surface was increased was used to transfer the worm eggs. The surface of the suspension in the centrifuge tube was touched with the flat end of the rod and any worm eggs or coccidia present would stay on it. They were then transferred to the water on the slide, covered with a cover slip and examined microscopically. In infested cases many ascaris eggs were seen.

Formula of the above mentioned sugar solution:

Sugar	454 grams
Water	355 grams
Phenol	1%.

Bang's Abortion Disease: The general technic in use was employed here. One cubic centimeter of a standardized abortion antigen was put in each of two tubes. To the first tube .02 cc of serum were added and .01 cc to the second tube. This made dilutions of 1 to 50 and 1 to 100 respectively. These tubes were incubated at 55° for 8 hours. If the fluid remained cloudy and there was no clumping in the bottom of either tube, the cow from which the sample of serum came was diagnosed as negative. If tube one showed complete clumping in the bottom and the supernatant fluid was clear, and tube two remained cloudy without any clumping, the cow was classified as suspicious. If there was a complete clumping in the bottom of both tubes and the supernatant fluid was clear, this cow was classified as having Bang's abortion disease.

Blackleg: Blood smears were stained with Gram's stain. The bacilli are Gram negative, short rods with rounded ends, occurring singly or in pairs and possessing numerous flagella. These bacilli did not possess spores as they had not been exposed to air. When spores are present they are either in the center or at the end. When they are at the end they give the bacilli a club shaped appearance (2).

Coccidiosis: Coccidiosis (Eimeria avium) was easily diagnosed by a microscopic examination of the intestinal contents of the infected birds. Cecal contents were used as coccidia are more prevalent there. A small amount of the material was mixed with approximately two drops of water on a glass slide and a cover glass applied. The coccidia are easily recognized on microscopic examination of the slide preparation. They have an oval appearance with a central, rounded, granular area surrounded by a wide, clear, transparent zone with double contoured border. They are considerably smaller than ascariadia, lineata and heterakis gallinae eggs. Fecal examination as outlined under ascariasis was used in many cases of suspected coccidiosis.

Fowl Cholera: This disease closely resembles many others in lesions and symptoms produced. Diagnosis was made by blood smears. These smears were stained with dilute aqueous fuchsin. The organism (Bacillus aviseptiens) is short, plump, and bipolar (3).

Fowl Typhoid: This disease was diagnosed by the history of the case, symptoms, lesions, and blood smears. The birds would usually become anemic, greatly prostrated, have some diarrhea, and would usually die within four days. The liver is enlarged and mottled with grayish foci.

The kidneys and intestines are usually congested. Blood smears were made and there was a great increase in leucocytes. The increase was in the polymorphonuclears.

Leukemia: Leukemia was diagnosed by macroscopic lesions and stained blood smears.

The lesions were a large, very pale liver andlarge spleen. Blood smears were made and stained with Wright's stain. In leukemia there is a great increase in white blood cells. (6). Lymphatic leukemia is usually found in poultry. In these cases the increase is in lymphocytes. In animals the leukemia is usually of a myelocytic type and here there is an increase in myelocytes.

Pullorum Disease: Most of the pullorum disease diagnostic work was in baby chicks, ranging in ages from one day to two weeks. The only practical and safe way to diagnose the disease is by isolation of the organism from the baby chicks. The feathers were burned off the chicks' legs and the leg cut off, with a sterile pair of scissors, just above the hock joint. A sterile platinum needle was passed down the marrow of the leg bone, and then smeared over agar slants, pH 6.6. These tubes were incubated at 37°C from 48 to 72 hours. In almost every case where an organism was found it

was a pure culture of Salmonella pullorum. Pullorum colonies resemble small dew drops on the surface of the agar. Sugars were inoculated with suspected colonies. The following reactions are considered diagnostic:

	Dextrose	Levulose	Mannite	Lactose	Maltose	Sacchrose
Acid	+	+	+	-	-	-
Gas	+	+	+	-	-	-

Taeniasis: The large taeniidae can be easily seen, but often the small ones are overlooked. The small intestine was incised longitudinally and placed in a glass base containing warm water. The small taeniidae were then easily seen extending from the intestinal mucosa. Fecal examinations as outlined under acariasis were used in many cases of suspected taeniasis.

Other Diseases: Diseases other than those already described were diagnosed by the symptoms of the bird or animal, lesions found on post mortem, and the history of the case.

Total 4035	353	3373	7	1	65	6	2	3	11	21
Diagnosis	Chickens	Cows	Dogs	Geese	Guinea Pigs	Hogs	Horses	Human	Sheep	Turkeys
Acariasis	1		5							
Actinomycosis		2								
Asthenia	4									
Avitanimosis	3				60					
Bang's abortion disease: Negative		2973					2	3		
Positive		452								
Suspicious		123								
Blackleg		1								
Botulism	2									
Coccidiosis	29	1								6
Contagious epitheliomia	3									1
Contusion	5									
Cholera						4				
Enteritis: Catarrhal	7	1			3					
Hemorrhagic	1									
Infectious Entero-Hepatitis										6
Fowl Cholera	6									
Fowl Typhoid	12									
Gout: Visceral	4									
Helminthiasis: Ascariasis	24								1	3
Filariasis			1						1	
Strongylisis-Gastric									3	
Pulmonomy		1								
T aeniasis	25								1	4
Hemorrhagic Septicemia						1				
Hepatitis									2	
J aundice	1									
Leukemia	12		1							
Lymphomatosis	2									
Mosquito Bites	1									
Neoplasms	5	3								

Continued on next page.

Continued from previous page.

Total 4035	353	3373	7	1	65	6	2	3	11	21
Diagnosis	Chickens	Cows	Dogs	Geese	Guinea Pigs	Hogs	Horses	Human	Sheep	Turkeys
Nemo-lymphomatosis	38									
Nemo-lymphomatosis and ascariasis	6									
Omphalo-phlebitis									2	
Osteomalacia	1									
Peritonitis	7	1								
Poison: Strychnine	9									
Pullorum disease	72									
Roup	6									
Ruptured egg in oviduct	6									
No diagnosis	60	8		1	2	1			1	1

RECOMMENDATIONS

The person in charge of laboratory diagnostic work should be prepared. The various media and stains likely to be needed should be kept ready for use. Some of these are:

Media:

Liver infusion agar plates and slants, Ph 6.6

Liver infusion broth in tubes, Ph 6.6

Plain agar plates and slants, Ph 6.6

Plain broth in tubes, Ph 6.6

Sugars

Stains:

Bismark brown

Wright's stain

Aqueous solution of Gentian violet

Alcoholic solution of Gentian violet

Carbol fuchsein

Gram's stains

Bromthymol blue

Eosin

All equipment should be kept in its allotted place so it may be found readily. Sterile glassware should be available at all times for immediate use.

For Bang's abortion disease diagnosis, fresh antigen should be prepared every thirty days. Antigen prepared by using phenolized 12% saline solution is recommended.

A minimum charge should be made for every diagnosis in order to justify the expenditure of and the purchase of necessary equipment for thorough examinations. The only charge for diagnostic work at present is ten cents for each sample tested for Bang's abortion disease. A total income of $212.80 was received for this work during the course of this study. More than one-half of the abortion diagnostic work was done on the college herds and no charges were made for this.

The practice of sending in dead animals, birds, or other perishable material for laboratory diagnosis should be discouraged because it is often impossible to make a satisfactory diagnosis with such material, due to decomposition changes.

Mimeographed sheets of some of the more numerous diseases should be prepared to send to the poultry and live stock owners. This would greatly reduce the amount of letter writing that has to be done. These mimeographed sheets should tell the cause, symptoms, lesions, prevention and treatment of the disease in question.

LITERATURE CITED

1. Mallory and Wright - Pathological Technique. Eighth edition, 1924.

2. Hiss and Zinsser - A Text Book of Bacteriology. Fifth edition, 1923.

3. Kaupp, B. F. - Poultry Diseases.

4. Ward and Gallagher - Diseases of the Domesticated Birds.

5. Hutyia and Marck - Pathology and Therapeutics of the Diseases of Domestic Animals.

6. Practical Bacteriology Blood Work Parasitology. Still, E. R. Eighth edition, 1923.