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VIRAL AGENTS ASSOCIATED WITH RETARDED GROWTH IN BROILER CHICKENS

(With 6 Tables)

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المسببات الفيروسية المرتبطة بتأخر النمو في بدارى التسمين

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تم عزل فيروس الجمبورو والريو من دجاج يعانى من نقص فى معدل النمو. تم التعرف على الفيروسات المعزولة باستخدام اختيار الترسيب فى الاجار. وكانت نسبة العزل الموجب فى مرض الجمبورو بنسبة 39% وفى حالة مرض الريو كانت النسبة 12%. تم عمل عدوى إصطناعية بفيروس مرض الريو فى الكتاكيت عمر يوم وفى الاسبوع الرابع حدث إنخفاض معنوى فى الأوزان المكتسبة بحيث كانت (1207.85 ± 11.6 , 458.33 ± 2.89 , 456.58 ± 3.96 , 355 ± 5.3 جم فى المجموعة المعديّة عن طريق الحقن العضلى والمعدية بالتقطير فى العين والمعدية بالفم والكنترول بالترتيب. كذلك فى السبوع الخامس كان معدل الانخفاض فى النمو معنوى جدا حيث كان (1544.28 ± 17.43 , 521.17 ± 8.01 , 479.84 ± 12.2 , 462.93 ± 4.19 جم) بنفس الترتيب السابق. تم عمل عدوى إصطناعية بفيروس مرض الجمبورو فى الكتاكيت عمر 20 يوم. وقد لوحظ حدوث إنخفاض معنوى جدا فى الأوزان المكتسبة فى الاسبوع الرابع من العمر بعد اسبوع من العدوى بفيروس مرض الجمبور حيث كانت (1207.85 ± 11.6 , 857.2 ± 7.6 , 842.35 ± 5.11 , 822.37 ± 9.16 جم) فى المجموعة المعديّة عن طريق التقطير فى العين والمعدية بالحقن فى العضل والمعدية بالفم والكنترول بالترتيب. أما الاسبوع الخامس فقد كان معدل النخفاض فى الأوزان المكتسبة معنوي جدا ايضا بعد اسبوعين من العدوى بحيث كان (1544.28 ± 17.43 , 934.16 ± 11.68 , 905.94 ± 30.07 , 895.25 ± 12.98 جم) فى المجموعة المعديّة عن طريق الحقن العضلى والمعدية بالتقطير فى العين والمعدية بالفم والكنترول بالترتيب. تم عمل دراسة هستوباثولوجية فى الدجاج المصاب بمرض الجمبورو وسجات الدراسة التغيرات الباثولوجية لأعضاء الجسم المختلفة المصابة من العدوى الصناعية حيث وجد تركز فى خلايا الليمف فى غدة فابريشس. أما الكتاكيت المصابة بمرض الريو فقد سجلت الدراسة التغيرات الباثولوجية لأعضاء الجسم المختلفة ووجد أن المعدة الغدية (proventriculus) بها تجمعات من كرات الدم البيضاء والهيترروفيل وخلايا الليمف.

SUMMARY

In the present study Reoviruses and IBDV were isolated from clinical cases of chickens showing retarded growth. The Percent of virus isolation was 12.1% for Reoviruses and 39.6% for IBDV. The titer of the selected Reov isolate was $10^{4.8}$ EIDSO/0.2ml while it was $10^{4.2}$ EIDSO/0.2ml for the selected IBDV isolate. Pathogenecity trials with the isolated viral agents revealed retarded growth with variable degrees where the decrease in body weight ranged from 66.3_70%, and 40_42% after Reovirus and IBDV infection respectively depending on the inoculation route

Key words:

INTRODUCTION

Malabsorption syndrome "MAS" is a widely spreaded problem in poultry industry with severe economic consequences caused by an enteric pathogen or by a combination of pathogens, mainly viruses (Zekarias *et al.*, 2002); Bayoumie 2004). The exact causative agent of "MAS" was the subject of great argue due to the isolation of a variety of agent s from the clinical cases of "MAS" (Page *et al.*, 1982; Meferran *et al.* (1983); Goodwin *et al.*, 1985; Decaesstecker *et al.*, 1986; Mcnulty *et al.* (1990)

Avian Reoviruses was proven to be a causative agent for "MAS" and the disease was successfully reproduced (Bayoumie, 2004). Recently IBDV isolates evolved as a viruses that can cause or contribute in infectious proventriculitis causing retarded growth (Skeels. *et al.*, 1995); Huff. *et al.*, 1997; Giambrane 2002a; Giambrane 2002b)

The present work aim to isolate and identify the viral agents from clinical cases of retarded growth, and to study their pathogenicity in chickens and their effect on performance parameters beside histological examination

MATERIALS and METHODS

Materials

1- Specimens

Samples from one hundred and seventy chickens suffering growth retardation were collected from field cases for virus isolation trails. Proventriculus, intestine and pancreas were used for preparation of intestinal homogenate (Kouwenhoven *et al.*, 1988) for Reoviruses Isolation attempts, while bursae, livers and spleens were used for IBVD isolation attempts

2- Day old chickens

One hundred and seventy day old broiler chicks "cobb" obtained from Cairo poultry company (CPC) were used in experimental trials. Birds were reared in floor pens under strict hygienic condition

3- Ration

starter broiler ration "3000 k cal energy, 21% protein and 3.6% fat" obtained from "EL eslamya company"

4- Embryonated chicken eggs (ECE)

9 - 11 day old (ECE) from native flocks were used for virus isolation, propagation and titration.

5- Reoviruses and IBDV antigens and antisera

Standard Reoviruses/ IBDV antigen and antisera were obtained from Animal Health Res. Inst - Dokki - Giza Egypt.

Methods

1- Preparation of specimen's for viruses isolation

Collected tissues were aseptically grounded, 1:10 dilution was prepared in phosphate buffer saline (PBS). The suspension was centrifuged at 3000 rpm for 15 min. The supernatant was membrane filtered then used for ECE inoculation as described by Kouwenhoven *et al.* (1988) and Senne (2008)

2- Agar gel precipitation test (AGPT)

The test was performed as described by Thayer and Beard (2008).

3-Viral titration

Viral titration was performed in ECE. The embryo infective dose 50 was calculated according to Read and Munch (1938)

4-Experimental design

Table (1) shows the distribution of experimental birds Used in the present study.

Table 1: Experimental design

Experiment	Number Of bird/ Group	Age of birds	Sub group	Number of bird/ subgroup	Infection				Serum samples
					Virus	Dose	Route	Age	
1	60	20 day old	A	20	IBDV	10 ^{4.2/0.2ml}	I/M	20 day of age	28, 3S, 42 day of age
			B	20			Orally		
			C	20			Eye drop		
2	60	Day old	A	20	Reo virus	10 ^{4.8/0.2ml}	I/M	One day old	7, 14, 21, day of age
			B	20			Orally		
			C	20			Eye drop		
3	50	-	C-ve(negative control)						

5- Histopathology

Specimens were fixed in 10% formalin, 5 µ paraffin section were prepared and stained with H&E, as described by Bancroft and Steven (1996), and was examined by light microscope

6- Performance parameters

The body weight (BWT), Body gain (BG), Feed intake (FI) and feed conversion rate (FeR) were used as indicators for performance parameters

7- Statistical analysis

Obtained data were statistically analyzed according to Tamhane and Dunlop (2000)

RESULTS

1- Virus isolation and identification

Harvested chorioallantoic membranes (CAM) of inoculated ECE with suspected materials were grounded, examined with AGPT for IBDV. and Reoviruses. The total positive samples for IBDV were 39.6% while it was 12.1% for Reoviruses. One isolated sample from each viral group was titrated.

2- Virus titration

The titer of selected Reovirus was $10^{4.8}$ EIOSO 10.2ml while the titer of the selected IBOV was $10^{4.2}$ EIOSO 10.2ml

3- Results of experimental infection with Reovirus isolate and performance parameter

The clinical symptoms, P.M. lesions post inoculation are shown in the Table 2. The mortality post inoculation varied according to the route of inoculation, it was 20% in subgroup (A) inoculated via 11M route, and 10% in subgroup (B) inoculated via oral route, while it was 5% in subgroup (c) inoculated via eye drops. The performance parameters at 4 weeks of age for subgroup (A) inoculated via 11M route showed the lowest Mean body weight gain 355 ± 5.3 gm followed by subgroups C and B (Table 3). There was also a significant difference in conversion rate when compared with control (5.37 ± 0.076 , 4.19 ± 0.029 and 4.26 ± 0.47 in subgroup A,B and C respectively (Table 3), while the performance parameters at 5th week revealed that chickens of subgroup(A) infected I/M showed the lowest Mean body weight gain followed by subgroup C and B. The difference in feed conversion was significant when compared with control as it was 6.9 ± 0.42 , 6.09 ± 0.11 and 6.65 ± 0.1 in subgroup A,b, and C respectively (Table 4).

Table 2: Pathogenicity of Reo virus, and IBVD infection in chickens:

Group	1 (Reo)			2(180)			Control ***
Subgroup	A	B	C	A	B	C	
Birds No	20	20	20	20	20	20	50
Route	I/m	Orally	Eye drop	I/m	Orally	Eye drop	
Mortality	4(20%)	2 (10%)	1 (5%)	6 (30%)	3 (15%)	2 (10%)	-
Survivors	16	18	19	14	17	18	50
1-clinical Signs	Vent pasting & trembling			Perfuse watery yellowish diarrhea and Severe body weight variation			No symptoms
2-p/m	Proventriculitis ,pale intestine Filled with gases & emaciation			Hemorrhages on thigh ,swollen bursae And congested kidneys			

*- $10^{4.8}/0.2ml$

** $10^{4.2}/0.2ml$

*** control

Table 3: The effect of Reo virus infection on body performance at 28

day old.

Group	Subgroup	Route	Body weight	Body gain	Feed in take	Feed conversion rate
Cove			1245.56 ± 9.18 a	1207.85±11.6a	1925.71±12.32 a	1.58 ± 0.02c
1	A	11M	393.75 ± 5.49 c	355±5.3c	1915.62±5.98 a	5.37 ± 0.076 a
	B	orally	498.33 ± 2.89 b	458.33±2.89b	1928.89 ± 7.2 a	4.19 ± 0.029 b
	C	Eye drop	496.58 ± 3.96 b	456.58±3.96b	1935.26 ±7.39 a	4.26 ± 0.047 b

Table 4: The effect of Reo virus infection on body performance at 35 day old.

Group I	Subgroup	Route	Body weight	Body gain	Feed in take	Feed conversion rate
C-ve			1594.44 ± 14.54 a	1544.28±17.43a	3142.85 ± 38.46 a	1.99 ± 0.029 c
1	A	1/M	502.93 ± 4.2 c	462.93±4.19c	3203.33 ± 10.3 a	6.9 ± 0.072 a
	B	Orally	561.18 ± 8.01 b	521.17±8.01b	3179.4± 16.64a	6.09 ± 0.11 b
	C	Eye drop	519.84 ± 12.19 c	479.84±12.2c	3168.95± 17.17a	6.65 ± 0.18 a

4- Results of experimental infection with IBDV isolate and performance parameters

The clinical symptoms, P.M. lesions, post inoculation with IBDV, are shown in (Table 2) mortality also varied in the different experimental groups. The mortality was 30% in subgroup (A) inoculated 1/M and 15% in subgroup (B) and 10% in subgroup (C) inoculated orally or via eye drops. Inoculated birds showed characteristics signs and lesions of IBDV. The observed performance at 4 week of age showed that group (C) inoculated via eye drops showed the lowest mean body weight gain followed by subgroup A then B (Table 5) The performance parameters at 3S day showed that subgroup injected 1/M showed the lowest mean body weight gain followed by group (B) and (C) (Table 5, 6)

Table 5: The effect of IBD virus infection on body performance at 28

day old:

Group	I	Subgroup	Route	Body weight	Body gain	Feed in take	Feed conversion rate
		C-ve		1245.56± 9.18a	1207.85±11.6a	1925.7 ± 12.32 a	1.58± 0.02 c
2	A	I/M		882.35 ± 5.11 b	842.35±5.11bc	1928.23± 6.7 a	2.28 ± 0.018 ab
	B	Orally		895.63 ± 7.89 b	857.2±7.6b	1923.68 ±6.03 a	2.23 ± 0.023 b
	C	Eye drop		860.26 ± 5.62 c	822.37±9.16c	1920.52± 7.02ba	2.34 ± 0.018 a

Means with different superscripts are significant at $p \leq 0.05$.

Chicks were infected at 20 day old

C-ve: negative control

Table 6: The effect of IBD virus infection on body performance at 35 day old:

Group	Subgroup	Route	Body weight	Body gain	Feed in take	Feed conversion rate
	C-ve		1594.44 ± 14.54 a	1544.28±17.43a	3142.85 ± 38.46 a	1.99 ± 0.023 b
2	A	I/M	940.87 ± 16 b	895.25±12.98b	3184.37± 16.27a	3.55 ±0.058 a
	B	Orally	972.61 ± 9.8 b	905.94±30.07b	3144.44 ±18.47a	3.42 ± 0.047 a
	C	Eye drop	974.16 ± 11.69 b	934.16±11.68b	3186.8± 17.45a	3.4 ± 0.04 a

Means with different superscripts are significant at $p \leq 0.05$.

Chicks were infected at 20 day old

C-ve: negative control

4- Results pf histopathological examination

The bursal lymphoid follicles of experimentally infected birds with IBDV showed lymphoid necrosis particularly in the medullary zone and was replaced by eosinophilic necrotic debris or became atrophied and shrunken together with vesicle formation in its covering epithelium. Lymphocytes showed pyknosis and karyorrhesis with hyperplasia of reticulo endothelial cells. Intense heterophil in the inter follicular tissues and within the cavities of some cystic follicles and fibroplasia was observed. Moreover, edema in interfollicular and subepithelial tissues with hyperemic capillaries were seen beside hyperplasia of epithelial covering. The splenic white pulps revealed intense lymphoid depletion and necrosis particularly in the germinal follicles and periarteriolar lymphoid sheath. Hyperplastic reticuloendothelial cells could be seen in the center of some white pulps around the adenoid sheath artery. The hepatic cells revealed

degenerative changes with peri vascular mononuclear cell infiltration.

The proventricular submucosa from birds experimentally infected with REOV revealed inter glandular edema accompanied by leukocytic aggregate mainly heterophils and lymphocytes., Other cases, showed disseminated leukocytic infiltration accompanied with degenerated and necrotic changes in the compound glands of the sub mucosa. The mucosa was edematous and infiltrated with leukocyte. The pancreas, showed Interstitial and perivascular leukocytic infiltration and mild lymphocytes infiltration was seen among pancreatic acini. The intestinal villi of the small intestine reduced in length and size with partial desquamation of their lining epithelium, the tips of the villi become rounded and thickened due to lukocytic infiltration in mucosal lamina pro pia and edema

DISCUSSION

Growth retardation is considered one of the most important problems facing poultry industry. Trials for virus isolation by inoculation of suspected samples in embroynated chicken eggs, and identification using AGPT revealed that the incidence of Reo virus was 12.1% in examined samples. Similar results were recorded by Reece et al. (1984) who reported that the incidence of runting varied from I to 20% and most of these birds were culled at the time of slaughter. While the rate of IBO viruses in collected samples were 39.6%. These results were nearly similar to that observed by Islam and Samad (2003), and Zeleke *et al.* (2005), who reported that the mortality rate of IBO in different poultry houses ranged from 45-50%.

The effect of reo virus infection in one day old chicks showed mortality rate ranged from 5-20%. These finding agreed with those mentioned by Rosenberger *et al.* 1989) who reported an outbreak in broilers characterized by serious mortality (10-18%) over a period of few days in chickens less than 14 day 0 I d. Chicks died post inoculation showed vent pasting, and trembling, Reece 1997). Postmortem examination revealed emaciated anemic carcass, pale intestine, filled with gases, enteritis, proventriculitis, and paleness of visceral organs. These findings were similar to Chooi and Chalan (1985); and Wood *et al.* (1997), ayoumie 2004) eported similar symptoms and P.M. lesions after experimental infection with reov but mortality was 5-10%

After 28 day of infection revealed significant decrease in body gain (BG) especially 11M infected groups 355 ± 5.3 gm when compared

with intraocular and orally infected groups and control (456.58 ± 3.96 , 458.33 ± 2.89 gm and 1207.85 ± 11.6 gm respectively). While no changes in feed intake in all groups. Significant decrease in FCR observed in all groups when compared with control group, FCR was 35.37 ± 0.076 , 4.19 ± 0.029 , 4.26 ± 0.047 , % and 1.58 ± 0.02 %) in all groups, while after 35 day of infection revealed very highly significant decrease in body gain (BG) especially 1/M infected groups 462.93 ± 4.19 gm when compared with intraocular and orally infected groups and control (479.84 ± 12.2 , 521.17 ± 8.01 gm and 1544.28 ± 17.43 gm respectively). While no changes in feed intake in all groups. Very highly significant decrease in FCR observed in all groups when compared with control group, FCR was 6.9 ± 0.072 , 6.09 ± 0.11 , 6.65 ± 0.18 , %, and 1.99 ± 0.029 %) in all groups these results agreed with those recorded by Reece and Frazier (1991) who stated that Small chickens are detectable by 4-6 days of age. Some birds ceased to grow and remained about 200 gm at 6-8 weeks of age (Runts) such chickens are usually culled. And Abdul-Aziz (1995) who stated that 10% of birds were affected between the 2nd and the 5th week of life experiencing retarded growth.

On the other hand the effect of IBO virus infection in 20 day old chickens showed mortality rate ranged from 10-30%, These findings were similar to those mentioned by Kurade *et al.* (2000).; and Giasuddin *et al.* (2005). Chicks died post inoculation showed depression anorexia, ruffled feather, watery yellowish- white diarrhea, body weight variation, trembling, and death, These finding similar to Islam and Samad (2003); and Hafz *et al.* (2003). Gross lesions was dehydrated emaciated carcass, haemorrhagic spots on thigh and breast muscle, swollen pale or congested kidneys. Bursa of fabricius was enlarged or atrophied in some cases. These findings agreed with EI-Batrawi (1990) and Islam and Samad (2003). Examination of infected chicken performance (BW, FI, FCR) after 28 days old (one week post IBOV infection) revealed significant decrease in body gain (BG) specially intraocular infected groups (822.37 ± 9.16 gm) when compared with 11M and orally infected groups and control (842.35 ± 5.11 , 857.2 ± 7.6 , and 1207.85 ± 11.6 gm respectively). While no changes in feed intake in all groups. Significant decrease in FCR observed in all groups when compared with control group, FCR was 2.28 ± 0.018 , 2.23 ± 0.023 , 2.34 ± 0.018 , %, and 1.58 ± 0.02 %) in all groups. While after 35 day old (two week post IBOV infection) revealed significant decrease in body gain (BG) specially 1/M infected groups (895.25 ± 12.98 gm) when compared with orally and intraocular infected groups and control (905.94 ± 30.07 , 934.16 ± 11.68 gm

and 1544.28±17.43gm respectively). While no changes in feed intake in all groups. Significant decrease in FCR observed in all groups when compared with control group, FCR was 3.55 ±0.058, 3.42 ± 0.047, 3.4 ± 0.04, % and 1.99 ± 0.023 %) in all groups these results agreed with Okoye and Aba Adulugba (1998).; and Paula *et al* (2004) they mentioned that birds survived the diseases lost weight from 1190 to 1320 g (Group I) than those broilers which did not have Gumboro (Group II; 1585-1620 g). Thus, there is a significant variation in body weight of Gumboro affected broilers due to existing and imposed vaccination program.

Histopathological changes after IBOV infection the bursa of fabricius revealed lymphoid necrosis practically in the medullary zone and replaced by eosinophilic necrotic debris or become atrophied and shrunken together with vesicle formation in its covering epithelium. Lymphocytes had pyknosis and karyorrhexis of their nuclei with hyperplasia of reticulo endothelial cells. Intense heterophil in inter follicular tissues and within the cavities of some cystic follicles beside fibroplasia were common. Moreover, edema in interfollicular tissues and subepithelial with hyperemic capillaries were seen beside hyperplasia of epithelial covering. These findings are similar to Khafagy *et al.* (1991) and Paul *et al.* (2003).

Histopathological changes after ReoVirus infection revealed inter glandular edema accompanied by leukocytic aggregates mainly heterophils and lymphocytes proventriculaur Submucosa. In the other cases, disseminated leukocytic infiltration accompanied with degenerated and necrotic changes in the compound glands of the submucosa were evident. The mucosa was edematous and infiltrated with leukocytes. These findings agreed with Goodwin *et al.* (1996) who mentioned that deep non purulent necrotizing proventriculitis (accompanied by

Interstitial leukocytic infiltration mainly mild lymphocytes were seen among pancreatic acini these finding similar to Riddell and Derow (1985), Reece and Frazier (1991). Also Focal hepatic necrosis with hemorrhages were common. Perivascular edema in some portal areas together with degenerated and necrotic changes in the hepatic were common in other cases. Glavits *et al.* (1984) *observed* dilated liver sinusoids and swollen endothelial cells. There was accumulation of heterophil granulocytes, monocytes, kupffer cells and there were signs of degeneration. Multinucleated giant cells were also demonstrated among the hepatocytes.

It is concluded that Reo virus and IBDV infections are highly serious diseases inducing severe decrease in body weight leading to high economic losses at the end of fattening period, where the decrease in body weight ranged from (66.370%, and 40-42% in Reo and IBDV respectively. From the previous results it is clear that vaccination against Reo virus and IBDV especially in breeder hens considered an utmost need to avoid the economic losses

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