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Field Survey of Important Causes of Poultry Diseases in Dus- Karmato City, Iraq.

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ABSTRACT

Background: The Study has been done to know the most popular causes disease in poultry that have been received by the fields consultants in City Dus- Karmato during the period from 1/1/2014 to13/12/2014.

Method: The results showed that a total of 250 sick and dead chickens were collected from 4 different poultry. Diagnosis of the cases have been done according to clinical history, clinical signs and postmortem findings of the affected birds whereas; confirmatory diagnosis was made by using cultural examination, Grams staining and different biochemical tests, Lacto phenol cotton blue, enzyme–Linked Immune sorbent assay, and antimicrobial sensitivity tests.

Results: In this study out of 120, 90(47.9%) chickens were clinically diagnosed as colibacillosis, 80(42.6%) as salmonellosis and 18 (9.6%) as Staphylococcus aureus. In the same way, out of 90 chickens, 30 (33.3%) were diagnosed as Infectious bronchitis (IB), 25(27.8%) as Infectious laryngotracheitis (ILT), 20(22.2%) as Infectious Bursal Disease (IBD), 10(11.1%) as Newcastle virus and 5(5.6%) as Reo virus. While the result of fungi test showed with, out of 40 chickens, 25(62.5%) as Aspergillus Fumigatus and 15 (37.5%) as Candidiasis.

Conclusion: Antimicrobial susceptibility test showed that most of the isolates in the study area Escherichia coli and salmonella spp. were found to be highly sensitive to Ciprofloxacin, Sulphanomide, Amoxcyline and Tetracyline, moderately sensitive to Neomycin and Resistant to Penicillin and Erythromycin. While showed with Staphylococcus aureus isolates sensitive to Ciprofloxacin, Penicillin and Erythromycin, moderately sensitive to Tetracyline and Resistant to Sulphanomide, Amoxcyline.

KEYWORDS: Poultry, Antimicrobials.

INTRODUCTION

In the last half century, significant increases in the productivity of modern poultry stocks have been achieved for both the meat and the egg production sectors of the global poultry industry. Synergies have resulted from advances made in all the major activities of poultry management and housing, nutrition and ration formulation, applying poultry genetics knowledge in commercial breeding programmes and better diagnosis and control of avian diseases. Of all these core elements, poultry health and disease can be the least predictable.[1]

Although poultry diseases from nutritional and metabolic causes can be of concern, the emphasis in this information note is on controlling diseases that are caused by infectious agents, which can exert damaging – and sometimes immediate – negative effects on the profitability of commercial operations. The development of an intensive poultry industry in many of the countries discussed here depends on the growth in number and size of small and medium-sized commercial poultry operations. The emphasis in this review is therefore primarily on optimizing poultry health for this scale of operations. Because of the importance of small-scale village-based production units in many developing countries, however, the poultry health implications for and such flocks are also included.[2]

Poultry that have an infection show a variety of symptoms, such as respiratory problems, diarrhoea and paralysis. It should be emphasized at the outset that prevention of infection in a poultry flock through sound management is very important. This is because although some infectious diseases can be treated, for many it is a waste of time and money and infected birds should be disposed of immediately.[3]

Poultry can be affected by many types of disease, and a wide variety of pests (and behavioral problems) including those Viruses, Mycoplasmas, Bacteria, Fungi,

Protozoa, Internal Parasites, External Parasites, Metabolic Disorders, environmental factors and Pests.[4]
Although the relative importance of poultry diseases may differ between countries and geographical areas, there are few important diseases that are unique to particular parts of the world, at the global level, however, differences in distribution among regions are now apparent, because genetic variants have emerged within some of the major specific pathogens of chickens. This has become important for attempts to prevent the spread of virulent strains through international movements of poultry products.[5]

MATERIALS AND METHODS

Study design and methodology

The study design used was cross-sectional study and it was carried out from 1/1/2013 to13/12/2013. the methods employed in this study clinical history, clinical signs and postmortem findings of the affected birds whereas; confirmatory diagnosis was made by using cultural examination, Grams staining and different biochemical tests, Lacto phenol cotton blue **,**enzyme–Linked Immune sorbent assay, and antimicrobial sensitivity tests.

Samples for the isolation of bacteria fungi and viral

For isolation and identification of bacteria and fungi different samples like liver, lung, heart, heart blood, spleen, peritoneal fluid, intestinal contents, tracheal swab, and egg follicles were collected from affected chickens. Blood samples (5ml) were obtained from each chicken by wing vein-puncture. Sera sample was stored at -20c for serological study.

LABORATORY DIAGNOSIS OF BACTERIAL, FUNGI AND VIRAL DISEASES

Culture and morphological staining of bacteria and fungi

The isolation and identification of the isolated colonies in different culture media was performed using standard bacteriological and fungi procedures.[6] The representative bacterial and fungi colonies were characterized morphologically using Gram's stain and Lacto phenol cotton blue.[7]

Motility test

The motility test was performed to differentiate motile and non-motile bacteria isolated from different suspected bacteriological samples.[8]

Reactions of the organisms in TSI agar slants

Triple sugar iron agar (TSI agar) was used to detect the lactose, sucrose and dextrose fermenters and also the bacteria which produce hydrogen sulphide. The organisms were seeded over the surface of the slants and stabbed into the butt where the cases changes after an incubation of 24 hours at 37°C.

Sugar fermentation test

The sugar fermentation test was performed using five basic sugars (e.g., dextrose, sucrose, lactose, maltose, and mannitol) separately according to the standard procedures.[9]

Catalase test and coagulase test

Slide catalase and tube catalase tests were performed to differentiate the isolated bacteria.[10] The results of coagulase test positive and negative samples were recorded according to the standard method.[11]

Indole test, Methyl red test and Voges-Proskauer test

These tests were used to differentiate the isolated bacteria from various bacteriological samples collected from sick and dead birds. The test was performed and result was interpreted according to the standard procedure.[11]

Agar slant and 25% sterile buffered glycerin

The organisms isolated were inoculated into the nutrient agar slants to preserve them as a stock culture for few months and in 25% sterile buffered glycerin for several years at -80ºC without any deviation of their original characters.[6]

Elisa Kits.

Identification of viral were used ELISA Kits for detection Abs in samples according to manufactured instruction by Biochek Smart Veterinary Diagnostics.

Antimicrobial sensitivity test

In vitro antibiotic sensitivity test (Kirby-Baur disc diffusion) method was carried out in order to identify the most effective drugs for mastitis treatment in the study area. A loop full of colony from the growth of isolates was transferred to the nutrient broth in tubes and incubated at 37°C for 5 h. Mueller-Hinton agar which was used as plating medium was inoculated with broth (bacterial suspension) by using cotton swab. Then antibiotic impregnated paper disc (Oxoid, UK) were applied and pressed onto the plate with forceps. Plates were incubated at 37°C for 18 h. The diameters of zones of growth inhibition were measured in millimeter and interpreted as sensitive, intermediate and resistant to different antibiotics.[12] The drugs used were Ciprofloxacin, Sulphanomide, Amoxcyline, Tetracyline, Neomycin, Penicillin and Erythromycin.

RESULTS & DISCUSSION

Results indicated that out of 250 sick and dead chickens, 120 (48%) were found to be positive for bacterial growth, 90 (36%) were positive for viral examination and 40 (16%) were positive for fungi examination in chickens Table 1.

Table 1: Results showing the prevalence of causative agent in Dus – Karmato chickens

	No of chickens	Porportion	
		Number	%
1	Positive for bacterial growth	120	48
2	Positive for Viral examination	90	36
3	Positive for Fungi examination	40	16
	Total Number examined	250	

These findings of the present study agree with the findings of previous studies.[14] Diseases occurs due to lack of proper care and management, inadequate nutritious feeding and some other factors.

RESULTS OF CLINICAL DIAGNOSIS AND LABORATORY DIAGNOSIS OF BACTERIAL, VIRAL AND FUNGI DISEASES.

Clinical diagnosis Colibacillosis

About 90 (47.9%) of the suspected chickens were diagnosed as colibacillosis. The most obvious clinical signs were diarrhoea, depression, soiling of cloaca with semisolid cheesy material, respiratory distress (coughing, sneezing), reduced egg production, loss of condition and death. Recorded postmortem lesions were omphalitis and fluid accumulation in the peritonial cavity of chickens, dark-colored swollen liver and spleen, fibrinopurolent airsacculitis, pericarditis, perihepatitis hemorrhagic enteritis with fluid accumulation in ligated intestinal loops and diarrhea, arthritis, panophthalmitis, and salpingitis in some cases. Similar types of findings were described in previous studies.[14,15]

Salmonellosis

Out of 80 (42.6%) suspected chickens were clinically diagnosed as Salmonellosis. Most common clinical signs of Salmonellosis were drowsiness, huddled together, poor growth, chalky white diarrhoea with pasted vent, dehydration, reduced egg production and death .After necropsy the gross lesions were observed as peritonitis, unabsorbed yolk ,discrete, small, white, necrotic foci in the liver which became swollen and fragile with distinctive coppery bronze sheen on the surface ,turbid yellow color fluids in the peritoneal cavity and irregular, haemorrhagic ova with prominent thicken stalks .These findings were supported by previous studies.[16,17]

Staphylococcus aureus

Out of 18 (9.6%) suspected chickens were clinically diagnosed as Staphylococcus aureus. Most common clinical signs of Staphylococcus aureus infections appear in three forms-septicemia (acute), arthritic (chronic), and bumblefoot. The septicemia form appears similar to fowl cholera in that the birds are listless, without appetite, feverish, and show pain during movement. Black rot may show up in eggs (the organism is passed in the egg). Infected birds pass fetid watery diarrhea. Many will have swollen joints (arthritis), follows the acute form. Birds show symptoms of lameness and breast blisters, as well as painful movement. Birds are reluctant to walk, preferring to sit rather than stand. Bumblefoot is a localized chronic staph infection of the foot, thought to be caused by puncture injuries. The bird becomes lame from swollen foot pads. These findings were supported by previous study.[18]

Infectious Bronchitis

Out of 30 (33.3%) suspected chickens were clinically diagnosed as Infectious Bronchitis Most common clinical signs of Chicks may cough, sneeze, and have tracheal rales for 10–14 days. Conjunctivitis and dyspnea and sometimes facial swelling, particularly with concurrent bacterial infection of the sinuses. Chicks may appear depressed and huddle under heat lamps. Feed consumption and weight gain are reduced. Infection with nephropathogenic strains can cause initial respiratory signs, then later depression, ruffled feathers, wet droppings, greater water intake, and death. After necropsy the gross lesions were observed as In the respiratory tract, the trachea, sinuses, and nasal passages may contain serous, catarrhal, or caseous exudates, and the air sacs a foamy exudate initially, progressing to cloudy thickening. If complicated by infection with *E coli*, there may be caseous airsacculitis, perihepatitis, and pericarditis. Infection with nephropathogenic strains results in swollen, pale kidneys, with the tubules and distended ureters.[19]

Infectious Laryngotracheitis (ILT)

Out of 25(27.8%) suspected chickens were clinically diagnosed as Infectious Laryngotracheitis (ILT). Most common clinical signs in chickens were acute infections, nasal discharge, moist rales, coughing and gasping. The neck may be extended ("pump handle respiration"). The eyelids can be red with increased ocular drainage. In severe cases there is marked labored breathing and coughing of blood-stained mucus, which covers the wing and breast feathers as a result of the bird shaking the head with violent coughing. Blood and yellow exudate in the trachea cause death by suffocation. In mild cases, after gross lesions are in the trachea and larynx. In severe cases, the trachea contains clotted blood. White fibrinonecrotic plugs can fill the glottis and trachea.[20]

Infectious Bursal Disease

Out of 20 (22.2%) suspected chickens were clinically diagnosed as Infectious Bursal Disease. Most common clinical signs in chickens were depression and ruffling of feathers, poor or lack of appetite huddling, unsteady gate, reluctance to rise, and diarrhea (sometimes, bloody). Immuno suppressed survivors may be affected with other disease agents, resulting in various secondary infections that can end in death, or manifest as respiratory or gastrointestinal disease. After post-mortem lesions (identified upon autopsy) indicative of IBDV infection. The bursa of Fabricius is the main organ affected, showing swelling from edema and hemorrhage during the early stages of the disease and then shrinking (atrophy) 7-8 days following infection. Bleeding in the breast and thigh muscles may be noted due to impaired blood clotting. Enlarged kidneys and spleen are also typical of IBDV infection. These findings were supported by previous study.[21]

Newcastle Disease

Out of 10 (11.1%) suspected chickens were clinically diagnosed as Newcastle Disease. Most common clinical signs in chickens were sudden death, depression,

inappetance, coughing, dyspnoea, diarrhoea, nervous signs, paralysis, twisted neck, severe drop in egg production and moult. After Post-mortem lesions, Airsacculitis, Tracheitis, Necrotic plaques in roventriculus, intestine, caecal tonsil, Haemorrhage in proventriculus and Intestinal lesions primarily occur in the viscerotropic form. These findings were supported by previous study.[22]

Reo virus (viral arthritis)

Out of 5(5.6%) suspected chickens were clinically diagnosed as Reo virus (viral arthritis) Most common clinically it is manifested by lameness and swellings affecting primarily tarsometatarsal joints and the feet. Many infected birds are in a good general condition, but some could be lethargic and exhausted. After post-mortem examination of affected birds, lesions always consisted of discrete swelling and oedema of the tendons, Swelling and accumulation of inflammatory fluid, Unilateral femoral head necrosis occurred in one cockerel, concurrently suffering from tenosynovitis These findings were supported by previous study.[23]

Aspergillosis

Out of 25 (62.5%) suspected chickens were clinically diagnosed as Aspergillosis. Most common clinically it is manifested by gasping and rapid breathing can be observed. Mortality is variable, from 5 to 50 %. Gross lesions involve the lungs and airsacs primarily. Yellow-white pin headsized lesions can be found. Sometimes all body cavities are filled with small yellow-green granular fungus growth. These findings were supported by.[24]

Candidiasis

Out of 15 (37.5%) suspected chickens were clinically diagnosed as candidia. Most common clinically it is manifested by present in only severely affected birds as depression, retardation of growth, stunted appearance, reduced food intake ruffling feathers, listlessness, diarrhea, may drooling mucus exudates of offensing odour from mouth . Drop of egg production in layers, pendulous crop in some of the affected birds may occur, when candidiasis occurs as a secondary infection, the signs of the predisposing disease may predominate the clinical signs. Lesions most frequently in the crop and to less extent in oesophagus and pharynx may extend to the buccal cavity and proventriculus. The lesion appear grossly as thickening of the mucosa with raised multifocal (circular) to confluent whitish cheesy material often there are pseudo or diphtheritic membranous necrotic batches that are peeled easily from the eroded mucosal surface sloughed ulcer formation may occur, when the proventriculus is affected its mucosa hemorrhagic with whitish membrane and appears swollen. Findings were supported by previous study.[25]

Table 2: laboratory diagnoses of different samples

	Caustive agent		Number	%
1	***Escherichia coli***		90	47.9
2	***Salmonella pullorum***		35	13.29
3	***Salmonella paratyphoid***		25	18.61
4	***Salmonella gallinarum***		20	10.6
5	***Staphylococcus aureus***		18	9.6
	Total	188		
6	***Infectious Bronchitis(IB)***		30	33.3
7	***InfectiousLaryngotracheitis (ILT)***		25	27.8
8	***Infectious Bursal Disease (IBD)***		20	22.2
9	***Newcastle Disease (ND)***		10	11.1
10	***Reo virus(viral arthritis)***		5	5.6
	Total	90		
11	***Aspergillosis***		25	62.5
12	***Candidiasis***		15	37.5
	Total	40		

On the basis of cultural and biochemical properties of the isolates, the result showed with188 bacterial isolates belonging to 5 species,90 virus belonging to 5 species and 2 fungi belonging 2 to species (Table2).

COLIBACILLOSIS CULTURAL EXAMINATION

The organisms were isolated from the samples on different agar media, where smooth, large, colorless, circular colonies on blood agar, rose pink colonies with precipitate on MacConkey agar, yellow to greenish-yellow colonies surrounded by an intense yellow-green zone on BGA, the blue-black colonies with characteristic green sheen on EMB agar, slight growth and pink to rose-red colonies with precipitate on SS agar media and yellow colored slant with the accumulation of gas bubbles in the butt on TSI agar slant were recorded which was corresponded with the findings of others.[26,27]

Gram's staining and motility test

The Isolated organisms of different pure culture media were gram-negative, pink colored, small rod shaped and

arranged in single, pairs or short chain which supports the findings of previous researchers.[6-8] The test organisms were found to be motile which is similar with the findings by previous study.[8]

Biochemical tests

The organisms produced both acid and gas by fermenting glucose, dextrose, maltose, lactose, sucrose and mannitol. Acid production was indicated by the color change of the sugar media from reddish to yellow and the gas production was noticed by the presence of gas bubbles in the inverted Durham's tube.[6] The organisms were positive to methyl red and negative to voges-proskauer reactions.[28] Hydrogen sulphide was not produced but Catalase and Indole test were positive which support the findings of previous study.[6,10] The result has been shown in Table 3.

Table 3: Biochemical properties of bacterial isolates

	Bacterial isolates	Carbohydrate fermentation tests					MR	VP	Indole	Catalase	H2S
		DX	ML	L	SU	MN					
1	*E.Coli*	AG	AG	AG	AG	AG	+	-	+	+	-
2	*Salmonella Spp*	AG/A	-/A	-	-	AG/A	+	-	-	+	+
3	*Staphylococcus aureus*	-	+	+	+	+	-	-	-	+	-

Table4. Results of antimicrobial sensitivity tests on the bacterial isolates.

	Pathogen	Total isolate	Cip	Sul	AMO	T	Neo	PEN	E
1	*Escherichia coli*	90	S	S	S	S	I	R	R
2	Sal. Spp	80	S	S	S	S	I	R	R
3	*Staphylococcus aureus*	18	S	R	R	I	R	S	S

T, Tetracycline (30 µg); Sul,Sulfamide, (25 µg); AMO, amoxicillin (10 µg); PEN, penicillin (10 IU); Cip, Ciprofloxacin (5 µg); E,erthyomycin(30 µg);Neo,Neomycin,(30 µg); S/R, sensitive/resistance.

SALMONELLOSIS CULTURAL EXAMINATION

The organism (*Salmonella* spp.) were grown on different media where they produced turbidity in nutrient broth; circular, smooth, opaque and translucent colonies on NA, colorless colonies with black centers on SS agar, pale pink color colonies against a pinkish background on BGA; Pale, smooth, transparent and raised colonies on MacConkey agar; large, colorless colonies on EMB agar media and on TSI agar slant *Salmonella gallinarum* produced black colony converting the slant to yellow color whereas *Salmonella pullorum* produced white colony and the slant converted to pink color which was corresponded with the findings of others.[6,29] (Table 3).

Gram's staining and motility test

The test organisms were gram-negative short, straight rod and mostly occurred singly occasionally paired which also corresponded with morphological characters of Salmonella as described by several authors. *Salmonella gallinarum* and *Salmonella pullorum* were non-motile whereas other poultry *Salmonella* spp. were found to be motile.[10]

Biochemical tests

Salmonella spp did not ferment lactose and sucrose but fermenting dextrose and mannitol produced both acid and gas which was corresponded with the findings of others.[30] *Salmonella gallinarum* produced only acid whereas *Salmonella pullorum* produced both acid and gas and all of the isolates were indole negative, methyl red positive and VP negative which are special biochemical characters for *Salmonella spp.* That previously suggested by a number of scientists.[30,31]

STAPHYLOCOCCUS AUREUS CULTURAL EXAMINATION

The organism (***Staphylococcus aureus***) were grown on different media where they produced turbidity in nutrient broth; round, smooth, shiny, opaque, golden yellow color on Mannitol salt agar media and nutrient agar, yellowish, smooth colonies with no hemolysis on blood agar which was corresponded with the findings of others.[32]

Gram's staining and Catalase activity test.

The test organisms were Gram-positive cocci and arranged in grape like clusters and positive, coagulase, catalase test with production of bubbles which was corresponded with the findings of others.[10,33]

Biochemical tests

The organisms produced both acid and gas by fermenting glucose, maltose, lactose, sucrose and mannitol. Acid production was indicated by the color change of the sugar media from reddish to yellow and the gas production was noticed by the presence of gas bubbles in the inverted Durham's tube.[34] The organisms were negative to methyl red and negative to voges-proskauer reactions.[34] Hydrogen sulphide was not produced**.**

ASPERGILLOSIS CULTURAL EXAMINATION

The organisms (*Aspergillus fumigates)* were isolated from the samples were grown on sabaroud dextrose agar (SDA); Blue – green, powdery and pale yellow on reverse and Lactophenol cotton blue staining of colony to see conidophores which are smooth, colorless to light

green near the vesicle. The conidiophore enlarges to form a flask-shaped vesicle. Conidiophore contains globase vesicles, phialides and radiate chains of conidia, which was corresponded with the findings of others.[35]

CANDIDIASIS CULTURAL EXAMINATION

The organisms were isolated from the samples were grown on sabaroud dextrose; white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells or blastoconidia and Lactophenol cotton blue staining of colony to see permitting visualization of pseudohyphae and budding yeast cells typical of many *Candida* species, which was corresponded with the findings of others.[36]

ANTIMICROBIAL SENSITIVITY TEST

A total of 188 isolates from were tested for sick and dead chickens. Their *in vitro* antimicrobial sensitivity result shows that escherichia coli and salmonella spp were found to be highly sensitive to ciprofloxacin, sulphanomide, amoxcyline and tetracyline, moderately sensitive to neomycin and resistant to penicillin and erythromycin which was corresponded with the findings of others.[37,38] While Staphylococcus aureus isolates were sensitive to ciprofloxacin penicillin and erythromycin, moderately sensitive to tetracyline and resistant to sulphanomide, amoxcyline which was corresponded with the findings of others.[39,40] The result has been shown in Table 4.

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