Detection of Infectious Causes of Arthritis in Breeder Chicken Flocks

A .El-H. Abbas*, M. M. Amer**, M.A. Bastami** and S. A. El Mahdi***
*Wadi Poultry Company, Cairo.
** Department of Poultry diseases, Faculty of Veterinary Medicine, Cairo University.
***Institute for Quality Control, Abbasia, Cairo.

SUMMARY. This study was carried out to investigate infectious causes of arthritis in hock joint in 10-16 weeks breeder chicken flocks clinically affected; 40 joints were subjected to isolation of bacterial causes (gram–ve and gram +ve),
Mycoplasma synoviae (MS) and Reovirus by culturing of prepared joint samples on Mac Conkey, nutrient and blood agar plates for bacterial causes, pplo broth followed by pplo agar plates for MS as well as inoculation of 9-10 days old chicken embryos via allantoic sac for reovirus. Results of this study proved isolation of E.coli (17.5%) Staph aureus (100%) proteus (7.5%) and reovirus isolates (70%) all examined samples were MS negative.
Furthermore trials were carried out to isolate MS from joints, lungs and tracheas of clinically positive cases of hock joint affection and serologically (ELISA) positive without success in MS isolation.
Representative isolates were inoculated in footpad of 1 day old chick groups (10 chicks each) to induce hock joint arthritis experimentally as group for each E.coli, Staph, Proteus, Reovirus and joint extract; arthritis induction has been proved in inoculated chicks at rate of 25%, 80%, 30%, 60% and 60% respectively.
Histopathological examination of joints from naturally affected and experimentally inoculated chicks were also carried out.

Key words: Detection; Infectious Arthritis; Breeder flock

Introduction:

Arthritis in hock joint of broiler breeder flocks have many causative agents; viral, avian reovirus is ubiquitous among poultry populations and thought to be the causative agent of viral arthritis, enteritis/diarrhea, and stunting or malabsorption syndrome (Rosenberger and Olson, 1991).

Mycoplasma, MS has the direct association with leg pathologies, which result in lameness (Wyeth, 1974; Mohamed et al., 1987; and Branton, et al., 1997a). And other bacterial agents, particularly Staphylococcus; Staphylococcus produces arthritis and tenosynovitis following septicemia causing common infections in tendon sheaths and joints especially tibiotarsal and stifle joints (Miner et al., 1968; Itakura et al., 1976; Ridell, 1980;Kibenge et al., 1982; Emslie and Nade, 1983; and Kibenge and Wilcox, 1983).

This study was carried out to investigate infectious causes of arthritis in hock joint in broiler breeder flocks showing signs of hock joint inflammatory affection.

Material and Method

Naturally infected joints: Joints; 40 legs of inflamed hock joint out of 40 broiler breeders aged 10 to 16 weeks of age with signs of lameness, inability to reach food or water and highly emaciated.

Experimental chicks and chickens:

· Ninety day old Ross commercial broiler chicks were used for foot pad inoculation and experimental infection with the isolates of reo virus, vaccinal live strains of reovirus, staphylococcus aureus and E. coli isolates.
· 80 Ross broiler breeder chickens at the 8th week of age (40 females and 40 males) were used in experimental infection by 3 routes of inoculation with the isolates of Reovirus, Staphylococcus aureus and E. coli.

Experiment 1:-
Whole legs with affected joints were separately collected under aseptic conditions in clean bags; Four sterile swabs were used for collection of samples for Mycoplasma isolation, from each bird, as 2 from trachea and 2 from joints. Joints from affected birds were separately collected aseptically, the collected joints were cut into one cm sections of each tendon, synovial sheath and membrane joint cartilage; flushed with antibiotic PBS and kept at 4º C for one hour; Joints from affected birds were fixed in 10% formol saline for histopathological examination. Nutrient agar, blood agar, Mac Conkey agar and modified Hayfilk's medium (Freundt, 1983 and Senterfit, 1983.). Mycoplasma broth medium, Mycoplasma agar medium Was prepared according to (Frey et al 1968) VERO cells (African green monkey cell line); kindly obtained from (CLEVB) Central Laboratory for Evaluation of Veterinary Biologics VERO cells were used for serum neutralization test. Dehydrated Eagl's Minimum Essential Medium (MEM) with Earls salts and L-glutamine without sod – bicarbonate was used.

Chemicals and reagents:
Faetal calf serum, trypsin solution (Difco 1:250), Buffers and other reagents Phosphate buffer saline, Formaldehyde solution, Haematoxylin and eosin stain (for histological sections), Gram stain (for bacterial isolation), Antibiotics, commercial antigens for Mycoplasma synoviae and REO virus; and ELISA kits for Reo and Mycoplasma synoviae.
Chemicals and reagents for serological tests Immune serum, Prepared serum, Reference antiserum, REO positive control serum, Antimycoplasma synoviae serum, Normal Control Serum used as negative control, agar medium for precipitation test, Nobel agar (Difco), Hank's balanced salt solution (HBSS).
Embryonated chicken eggs (ECE), chicken embryo cell culture (CEF).
Bacterial Isolation :-
Aseptically collected joints were opened a loopfull from each of exudates in articular surface, Synovial sheath, tendon sheath and around joints were separately taken and streaked; each sample separately on 2 plates of nutrient agar, 10% sheep blood agar and Mac Conkey agar; The inoculated plates were incubated for 24 hours at 37ºC after inoculation, the bacterial growth was examined and subjected for further studies for identification.
Mycoplasma in broth:-
The collected swabs (2 from joint and 2 from trachea / affected bird) were separately dipped in modified Hayflick's broth, followed by culturing in Mycoplasma broth base with serial ten fold dilution up to 10-3 , platted on Mycoplasma agar broth, incubated at 37ºC under Co2 in candle jar for one week. Mohamed et al., (1986c) Metwalli (1989).

Mycoplasma on agar the 7 days incubated broth was subcultured on agar plates (2 plates / broth tube); all inoculated plates were incubated at 37ºC under Co2 in candle jar for 3 weeks with examination under dissecting microscope.
All the collected samples were prepared for bacterial, Mycoplasma and viral isolation and for serological examinations.

Results:

1- histopathological examination
joints of 16 weeks chicken show necrosis with massive number of leucocytic cells infiltration were noticed in the tendon sheath, mononuclear leucocytic inflammatory cells infiltration in the periosteum, tendon sheath and synovial membrane, osteomalacia was observed in the bony structure of the chondyle. Joints of 22 weeks chicken naturally infected show oedema, inflammatory cells infiltration and necrosis were detected in the tendon sheath and synovial membrane, The necrosed area had a mash of fibrin threads intangled in it massive number of leucocytes, periosteum showed stratification with inflammatory cells infiltration,The bony structure of the joint had osteomalacia In general the joint with signs of natural infection had shown soft tissue surroundings the tendons, sheath was infiltrated by leucocytic inflammatory cells as well as penetrated by adipose tissue in focal manner. Joints of apparent normal control group chicken show the normal the normal histological structure.
isolates Identification of REO by: -

1-AGPT using known hyperimmune serum of the reference strain S-1133, clear lines of precipitation were detected at the dilution 1:4 from embryos died at the 5th day post inoculation. 2- by the inoculation of the CEF tissue culture, clear giant cell was formed in 72 hours post inoculation. 3- REO virus; Agar gell precipitation test results showed that only 13/40 positive (32.5%) as they give precipitation lines in 3 days after filling with diluted serum; 1/8 to 1/64; as the highest two fold dilution of serum giving a precipitating response in the AGPT, Neutralization test; 10 samples (25 %) showed titers over 10 in the SNT; it was observed that positive samples for AGPT were also positive for SNT, ELISA; titer of the 40 samples were also shown in the following table ( ) MS All tested samples show negative results for Mycoplasma synoviae in both serum plate agglutination and ELISA tests; optic density for MS ELISA was ranged between 0.13-0.40 as represented by 0 titers._Histopathological Examination; The ECE inoculated by prepared joint extract were examined histpathologically and compared with ECE inoculated by serially passaged extract and REO reference strain S1133; lesions score as follows in table (1)
Experiment 2:-
Experimental induction of arthritis in day old chicks using the isolated agents; 80 commercial Ross broiler day old chicks were used; the chicks were randomly divided into 8 groups; 10 chicks each, Chicks of groups 1-7 were infected with 0.1 ml via foot pad route as follows: - Group 1= chicks were infected by isolated staph aureus in a dose of 0.1 ml of 107.5 CFU/ml. Group 2 = chicks were infected by 0.1 ml containing 104.5 PFU/ml of the 3rd passage reovirus. Group 3 = chicks were infected by 0.1 ml of isolated staph aureus and viral arthritis. Group 4 = chicks were infected by 0.1 ml from extract of naturally infected joint. Group 5 = 0.1 ml of joint extract and isolated proteus

of 107 CFU/ml infected chicks. Group 6 = chicks were infected by 0.1 ml of staphylococcus aureus and isolated proteus. Group 7 = chicks were
infected by 0.1 ml 10^7 CFU/ml proteus only. Group 8 = chicks were left as non-infected control group. All groups were subjected for daily observation up to 12 days for clinical signs, mortalities and trials of reisolation of the inoculated organism at 1,3,6,9,12 days post infection as well as histopathological examination of samples from organs showing lesions; expressed as lesion score in (tables and), Results of the experiment are shown in tables (1 and 3).

Results:-

In group 1 signs; huddling, chevering, inability to move; was seen within 24 hours post inoculation in 8 out of the inoculated 10 chicks (80%) and lasted for 3 days. chicks showed 5% mortality in 3 days as mortality started in the 2nd day PI. Lesions; sever congestion with hotness and swelling of the whole infected leg (gangarenous changes occur inchick. Histopathological; the hock joint show thickening with focal lymphocytic cells aggregations in the synovial capsule, while foot pad shows focal necrosed area surrounded by heterophils and mononuclear leucocytes mainly lymphocytes were observed in the connective tissue and muscular tissue adjacent the foot pad focal circumscribed round granuloma like formation was detected in the periosteum. Staphylococcus aureus was reisolated from heart blood, liver and footpad, only. **In group 2** signs; varied from mild to moderate lameness, started in 3- 5 days PI in 60% of the chicks and lasted for about 5 days, without any mortality. Lesions; was started as mild swelling of footpad and hock, this swelling slowely decreased by time. Histopathologically examined hock joint shows thickening with focal lymphocytic cells aggregation in the synovial capsule. Associated with foccal necrosis. Lymphocytes were also infiltrated the synovial capsule in diffuse manner, mononuclear leucocytes were infiltrated the skeletal muscle surrounding the joint, while the footpad mononuclear leucocytic inflammatory cells were infiltrated the deep connective tissue of the dermis. Lymphocytes were also aggregated in focal manner at the interdigital tissue. Surrounding the blood vessels and in the skeletal muscle surrounding the foot , massive number of lymhocytes were infiltrated the periosteum of the footpad. This group of chicks showed negative reisolation from died birds or birds with signs. **In group 3** signs; of sever lameness and inability to move
started 24 hours PI in 40% of the inoculated chicks and lasted for 5 days only, while only deaths of 2 birds (20%) was occurred the 2nd day post infection. Rests of affected birds (20%) were recovered by the end of the 5th day. Died birds showed sever gangrenous swelling of the whole leg with sever septicemia and congestion of the muscles and viscera of the affected birds.

Histopathological examination shows that the hock joint synovial capsule was infiltrated by mononuclear leucocytic inflammatory cells associated with extravasted red cells (fig 28) there was thickening with inflammatory cells infiltration in the periosteum; at the 2nd and 3rd days PI. While footpad showed only mild thickening noticed in the interdigital tissue. Only staphylococcus aureus was reisolated from birds showing symptoms; while; the others were negative. **In group 4** signs of mild lameness started 6 days PI in 60% of infected chicks lasted till the 7th day, where all birds were recovered. Affected joints show only slight swelling. No mortality or marked lesions could be detected. Histopathological examination of this group revealed the following; liver

dilatation of the central veins and sinusoids associated with diffuse proliferation of Kupffer cells , and focal lymphocytic cells aggregations in hepatic tissues, heart; myeloid cells were aggregated in focal manner in between the myocardial bundles, while the lymphocytes were infiltrated the myocardium in diffuse manner. Lung, interlobular blood vessels were severely dilated and encorged with blood associated with perivascular oedema . Spleen; focal circumscribed round aggregations of hyperplastic lymphoid cells were observed in diffuse manner all over the spleenic tissue. Bursa of fabricius; follicles showed lymphoid cells hyperplasia with sever increase in the size and width. Footpad; lymphocytes were infiltrated the digital, interdigital and foot pad connective tissue in diffuse manner with focal hyalinization . while hock joint showed no charactrestic alteration. Moreover, reisolation revealed negative results. **In group 5** signs; were slight swelling in foot pad in 20% of the infected birds, and no mortality occurred in this group. Proteus was reisolated from footpad, only from 30% of the inoculated chicks. Lesions; only mild swelling of the footpad were in 30% of chicks. Histopathological lesions were oedema in footpad with mononuclear leucocytic inflammatory cells infiltration were noticed in the periosteum and connective tissue of the foot pad, while no lesions could seen in the hock joint. **In group 6** signs; were sever acute swelling of the leg following first 24 hours PI in 70% with 40% mortality in 2 days, signs lasted for 3 days and began to decline in the survives. Lesions were acute ulcerative gangrenous swelling of the whole leg particularly the footpad, acute septicemic picture of the whole muscles and viscera.

Histopathological findings were no alteration in hock joint tissues, while footpad showed the following, focal circumscribed round areas of lymphocytic cells aggregations as well as diffuse infiltration of the lymphocytes with newly formed blood capillaries and fibrous tissue was observed in the thickened periosteum. Massive number of lymphocytes infiltrated the connective tissue and muscular tissue surrounding the osseous tissue of the footpad. Staphylococcus, proteus, were reisolated and also E-coli out of the heart blood, liver and foot pad of 100% of birds showing signs (70% from total). **In group 7** birds of this group showed only mild signs in 30% of the chicks at the 2nd day PI, without mortality. Signs started to disappear at the 4th day. Slight swelling in the hock joint was the only recorded lesion.

Histopathological examination of these chicks revealed that hock joint tissues were surrounded by mnonuclear leucocytic inflammatory cell infiltration. **Birds of group 8** (non-infected control) showed no detectable signs, lesions and negative cultures. Both joint and foot pad showed normal histological structure.

Table (1) Lesions histopathological score of chicken experimentally infected in the next table:-

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Hock joint s.	+	++++	---	+	±	-	-	-
h.	++	+	---	-	-	-	-	-
m.	---	+	+	-	-	+	+	-
Foot bad d.	+++	+++	++	++	+	±	-	-
p.	+++	+++	++	++	+	±	-	-
m.	+++	+++	+	±	+	+++	-	-

Abbreviation key: s = synovitis h = hyperemic, haemorrhagic; p = periostitis; d = dermatitis; and m = myocitis.

Table (2): Results of ELISA, SNT, AGP tests and Reo virus isolation from naturally infected joints.

No	ELISA OD	ELISA titer	SNT	AGP	virus	No	ELISA OD	ELISA titer	SNT	AGP	virus
1	0.32	1618	0	-	-	21	1.16	8851	32	32	±
2	0.33	1698	0	-	-	22	0.29	1339	0	0	-
3	0.30	1442	0	-	-	23	0.91	6951	0	0	-
4	0.54	3458	8	-	-	24	0.47	2870	4	4	-
5	0.64	4276	8	-	-	25	0.68	4577	16	16	-
6	0.14	0	0	-	-	26	0.82	5794	16	16	-
7	0.29	1379	0	-	-	27	0.47	2870	4	4	-
8	0.86	6225	8	-	-	28	1.27	9868	32	32	±
9	0.42	2398	4	-	-	29	0.66	4439	8	8	-
10	0.42	2414	4	-	-	30	0.61	4036	8	8	-
11	1.34	10507	32	+	±	31	0.48	2878	4	4	-
12	1.18	9068	32	+	±	32	0.32	1642	4	4	-
13	0.21	758	0	-	-	33	0.49	3037	8	8	-
14	1.06	7987	32	+	±	34	0.80	5628	16	16	-
15	0.51	3129	4	-	-	35	0.41	2299	4	4	-
16	0.52	3213	4	-	-	36	0.48	2945	4	4	-
17	0.79	5558	16	-	-	37	0.20	698	0	0	-
18	0.46	2758	4	-	-	38	0.60	4130	4	4	-
19	1.50	12005	64	+	+	39	0.30	1488	0	0	-
20	0.51	3180	4	-	-	40	0.19	0	0	0	-

Abbreviation key:- SNT = Serum neutralization test, AGP = Agar gel precipitation test,
OD = Optic density, ELISA = Enzyme linked immunosorbent assay.

Table (2) Pathological Lesion Score in Chicken Embryos Groups Experimentally Infected:-

Organ		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Liver	h.	+++	++	+++	±	±	+	-	-
	d.	-	++	+++	--	--	-	-	-
	n.	-	++	+++	--	--	-	-	-
Kidney	h.	+++	++	++	+	-	+	-	-
	d.	-	--	++	-	-	-	-	-
	n.	-	++	++	-	-	-	-	-
Heart	h.	+++	++	++	+	+	+	±	-
	d.	-	--	--	-	-	-	-	-
	n.	-	--	--	-	-	-	-	-
Lung	h.	++	NE	±	±	-	-	-	-
	d.	-	NE	--	--	-	-	-	-
	n.	-	NE	--	--	-	-	-	-
Provent.	h.	-	++	--	+	-	-	-	-
	d.	-	--	--	-	-	-	-	-
	n.	-	--	--	+	-	-	-	-
Intestine	h.	-	++	+	+	-	-	-	-
	d.	-	--	-	-	-	-	-	-
	n.	-	--	-	-	-	-	-	-
S muscles	h.	+++	+	++	-	-	-	-	-
	d.	-	-	--	-	-	-	-	-
	n.	-	-	--	-	-	-	-	-
Brain	h.	-	+	-	+	-	-	-	-
	d.	-	-	-	+	-	-	-	-
	n.	-	-	-	+	-	-	-	-
CAM	h.	-	++	++	+	-	-	-	-
	d.	-	--	++	-	-	-	-	-
	n.	-	--	++	-	-	-	-	-

Abbreviation key:- h = hyperemic, haemorrhagic; d = degenerated; n = necrosed; and NE = not examined.