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STUNTING SYNDROME IN BROILER CHICKENS CLINICAL FIELD AND EXPERIMENTAL INVESTIGATION

(With 4 Tables & 3 Fig.)

By

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ظاهرة اعاقة النمو فى بدارى كتاكيت التسمين دراسة حقلية وتجريبيه

كمال الزناتى

تم دراسة ظاهرة اعاقة النمو فى قطعان مختلفه من بدارى كتاكيت التسمين فى منطقة مصر العليا فى الفترة من سبتمبر ١٩٩٠ - أبريل ١٩٩٢ وفى تلك الظاهرة لوحظ نقص الأوزان وعدم التساوى فى النمو بين الكتاكيت فى قطعان التسمين المصابه عند عمر ١٠ - ١٥ يوم وكان أكثر وضوحا عند عمر ٤ أسابيع تم توصيف الأعراض الاكلينيكيه والباثولوجيه وتسجيل الوفيات فى هذه القطعان المصابه حقليا. تم عزل فيروسات من العينات المأخوذه من القناه المعديه - المعويه وكذلك من الأعضاء الداخليه من الكتاكيت المصابه حقليا على أغشية أجنة البيض (C A M) والتى أظهرت بثرات مميزه على هذه الأغشيه وكذلك وفيات فى الأجنه من ٣ - ٧ أيام بعد الحقن. وبدراسة الصفات الفيزوكيميائيه والبيولوجيه والسرولوجيه تبين أن جميع الفيروسات المعزوله هى فيروسات الريو حيث كانت سلبيه فى اختيار تلازن الدم وغير حساسه للمعامله بالكلوروفورم وكانت ايجابيه فى اختيار الترسيب (A G P) ضد سيرم فيروس الريو. تم المقارنه الباثولوجيه بين فيروسات الريو المعزوله من القناه المعديه - المعويه فى أجنة البيض. وقد أجريت العدوى الصناعيه لاحداث الظاهره باستخدام أحد عترات فيروس الريو المعزوله من القناه المعديه - المعويه من نفس الحالات التى تم منها عزل فيروس الريو والمستخدم فى التجربه - تم تسجيل الأعراض الاكلينيكيه والباثولوجيه والوفيات وكذلك تم اعادة عزل الفيروس.

SUMMARY

In September, 1990 - April, 1992 a stunting syndrome was observed on most of broiler chicken farms located in Upper Egypt which was characterized by an unusual high incidence of visible unevenness in growth between 10-15 days old with maximum difference in size was seen at four weeks old. Different clinical and pathological features of the natural syndrome were described. Several reoviruses were isolated from gastrointestinal homogenate and visceral organs of severe clinically diseased broiler chicks. All reovirus isolates produced characteristic pocks on chorio-allantoic membrane (CAM) of embryonated chicken eggs (ECE) and mortalities between 3-7 days posinoculation (PI). All isolates were insensitive to chloroform treatment, did not hemagglutinate chicken erythrocytes and positive in agar gel precipitation (AGP) test against standard reference reovirus antiserum. Pathogenicity comparison between the six islated gastrointestinal reoviruses in ECE was carried out. Pathogenicity of gastrointestinal homogenate and reovirus isolated from the same field cases into 1-day-old broiler chicks was done. Clinical signs, pathological findings, mortalities in experimentally infected chicks and virus reisolation were reported.

INTRODUCTION

A disease syndrome of broiler chicks characterized by stunted growth elevated feed conversion ratios and poor feathering was initially reported in the Netherlands *(KOUWENHOVEN* et al., *1978a)*. Subsequently, similar conditions have since been noted in several countries *(BRACEWELL & WYETH, 1981; PAGE* et al., *1982 and PASS* et al., *1982)*. Due to the variety of clinical and post-mortem findings observed, the condition has been described under various names including infectious proventriculitis *(KOUWENHOVEN* et al., *1978b)*, helicopter disease, pale bird syndrome *(VAN DER HEIDE* et al., *1981)*, infectious stunting and runting syndrome *(BRACEWELL & WYETH, 1981 and PASS* et al., *1982)* and malabsorption syndrome *(PAGE* et al., *1982)*. Reovirus, enterovirus and parvovirus have been isolated from chickens with this syndrome and the

correlation between the outbreak of stunting and the etiological role of these viruses has been examined (*DECAESSTECKER et al., 1986; HIERONYMUS et al., 1983; KISARY, 1985; KISARY et al., 1984; McNULTY et al., 1984, and McNULTY et al., 1987*).

However, the etiological role of these viruses is still questionable. Reproduction of the syndrome with reoviruses isolated from affected birds has been inconsistent (*OLSON, 1984; PAGE et al., 1982; RUFF & ROSENBERGER, 1985 and BEKHIT et al., 1990*).

The present study describes: The clinical and pathological findings in naturally affected broiler chicken flocks. Isolation and identification of reoviruses from severe clinically affected broiler chickens. Comparison study of the pathogenicity between different gastrointestinal reovirus isolates in ECE. Pathogenicity of isolated reovirus and infectious intestinal homogenates from clinically infected broiler chicks for 1-day-old chicks. Clinical signs, pathological finding and mortalities as well as trial for virus reisolation from experimentally infected chicks.

MATERIAL AND METHODS

History of clinically affected broiler chicken flocks:

A stunting syndrome have been seen simultaneously in the major broiler growing chickens in private and governmental farms in Assiut and Sohag Provinces. Producers first noticed unusual high incidence of unevenness (5-15%) in the size of birds which was the most common complains during the second week of life (10-15 days). Affected birds being markedly smaller than others and the proportion of affected birds on each flock varied but the other features of the syndrome were very similar throughout. Six broiler chicken flocks were undertaken in this study. Clinical signs and pathological findings are mentioned below.

Specimens:

Two-Cm-sections of the proventriculus, duodenum, jejunum and ileum with their content as gastrointestinal sample were collected from clinically affected birds (5 birds/sample). 10% suspension was made using physiological buffer saline (PBS) containing 10,000 IU penicillin, 10,000 ug streptomycin and 250 ug amphotericin/ml (suspension was left for one hour at -4 C), homogenized, centrifuged and supernatant divided into two parts (stored at-20 C till used), one part for virus isolation trials, other part for pathogenicity test. Along with

gastrointestinal samples, part from liver, spleen, pancreas, kidneys, bursa and thymus (5 birds/sample) from the same clinically affected cases were collected as visceral organ samples, 10% suspension was made with PBS-antibiotic, homogenised, centrifuged and the supernatant as visceral organs inoculum stored at-20 C till used for virus isolation. Loopful from heart blood, Liver and spleen were taken from the same cases at the same time for bacteriological examination.

Embryonated chicken eggs (ECE):

10-day-old ECE were obtained from Agriculture Faculty Farm, Assiut University (pretested for freedom from reovirus antibodies), used for virus isolation, propagation, titration and pathogenicity comparison of the isolated reoviruses.

Chicks:

Newly hatched chicks (Hubbard) from private farm pretested for freedom from reovirus antibodies were reared in isolators, fed on commercial broiler chicken ration and used in pathogenicity test.

Virus isolation:

Virus isolation was accomplished by inoculating 0.1 ml of sample supernatant into four 10--day-old ECE via chorio-allantoic membrane. Eggs were candled twice daily for 8 days. mortalities and lesions were recorded. All deaths occurring within 24 hours postinoculation (PI) were regarded as non specific. From embryos dying between 1 and 8 days PI, CAM was harvested aseptically and stored at-20 C.. At least three blind passages were made before a sample was considered negative.

Virus identification was performed by testing affected CAM material in an AGP-test against reovirus (S-1133 strain) antiserum, HA activity and chloroform sensitivity.

Reference reovirus:

Reovirus S-1133 (52-egg passage) was kindly supplied from the Institute of Poultry Dis., Free Univ., Berlin. The antigen was a homogenised suspension of CAM infected with the S-1133 strain, harvested 3 to 5 days after CAM infection, homogenized, frozen & thawed three times, centrifuged and the supernatant was used in the AGP test against reovirus-specific serum as control.

Reference antiserum:

Chicken immune sera against reovirus S-1133 was kindly supplied by Prof. Von Bulow, Institute of Poultry Dis., Free Univ., Berlin.

Hemagglutination (HA) test:

Allantoic fluid of all virus isolates were tested for HA activity by rapid slide and slow plate HA test after *Anon., 1971* using 10% and 0.75% washed chicken erythrocytes, respectively.

Agar gel precipitation (AGP) test:

The test was done as described by Bulow and Biggs, 1975. Previously infected homogenized CAM were frozen and thawed three times, centrifuged and the supernatant was then used as antigen in the AGP test against reference reovirus (S-1133) antiserum. The test was read 24-48 h. post incubation at 37 C.

Chloroform sensitivity:

Sensitivity of virus isolates to chloroform was done after *LUKERT, 1989.* A loss of infectivity of 1 Log_{10} or greater indicates an enveloped virus (*FELDAMAN and WANG, 1961).*

Comparison of the pathogenicity between gastrointestinal isolated reoviruses in ECE:

A comparative study of the pathogenicity among gastrointestinal isolated reoviruses (one reovirus from each observed six flocks, designated RB_1, RB_2, RB_3, RB_4, RB_5, RB_6), was carried out onto CAM of ECE using 0.1 ml as inoculum. Virus titre (ELD_{50}/ml) was calculated (*REED and MUENCH, 1938),* mean death time in days was determined *(HANSON, 1975)* and rate of mortality of embryos was recorded.

Pathogenicity test in 1-day-old chicks:

Ninety newly hatched commercial broiler chicks from a source pretested for freedom from reovirus antibody were divided into three equal groups (30 chicks/group), reared in isolators and fed ad libidum on a commercial broiler chicks ration. At one-day-old, the chicks in the 1st and 2nd group were orally inoculated with 0.5 ml/chick antibiotic treated gastrointestinal supernatant (from which reovirus RB_5 was isolated) and 0.5 ml ($10^{5.75}$ ELD_{50}) reovirus RB_5 /chick respectively, while chicks in the 3rd group were orally inoculated with 0.5 ml/chick sterile PBS and kept as control

group. All chicks were observed for clinical signs, mortality and PM lesions. At 7, 14,21,28 and 35 days, three chicks from each group were killed by cardiac puncture, necropsied for post-mortem examination and to obtain sample for virus reisolation and identification in the same way mentioned previously. The remaining chicks in each group were weighed and the mean body weight of each group was recorded. At the end of experiment the remaining chicks of each group were necropised for PM examination

RESULTS

Clinical manifestations in naturally affected broiler chicks:

Unevenness in growth of chicks at the initial outbreaks (2-3 weeks old) was the first visible, most and constant sign (Fig. 1). The proportion of brids affected on each farm varied Table (1) but the other features of the syndrome were very similar throughout the flocks. Difference in feathering between normal and stunted birds are apparent at 2-3 weeks and maximum at four weeks of age. The development of adult plumage is delayed in some affected birds, so that they still have yellow down on their heads which may persist even to 5-weeks of age by which time the unaffected birds are completely white feathered. In three of six observed flocks, the wing primaries is delayed and irregularly angled, resulting in the helicopter chick. Diarrhea and undigested feed in the fecal material was noticed in some cases of affected flocks. The appetite of stunted birds is well maintained and they are usually quite active. Affected birds often have pendulous, sometimes hard and distended abdomen. A partial recovery occurred after five weeks old in affected flocks (Table 2). but at the time of slaughter, the affected birds were still very under weight and many carcasses being too small. Deaths in affected flocks were illustrated in Fig. (2).

Post-Mortem findings: The carcases of most stunted broiler chicks examined have been well fleshed for their size but have tended to show abdominal distention. The intestine are dilated, friable, pale throughout their length, distented with undigested food especially in the lower and in some cases the caecal tubes are distented with frothy material. Enlargement of the proventriculus, reduction in the size of the gizzard and hydropericardium are frequently noticed in affected broiler chickens. Atrophy of the pancreas, spleen, thymus and bursa of Fabricious to a varying degree was seen commonly in stunted chickens particularly at 4-5 weeks old. The thymus often being

reduced to a small strip of tissue. Spleen was dark and in some carcases small.

Bacteriological examination of visceral organs: revealed non specific bacteria.

Virus isolation: A total of 23 virus isolates, 15 (48.4%) from 31 gastrointestinal samples and 8 (25.8%) from 31 visceral organ samples were harvested. All virus isolates produce in the 2nd or 3rd egg passage on the CAM, small localized white pock lesions, the CAM became generally thickened and oedematous with congested blood vessels. Embryos were congested, hemorrhagic and died between 3-7 days PI.

HA- test: All virus isolates failed to agglutinate chicken erythrocytes.

AGP-test: Affected CAM, produce precipitin lines against reference reovirus antiserum 24-48 hours post incubation.

Chloroform sensitivity: All virus isolates were insensitive to chloroform indicating that these viruses were non-enveloped.

Pathogenicity comparison of gastrointestinal reovirus-isolates in ECE: Virus titre (ELD_{50}/ml), mortality percent and MDT (days) were shown in Table 3.

Pathogenicity test in 1-day old chicks: Clinical signs in the 1st and 2nd inoculated group chicks are in general similar to that observed in the naturally affected birds but are more obvious, appeared earlier and severe in the 1st group than the 2nd inoculated group chicks. Diarrhea was the first clinical sign observed from day 3 and day 5 in the 1st and 2nd inoculated group chicks respectively and lasted about 10 days PI in the two groups. At 2- weeks old, most of inoculated chicks in the 1st and 2nd groups have distented abdomen particularly chicks in the 1st group. Growth impairment could be noticed by one week of age in the 1st and 2nd inoculated group chicks. At 7, 14, 21, 28 and 35 days old PI, the mean weights of the 1st group inoculated chicks were less than those of the controls by 27.2, 43.6, 59.5, 57.1 and 48.7 percent, respectively, whereas in the 2nd group inoculated chicks, the mean weights were less than those of the controls by 23.6, 37.8, 42.9, 47.3, 33.5 percent, respectively. Differences in feathering between the inoculated chicks (1st and 2nd groups) and control are apparent 3-4 weeks-old and resemble to the naturally affected chicks. The mortality rate during the observation period was 16.7 and 13.3% in the 1st and 2nd groups, respectively. No abnormal signs were observed in the control group chicks.

Post-Mortem findings: No gross post-mortem abnormalities are apparent at 7 days PI. Gross lesions are more or less similar in the 1st and 2nd inoculated group chicks, but differ in the time of appearance (earlier in the 1st than 2nd group) and the number of organs with lesions (Table 4). In the first two inoculated groups, the intestinal tracts were dilated (2-week PI), sometimes swollen, pale throughout their length and contained poorly digested feed Fig.3. The caeca are usually distended with gas and frothy-fluid ingesta. Decrease in intestinal tract length (from the end of gizzard to rectum) was noticed. At 28 and 35 days PI, the mean intestinal tract lengths of the 1st inoculated group chicks were decreased than those of controls by 15.92, 27.62% and in the 2nd group by 11.32, 18.86%, respectively. Slight decrease in size of pancreas, BF, thymus, spleen, gizzard was noticed at 2nd and 3rd week PI in the 1st and 2nd inoculated group chicks. By the 4th and 5th week PI, the decrease in size of these organs is more pronounced in the 1st than the 2nd inoculated group chicks. By this time, pancreas was diminished in size, became much pale and firmer in texture and all pancreatic lobes were thinner, BF and thymus were markedly smaller and atrophied, the thymus is reduced to a small strip of tissue and thymic lobes were reduced to small, translucent nodules and became hyperaemic. Enlargement of the proventriculus, the wall being thickened, more turgid and hydropercardium were observed in some affected chicks in the 1st and 2nd group chicks from the 4th week PI. Obvious reduction in the size of spleen was seen at 5th week PI in the 1st group chicks than the 2nd one.

DISCUSSION

The general aspects of natural outbreaks of stunting syndrome reported here (diarrhea, stunting, proventriculus enlargement, decrease in the gizzard size, hydropercardium) were similar to great extent those described by *KOUWENHOVEN et al., 1978b; VERTOMMEN et al., 1980; BRACEWELL and WYETH, 1981; VAN DER HEIDE et al., 1981; PAGE et al., 1982 and PASS et al., 1982,* but signs of lameness reported by them were not observed in this study. pancreatic lesions described in this study were previously reported by *RANDALL et al., 1981 and PASS et al., 1982,* but did not by *BRACEWELL and WYETH, 1981 and PAGE et al., 1982.*

The biological, physicochemical characteristics of the isolated viruses indicated that they were reoviruses as

described previously by (*DESHMUK and POMEROY, 1969; HIERONYMUS et al., 1983*). Avian reoviruses were frequently recovered from tissue of naturally affected birds (*VAN DER HEIDE et al., 1981; PAGE et al., 1982; PASS et al., 1982; HIERONYMUS et al., 1983*).

Pathogenicity of six isolated gastrointestinal reoviruses (RB_1, RB_2, RB_3, RB_4, RB_5 & RB_6) in embryos indicated that reovirus RB_4 and RB_5 should be considered to be of low and high virulence respectively according to description of *TAKASE et al., 1987. KIBENGE and DHILLON (1987)* reported that the low virulence reovirus was rarely to induce growth retardation in chicks, thus the high virulence RB_5 reovirus was choice in the pathogenicity test.

Experimentally, oral inoculation of one-day-old chicks with gastrointestinal homogenate from affected birds showed clinical signs and post mortem lesions identical to natural affected birds. The syndrome was transmitted experimentally to broiler chicks with an intestinal homogenate prepared from affected birds by several workers (*BRACEWELL and WYETH, 1981; KOUWENHOVEN et al., 1978a; VERTOMMEN et al., 1980; PAGE et al., 1982; KOUWENHOVEN et al., 1988*).

Results of reproduction the syndrom in one-day-old chicks by oral inoculation with isolated reovirus, are inagreement to some extent with those reported by (*PAGE et al., 1982; ROSENBERGER et al., 1989*), as signs and lesions similar to those seen in natural affected chickens were reported by them when isolated reoviruses were inoculated into day-old chicks, at the other hand our results are in contrast with *KOUWENHOVEN et al., 1988*, as they reported that the syndrome could not be reproduced by oral inoculation of newly hatched chicks with reoviruses isolated from naturally affected birds. *SHIRA et al., 1990* suggested that avian reovirus is the predominant etiological agent of the stunting syndrome which agree with our findings.

In conclusion, as the results of virus isolation, only avian reoviruses could be isolated in ECE affected chickens which indicate the wide spread of these viruses in commercial broiler chicken flocks. The role of these reoviruses was investigated by reproducing the syndrome. It is possible that other viral pathogen (s) may be involved in these outbreaks but this pathogen (s) could not be propagated and isolated in ECE or the predominant lesions of isolated reoviruses in ECE may reduce the possibility for their identification.

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Fig. 1:
R. Stunted chicken 3-Week old.
L. Unaffected chicken of the Same age.

Table (1): Field outbreaks of stunting syndrome and reovirus isolation

Flock No.	No. of chickens per flock	Age* (weeks)	Chickens with SS** at the initial of outbreak		Stunted chickens at 8 week-old (slaughter time)		No. of deaths	Mortality %	Isolation of reoviruses					
									Gastrointestinal samples			Viscral organ samples		
			No.	%	No.	%			No. of samples	No. of isolated viruses	reovirus isolation %	No. of samples	No. of isolated viruses	reovirus isolation %
1	11230	3	937	8.30	2127	18.94	1352	12.04	6	2	33.3	6	1	16.7
2	9470	2	1249	13.19	2827	29.85	1319	13.93	5	2	40.0	5	1	20.0
3	12450	2	903	8.92	1890	18.66	1115	11.01	7	3	42.9	7	2	28.6
4	9470	3	676	5.42	2163	17.37	1539	12.36	3	2	66.7	3	1	33.3
5	9230	2	1018	11.03	2472	26.78	1341	14.53	6	3	50.0	6	2	33.3
6	10020	2	782	7.80	1590	15.86	1008	10.06	4	3	75.0	4	1	25.0

* = Age in weeks at which the first signs of stunting syndrome (unevenness in size) are observed.
SS** = Stunting syndrome

Table (2) The percentage decrease of the mean body weights of naturally affected chicken flocks than those nonaffected ones at 7,14,21,28,35,42 days old.

Flock No.	Age in days					
	7	14	21	28	35	42
1	43.7	60.2	64.0	57.6	52.3	45.5
2	22.3	42.0	51.4	34.2	28.8	26.3
3	35.9	59.3	65.6	48.7	45.6	41.9
4	39.2	73.6	78.5	52.6	57.2	48.7
5	28.8	53.4	60.1	63.4	58.8	45.2
6	37.1	62.6	68.7	43.0	37.8	32.9

Fig. 2: Weekly deaths in the naturally affected flocks

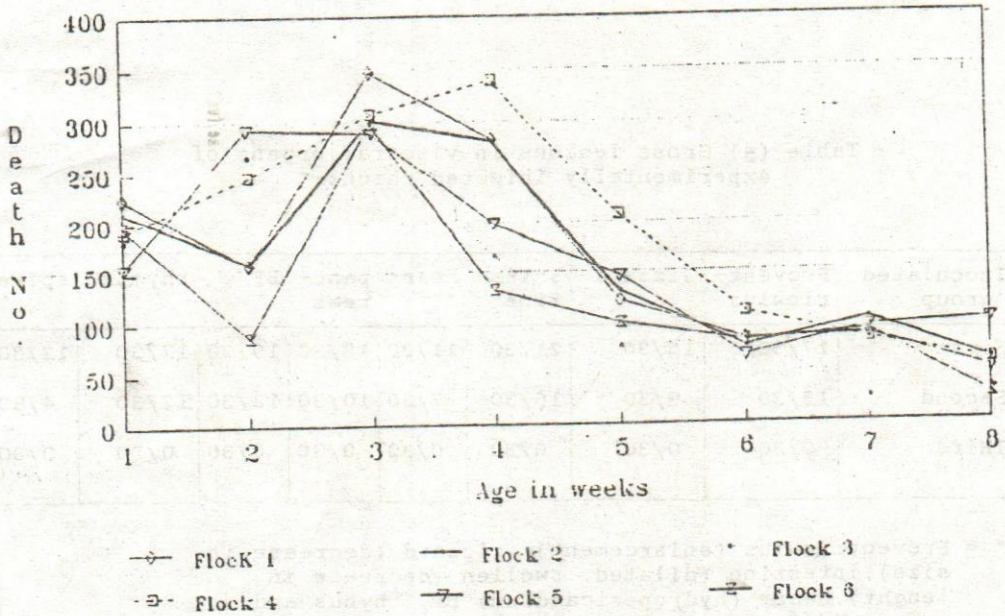

Table (3): Comparison the pathogenicity among six isolated gastrointestinal reoviruses in ECE.

reovirus isolate	ELD 50/ml log 10	Mortality %	MDT (days)
RB1	5.75	53.06	5.87
RB2	7.00	47.92	6.08
RB3	6.75	63.75	5.75
RB4	5.25	36.17	6.62
RB5*	6.25	72.25	4.96
RB6	5.65	44.89	6.58

* = reovirus isolate RB5 produce high embryos mortality rate and lowest MDT, it was used in the pathogenicity test.

Table (4) Gross lesions in visceral organs of experimentally infected chicks.*

Inoculated group	Proventriculus	Gizzard	Intestine	heart	pancreas	BF	thymus	spleen
First	17/30**	15/30	21/30	11/30	13/30	19/30	17/30	12/30
Second	13/30	9/30	16/30	7/30	10/30	13/30	12/30	4/30
THird	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30

* = Proventriculus (enlargement), gizzard (decrease in size), intestine (dilated, swollen, decrease in lenght), heart (hydropericardium) BF, thynus and spleen (decrease in size & atrophy).

**= Number with gross lesions/number of inoculated chicks.

Fig. 3:
Intestine was dilated and Swollen.