Dept. of Avian and Rabbit, Medicine,
Fac.of Vet. Med., Zagazig Univ.

VIRAL AGENTS ASSOCIATED WITH RETARDED GROWTH IN BROILER CHICKENS

(With 6 Tables)

By

A.M.A. HEGAZY; H.A.A. BAYOUMIE; O.A. SAMAHA***

*Technical Manager of NLQP-Sharkia,
** Goves Zagazig

(Received at 20/4/2009)

المسببات الفيروسية المرتبطة بتأخر النمو فى بدارى التسمين

أحمد الصادق محمد حجازى ، هشام أحمد عبد البديع ، أسامة أحمد سماحة

تم عزل فيروس الجمبورو والريو من دجاج يعانى من نقص فى معدل النمو. تم التعرف على الفيروسات المعزولة بإستخدام اختبار الترسيب فى الاجار. وكانت نسبة العزل الموجب فى مرض الجمبورو ٣٩,٦ % وفى حالة مرض الريو كانت النسبة ١٢,١%. تم عمل عدوى إصطناعية بفيروس مرض الريو فى الكتاكيت عمر يوم وفى الاسبوع الرابع حدث إنخفاض معنوى فى الأوزان المكتسبة بحيث كانت (355±5.3, 456.58±3.96, 458.33±2.89, 1207.85±11.6جم) جم فى المجموعة المعدية 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 (462.93±4.19, 479.84±12.2, 521.17±8.01, 1544.28±17.43جم) بنفس الترتيب السابق. تم عمل عدوى إصطناعية بفيروس مرض الجمبورو فى الكتاكيت عمر ٢٠ يوم. وقد لوحظ حدوث إنخفاض معنوى جدا فى الاوزان المكتسبة فى الاسبوع الرابع من العمر بعد اسبوع من العدوى بفيروس مرض الجمبور حيث كانت (822.37±9.16, 842.35±5.11, 857.2±7.6, 1207.85±11.6 جم) فى المجموعة المعدية عن طريق التقطير فى العين والمعدية بالحقن فى العضل والمعدية بالفم والكنترول بالترتيب. أما الاسبوع الخامس فقد كان معدل الانخفاض فى الاوزان المكتسبة معنوي جدا ايضا بعد اسبوعين من العدوى بحيث كان (895.25±12.98, 905.94±30.07, 934.16±11.68, 1544.28±17.43 جم) فى المجموعة المعدية عن طريق الحقن العضلى والمعدية بالتقطير فى العين والمعدية بالفم والكنترول بالترتيب. تم عمل دراسة هستوباثولوجية فى الدجاج المصاب بمرض الجمبورو وسجلت الدراسة التغيرات الباثولوجية لأعضاء الجسم المختلفة المصابة من العدوى الصناعية حيث وجد تكرز 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 والهيتيروفيل 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 $10^{4.8}$ EID50/0.2ml while it was $10^{4.2}$EID50/0.2ml for the selected IBDV isolate. Pathogenecitity 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: *Chicken, virology, Reovirus, malabsorption syndrome.*

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 agents 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 aimed 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 trials. 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 15min. 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 I: 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 (FCR) 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 $10^{4.8}$ EID50/0.2ml while the titer of the selected IBDV was $10^{4.2}$ EID50/0.2ml

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

The clinical symptoms, P.M. lesions post inoculation are shown in Table 2. The mortality post inoculation varied according to the route of inoculation, it was 20% in subgroup (A) inoculated via 1/M 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 1/M 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(IBD)**			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
C-ve			1245.56 ± 9.18 a	1207.85±11.6a	1925.71±12.32 a	1.58 ± 0.02c
1	A	1/M	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

Means with different superscripts are significant at $p \leq 0.05$.
Chicks were infected at one day old C-ve: negative control

Table 4: The effect of Reo 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 ± 0.029 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

Means with different superscripts are significant at $p \leq 0.05$.
Chicks were infected at one day old C-ve: negative control

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 35 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	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

5- Results of 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 karyorrhexis 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 interfolicular and subepithelial tissues with hyperemic capillaries were seen beside hyperplasia of epithelial covering. The spleenic white pulps revealed intense lymphoid depletion and necrosis particularly in the germinal follicles and periarteriolar lymphoid sheath. Hyperplasic 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 became rounded and thickened due to lukocytic infiltration in mucosal lamina propia 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 the examined samples. Similar results were recorded by Reece *et al.* (1984) who reported that the incidence of runting varied from 1 to 20% and most of these birds were culled at the time of slaughter. While the rate of IBD viruses in collected samples was 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 IBD 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 findings 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 than14 day old. 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); Wood *et al.* (1997). Bayoumie (2004) reported 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 I/M infected groups 355±5.3gm when compared with intraocular and orally infected groups and control (456.58±3.96, 458.33±2.89gm and 1207.85±11.6 gm respectively). While no changes in feed intake in all groups. Significant decrease in FCR was 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 I/M infected groups 462.93±4.19gm 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 was observed in all groups when compared with control group, FCR 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 recoded 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 IBD 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 findings are similar to Islam and Samad (2003); and Hafz *et al.* (2003). Gross lesions were 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 IBDV infection) revealed significant decrease in body gain (BG) specially intraocular infected groups (822.37±9.16gm) when compared with 1/M 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 was 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 IBDV infection) revealed significant decrease in body gain (BG) specially I/M infected groups (895.25±12.98gm) when compared with

orally and intraocular infected groups and control (905.94±30.07, 934.16±11.68gm and 1544.28±17.43gm respectively). While no changes in feed intake in all groups. Significant decrease in FCR was 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 IBDV infection the bursa of fabricius revealed lymphoid necrosis practically in the medullary zone and replaced by eosinophlic 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 interfolicular 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 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 purlulent necrotizing proventriculitis accompanied by adenoepithelail hypertrophy and hyperplasia was the most common light microscopic diagnosis in the proventriculi examined. Degenerating and necrotic alveolar secretory cells had amorphous, granular or vacuolated cytoplasm. Nuclei usually were either pyknotic, karyorrhectic or karyolitic.

Interstitial leukocytic infiltration mainly mild lymphocytes were seen among pancreatic acini. These findings are 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 Reovirus 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 is considered an utmost need to avoid the economic losses

REFERENCES

Abdul-Aziz, T.A. (1995): Field outbreak of infectious stunting syndrome in broiler chickens in Jordan. Tropical-Animal-Healthand-Production, 1995, 27: 173-174.

Bancrefr, J.D. and Stevens, A. (1996): Theory and practice of histological technique. 4th Ed .churchuill. Living stone, Edinburgh. London Melbaurne and New York.

Bayoumie, H.A.A. (2004): Studies on Reovirus infection in chicken. PH.D thesis in poult.dis. fact. Vet. Med. Zagazig

Chooi, K.F. and Chulan, U. (1985): Broiler ranting / stunting syndrome in Malaysia. Vet. Rec. 350: 354.

Decaesstecker, M.G.; Chariler, G. and Meulemans, G. (1986): Significance of Parvo viruses, Entro-like viruses and Reoviruses in the aetiology of the chicken mal absorption syndrome Avian path.15: 769-782

El-Batrawi, A.M. (1990): Studies on severe outbreaks of infectious bursal disease. The natural and experimental disease. Proceedings 2nd Scientific Conference of the Egyptian Veterinary Poultry Association, 12-14 March, Cairo: 239-252.

Giambrone, J.J. (2002a): Infectious bursal disease virus: an old foe changes its face again. Educational Development Magazine, Feb pp: 10

Giambrone, J.J. (2002b): Working group 3: vaccination" passive protection and vaccination (current and future possibility) in the presence of maternally derived antibody" third meeting Pulawy, Poland, keynote lecture.

Giasuddin, M.; Alam, J.; Islam, M.R; Taimur, M.J.F.A. and Rahman, M.M. (2005): Epidemiological investigation of infectious bursal disease in Bangladesh. Indian. J. Poult. Sci. 2005; 40: 99-101.

Glavits, R.; Molnar, E.; Ratz, F.; Saghy, E.Q.; Fehervarie, T. and Meder, M. (1984): pathological studies in chicken embryos and day old chickens experimentally infected with avian Reoviruses. Acta. Vet. Hungurie 32: 33-37

Gohary, A.E. (1991): Very virulent infectious bursal disease. Vet. Med. J. Giza, 39; 299-317

Goodwin, M.A.; Dekish, M.; Latimer, K.S. and Fletcher, O.J. (1985): Quantitation of intestinal D-xylose absorption in normal broilers and in broilers with bale bird syndrome. Avian Dis 24: 630-639.

Goodwin, M.A.; Hafner, S.; Bounous, D.I.; Latimar, K.S.; Player, E.C.; Niagro, F.D.; Campagnali, R.P. and Brown, J. (1996): Viral proventriculitis in chickens. Avian path, 25: 369-379.

Haddad, E. (1995): The involvement of infectious bursal disease Virus as a cause of proventriculitis in chickens, 46th North Central Disease conference Minneapolis, M.

Hafez, H.M.; Prusas, C. and Raue, R. (2003): Very virulent infectious bursal disease virus (vvIBDV) in vaccinated broiler flock: course of the disease, identification and characterisation of isolated strain. Archiv-fur-Geflugelkunde. 2003; 67: 2-5

Huff, G.R; Huff, W.E.; Balog, J.M.; Rath, N.C. and Zheng, Q. (1997): Studies of infectious proventriculitis Proc. 32nd National meeting on poultry health & processing October 15-17, Sheraton Ocean City, Ocean City Maryland. pp 39-43

Islam, M.T. and Samad, M. (2003): Outbreaks of infectious bursal disease in vaccinated and unvaccinated commercial cockerel farms in Bangladesh. Bangladesh J. Vet. Med 1: 21-24.

Khafagy, A.K.; Assia, M. El-Sawy; Kouwenhoven, B.; Vielitz, E.; Ismail, A.A.; Sultan, H.A. and Elgohary, A.E. (1991): Very virulent infectious bursal disease. Vet. Med. J. Giza, 39; 299-317.

Kouwenhoven, B.; Vertommen, M. and Goren, E. (1988): Investigation into the role of reovirus in the malabsorption syndrome. Avian Path. 17: 879-892.

Kurade, N.P.; Bhat, T.K. and Jithendran, K.P. (2000): Occurrence of infectious bursal disease and its pathology in the birds of Himachal Pradesh. Indian J. Vete. Path. 24: 133-134

Mcferran, J.B.; Mc Nulty, M.S.; Me Cracken, R.M. and Creene, J. (1983): Enteritis & associated problems in: disease prevention and control in poultry production Edited by Hugnerpford, T.G; Sydney, University Press

Mc Nulty, M.S.; Connor, T.J.; Mc Neitly, F. and Mc Ferran, J.B. (1990): Biological characterization of an avian enetroviruses like viruses. Avian Path. 19: 75-87

Okoye, J.O.A. and Aba Adulugba, E.P. (1998): Comparative study of the resistance or susceptibility of local Nigerian and exotic ch ickens to infectious bursal disease. Avian path. 27: 168-174

Page, R.K.; Fletcher, O.J.; Rowland, G.N.; Grandy, D. and Vilegas, P. (1982): Malabsorption syndrome in broiler chickens. Avian Dis. 26: 618-624

Paul, B.K.; Das, S.K; Badhy, S.C.; Amin, M.R.; Amin, K.M.R. and Banik, S.C. (2003): Effect of existing and imposed vaccination on body weight against Gumboro in broilers under farm conditions. Int J. Poult. Sci. 3: 655-657.

Paula, M.B.C.; de., Yokosawa, J.; Coutinho, M.D.B.; Silva, P.L.; Ferraz, R.A.; Oliveira, T.F.M. and Queiroz, D.A.O. (2004): Identification and molecular characterization of the infectious bursal disease virus (IBDV) from an outbreak in a broiler flock in midwestem Brazil Brazilian-Joumal-of-Microbiology. 35: 352-358.

Reece, R.L.; Hooper, P.T.; Tate, S.H.; Beddome, V.D.; Forsyth, W.M.; Scott, P.C. and Barr, D.A. (1984): Field, clinical and pathological observations of a runting and stunting syndrome in broilers. Vet. Rec. 115: 483-485.

Reece, R.L (1997): Infectious stunting syndrome. In : Poultry Diseases. 4th edition pp. 375-388 edited by Jordan, F.T.W.; Pattison, M. Saunders W.B. Co, Ltd., London, Philadelphia, Toronto, Sydney, Tokyo.

Reece, R.L. and Frazier, J.A. (1991): Infectious stunting syndrome of chickens in Great Britain: Field and experimental studies Avian Path. 19: 723.

Reed, L.J and Muench, H. (1938): A simple method for estimating fifty percent end points. Am. J. Hyg, 27: 496

Riddell, C. and Derow, D.C. (1985): Infectious stunting and pancreatic fibrosis in broiler chickens in Saskatchewan. Avian Dis.29: 107-115

Rosenberger, J.K.; Sterner, F.J.; Botts, S.; Lee, P.K. and Margolin, A. (1989): In Vitro and In Vivo characterization of avian Reoviruses. I. Pathogenicity and antigenic relatedness of several avian Reovirus isolates. Avian Dis. 33: 535-544.

Senne, DA. (2008): Viruses propagation eggs. pp. 204. In, isolation, identification and characterization of avian pathogen. 5th Ed. Edited by Zavala, L.D.; Swayne, D.E.; Glissone, J.R.; Peasson J.E. M.W., Woolcock PR.published AAAP 953 collage station Road aschens GA 30602-4875.

Skeeises, J.k.; Bayyari, G.R.; Newberry, L.A.; Wilson, M.A.; Huff, W.E.; Beasley, J.N.; Clark, F.; Whitfill, C.E and Haddad, E. (1995): The involvement of infectious bursal disease Virus as a cause of proventriculitis in chickens ,46th North Central Disease conference Minneapolis, M.

Tamhane, A.C. and Dunlop, D.D. (2000): Statistics and data analysis from elementary to intermediate. Upper saddle River, USA.

Thayer, S.G. and Beard, C.W. (2008): Serological procedure. Pp222 In, isolation, identification and characterization of avian pathogen. 5th Ed. Edited by Zavala, L.D.; Swayne, D.E.; Glissone, J.R.; Peasson, J.E. M.W., Woolcock PR.published AAAP 953 collage station Road aschens GA 30602-4875.

Wood, G.W.; Mackenzie, G.; Dagless, M.D.; Pearson, D.B. and Delissen, G. (1997): Reemergence of a stunting syndrome in broiler chicken.Vet. Rec: 463.

Zekarias, B.; songserm, T.; Post, J.; Kok, G.L.; Pol, J.M.A.; Engel, B. and Ter–Huurne, A.A.H.M. (2002): Development of organs and intestinal mucosa leukocytes in four broiler lines that differ in susceptibility to malabsorption syndrome Poultry-Science. 2002, 81: 9, 1283; 27.

Zeleke, A.; Gelaye, E.; Sori, T.; Ayelet, G.; Sirak, A. and Zekarias, B. (2005): Investigation on infectious bursal disease outbreak in Debre Zeit, Ethiopia. Inter. J. Poult. Sci. 2005; 4: 504-506.