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Detection of Chicken Anemia and Reo Viruses in broiler chickens in Behera governorate

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Abstract

Chicken Anemia virus (CAV) and Avian Reo virus (ARV) are common immunosuppression viruses that affect commercial boilers through either vertical or lateral transmission routes causing severe economic loss. In Egypt during last 10 years, there was field observation of high mortality rates, and poor weight gain in commercial broiler sectors where immunosuppression risks suspected to play role. In this study we conducted a retrospective analysis for CAV and Reo viruses during 2015-2018 in broiler sector in Behera governorate.

Serological examination for 103 broiler chicken flocks was conducted at 3-8 weeks age using ELISA test. High sero-positivity rate (81%, 94%, 88% and 83%) for CAV were detected during 2015, 2016, 2017 and 2018, respectively. Sero-positivity rate for Reo virus antibodies were detected (83%, 91%, 93%, 87% and 87%) during 2015, 2016, 2017 and 2018, respectively. Furthermore, we have recorded sero-positivity of both CAV and Reo viruses in 81% (69/85) of examined farms.

Molecular detection has conducted on thymus, liver, spleen, proventriculus and intestine of (75) diseased flocks' (27 examined for CAV, 19 examined for ARV and 29 examined for two viruses) at age 1-to-44 days. CAV virus PCR detection rate was 10.7% (6 positive farm out of 56), the detection rate differ from year to year as following (0, 20%, 6.2%, and 9.1%) during 2015, 2016, 2017and 2018, respectively. Reo virus PCR detection rate was 12.5% (6 positive farms out of 48), the detection rate differ from year to year as following (25%, 14.3%, 11.1%, and 10.7%) during 2015, 2016, 2017 and 2018, respectively. In conclusion, PCR detection and sero-survillance data results indicate early exposure of both CAV and/or Reo virus. These observations required further investigation to understand the causes of high prevalence of both viruses for broiler sector in Egypt.

Keywords: *Chicken Anemia virus (CAV), Avian Reovirus (ARV), serology and PCR.*

Introduction

Chicken Anemia Virus (CAV) is single-stranded DNA virus belonging to the family Circoviridae **(Fenner *et al.,* 1993).** CAV disease exclusively affects chickens between 2 -3 weeks old, but it is difficult to detect CAV in adult birds. The main high risk of CAV is its capability to transmition by vertical **(Yuasa and Yoshida, 1983)** and horizontal **(Yuasa *et al.,* 1979)** routes. The main clinical importance of CAV infection in young chicken is the immunosuppression pictures, aplastic anemia and growth retardation due to generalized lymphoid atrophy **(Schat, 2009).** While it only shows subclinical pictures in adult birds which characterized by immunosuppression, daily weight reduction and increase risk of other infections **(Dhama *et al.,* 2008)**.

In Egypt, CAV infection has been reported in 90th of last century **(El-Lethi *et al.,* 1990)**, the mass exposure of commercial chicken has been confirmed (**Zaki and El- Sanousi 1994; Sabry *et al.,* 1998**). In addition, characterized of CAV from infected broiler-breeder flocks **(Aly, 2001; Hussein *et al.,* 2002**), and was previously confirmed (26.6%) broiler chickens

(Mohamed, 2010), genetic analysis of a field CAV was obtained from broiler flocks during 2010 and this virus was unrelated to vaccine strains **(Abo Elkhair *et al., 2014*).** Furthermore, CAV was reported in Sharkia province by molecular and serological methods **(Hegazy *et al.,* 2014).** Molecular and Pathological analysis of CAV isolated from broiler chicken flocks during 2014-2015 from Three Egyptian Provinces (El-Behera, Matrouh and Kafr El-Sheikh) **(Hussein *et al.* 2016).** Characterization of full genome sequences of chicken anemia viruses circulating in Egypt, the results showed evidence of substitution and recombination among the viral genome **(Erfan *et al.,* 2018; Abdel-Mawgod *et al.* 2018).**

Avian Reo (ARV) viruses are the members of the genus Orthoreovirus in the Reovirus family **(Mathews, 1982).** Avian Reovirus (ARV) infections are mixed syndromes which are induced by ARV, and affect chickens including viral arthritis or tenosynovitis, stunting syndrome, enteric disease, malabsorption Syndrome, immunosuppression and slow feathering disease or abnormal feathering disease (Helicopter disease) **(McNulty, 1993; Rosenberger, 2003)**. ARV could transmitted vertically and horizontally, adult birds don't show clinical singes when infected **(McNulty and McFerran, 1996)**.

In Egypt, ARV was firstly reported in 1984 **(Tantawi *et al.,* 1984).** Further, many studies have confirmed ARV infection in Egypt **(Madbouly *et al.,* 1997; Madbouly and El-Sawah, 1999; Madbouly *et al.,* 2001 and Madbouly *et al.,* 2009).** Recently **Mansour *et al,* (2018)** isolated ARV from broiler chickens showing different clinical signs.

We have conducted this study to clarify the current prevalence of CAV and ARV as a possible cause of early immunosuppression and high mortality in commercial broiler chicken flocks in Behera governorate from 2015 to 2018.

Materials and Methods

Studied flocks history

The study was conducted on total 178 broiler chicken flocks from different districts in Al Behera governorate during 2015-2018. All flocks were showing history of growth retardation, variance in live body weights, high mortality. The reported cases were representing different chicken broiler breeds, the white foreign breeds, Saso and Balady broiler chickens, none of the farms vaccinated against CAV or ARV. The investigated flocks samples were collected and reported to the reference laboratory for veterinary quality control on poultry production – Damanhur branch (RLQP).

Samples for laboratory investigation

A total 1030 blood samples were collected for serological examination, from 103(11 examined for CAV, 7 examined for ARV and 85 examined for two viruses) commercial broiler chickens flocks (10 samples/flock), the collected sera were kept at -20°C until tested. On other hand, different organs were collected (Thymus, bursa of fabricious, liver, intestine and spleen) for PCR from 75 (27 examined for CAV, 19 examined for ARV and 29 examined for two viruses) broiler flock.

Enzyme-Linked Immusorbent Assay (ELISA):

A commercial test kit was used to detected specific antibodies against CAV and ARV based on indirect ELISA obtained from (Synbiotics Corporation, USA). ELISA procedures were conducted according to manufacture instructions. Optical density value was read at 405 nm wave length using Tecan Sunrise ELISA reader.

Polymerase chain reaction (PCR)

Whole nucleic acid extraction from tissue samples were performed using the QIAamp Mini Elute virus spin kit (Qiagen, Germany, GmbH). Procedures were conducted according to manufacture instructions.

Oligonucleotide Primers: Supplied from **(Metabion Germany)** as following;

Agent	Gene	Primer sequence (5'-3')	Amplified product (bp)	Reference
REO	S2	CCC ATG GCA ACG ATT TC	399 bp	**Bruhn *et al.,* (2005)**
		TTC GGC CAG GTC TCA AC		
CIA	VP	AAT GAA CGC TCT CCA AGA AG	583 bp	**Hegazy *et al.,* (2010)**
		AGC GGA TAG TCA TAG TAG AT		

PCR amplification

CAV PCR: Primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Primary denaturation step was done at 95°C for 5 min, followed by 35 cycles of 94°C for 30 sec., 56°C for 40 sec. and 72°C for 45 sec. A final extension step was done at 72°C for 10 min.

ARV PCR: Primers were utilized in a 25µl reaction containing 12.5 µl of Quantitect probe rt-PCR buffer (QIAgen, GmbH), 1 µl of each primer of 20 pmol concentration, 0.25 µl of rt-enzyme 4.25 µl of water, and 6 µl of template. The reaction was performed in an applied biosystem 2720 thermal cycler. Reverse transcription was applied at 50 ^{o}C for 30 min, a primary denaturation step was done at 95 ^{o}C for 5 min, followed by 35 cycles of 94°C for 30 sec., 55°C for 45 sec. and 72°C for 45 sec. . A final extension step was done at 72°C for 10 min.

PCR Products analysis: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gelpilot 100 bp DNA ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Results

Clinical sings and Post mortem findings

The main recorded clinical signs were anorexic and depressed birds, ruffled feathers, pale comb and wattles, increase daily mortality, growth retardation, variance in live body weights, diarrhea and respiratory manifestations.

The post mortem lesions that observed were massive atrophy and congestion of thymus, generalized anemia (pallor), a reduction in the size of the spleen and the bursa of Fabricius, pale liver or enlarged livers with multiple white to yellow foci, spleenomegaly with discoloration and hard consistency, hydropericardium, the intestines of the stunted chicks were pale and dilated with gaseous and watery contents or poorly digested food materials also enlarged proventriculus and pancreas atrophy.

Serological monitoring

The overall Sero-prevalence rate of CAV during the 4 years of study period was 88% (84/96) in tested farms. However this sero-prevalence was differ from year to other as following 81% (25/31), 94%(34/36), 88%(15/17) and 83%(10/12) during 2015, 2016, 2017 and 2018 respectively, as shown in Table (1). In general, the antibody-positive % among tested chickens/flocks was ranged from 10-100% and Geometric Mean Titer (GMT) ranged from (20 -to-8054).

The overall Sero-prevalence rate of ARV during the 4 years of study period was 88% (81/92) in tested farms. However this sero-prevalence was differ from year to other as following 83% (24/29), 91% (31/34), 93% (13/14) and 87% (13/15) during 2015, 2016, 2017 and 2018 respectively, as shown in Table (2). In general, the antibody-positive % among tested chickens/flocks was ranged from 10-100% and Geometric Mean Titer (GMT) was ranged from (20-to-3900).

One of the main observations of this study was evidence of seroconvertion for both CAV and ARV antibody responses in 81%(69/85) of tested farms.

The sampled farms were from different districts inside Al Behera governorate as shown in table (4).The results provide evidence of widespread distribution of the CAV and ARV viruses in commercial broiler chicken flocks in the governorate.

Table (1). Sero-prevalence of CAV during 2015-2018

Year	Total examined farms	No. of CAV positive farms	Sero-positive %*
2015	31	25	81%
2016	36	34	94%
2017	17	15	88%
2018	12	10	83%
Total	**96**	**84**	**88%**

* Sero-positive %; calculated as (No. of Sero-positive farm/total tested farms) X 100.

Table (2). Sero-prevalence of ARV during 2015-2018

Year	Total examined farms	No. of ARV positive farms	Sero-positive %*
2015	29	24	83%
2016	34	31	91%
2017	14	13	93%
2018	15	13	87%
Total	**92**	**81**	**88%**

* Sero-positive %; calculated as (No. of Sero-positive farm/total tested farms) X 100.

Table (3). Detection of both CAV and ARV antibody responses in different districts of Behera governorate.

Districts	Total No. of Examined farms	Farms examined for both antibodies	No of Sero-Positive farms
Abo Elmatamer	29	26	21
Kafr Eldawwar	17	15	8
Aldelengat	10	7	7
Abo Homos	10	8	7
Etay Elbaroud	9	8	7
Damanhur	8	8	7
HoshEsa	8	5	5
El Mahmoudia	3	1	1
Edko	2	2	2
El Rahmania	2	1	1
Rashid	2	2	1
El Noubaria	1	1	1
Kom Hamda	1	-	-
Shabrakhet	1	1	1
Total	**103**	**85**	**69**

Molecular detection

However, we have tested the following different organs (Thymus, bursa of fabricious, liver, spleen, intestine) from a total 75suspected farms, and these farms were representing different age starting from 1st –to-44thdays ages for molecular examination, the molecular detection was as following;

Molecular detection of CAV virus was only 10.7% (6/56) during the whole 4 years. This detection rate was variable from year to other as following; 0% (0/3), 20% (3/15), 6.2% (1/16), 9.1% (2/22) during 2015, 2016, 2017 and 2018, respectively. Almost similar results were obtained in case of ARV detection, the total detection rate was 12.5% (6/48). This detection rate was variable from year to other as following; 25% (1/4), 14.3% (1/7), 11.1% (1/9), 10.7% (3/28) during 2015, 2016, 2017 and 2018, respectively as shown in Table 4.

The locality and age of positive farms of CAV and ARV by PCR test where explained in Table 5.

Table (4). Molecular detection of CAV and ARV during 2015-2018

	CAV			ARV		
Year	**Total examined farms**	**No. of positive farms**	**Positive %***	**Total examined farms**	**No. of positive farms**	**Positive %***
2015	3	0	0%	4	1	25%
2016	15	3	20%	7	1	14.3%
2017	16	1	6.2%	9	1	11.1%
2018	22	2	9.1%	28	3	10.7%
Total	**56**	**6**	**10.7%**	**48**	**6**	**12.5%**

* Positive %; calculated as (No. of positive farm/total tested farms) X 100.

Table (5). Positive farms of CAV and ARV by PCR test during 2015-2018

Farm				PCR results	
Serial	**Year**	**Districts**	**Age (Day)**	**CAV**	**ARV**
1	2015	Unknown	1	Nd*	pos
2	2016	Unknown	32	Nd*	pos
3	2016	Unknown	1	pos	Nd*
4	2016	Unknown	2	pos	Nd*
5	2016	Unknown	1	pos	Neg
6	2017	Damanhur	13	pos	Neg
7	2017	EtayElbaroud	18	Neg	pos
8	2018	HoshEsa	11	pos	Neg
9	2018	Aldelengat	Unknown	pos	Neg
10	2018	Abo Elmatamer	4	Neg	pos
11	2018	Abo Elmatamer	3	Neg	pos
12	2018	El Amereia	13	Neg	pos
Total	**12**			**6**	**6**

*Nd: not done

Discussion

Since the early detection and confirmation of both CAV (**El-Lethi *et al.,* 1990**) and ARV (**Tantawi *et al.,* 1984**) circulating in Egypt, no one can deny the economic importance and immunosuppression risks of two viruses especially in broiler production sectors. Furthermore, the difficulty of control of these two viruses as the only effective way to decrease their Biorisk for broiler sector is using potent, effective vaccines and vaccination regimes for breeder flocks to prevent the vertical transmission to one day old chicks **(Engstrom, 1999).**

In this study, we focused on the prevalence of both CAV and Reo virus in Al Behera governorates during 2015-2018 among commercial broiler chicken. However, the simple clinical diagnosis of CAV and ARV infections depending on the clinical signs, gross pathological findings of affected birds **(Rosenberger and Cloud, 1998),** nevertheless the recent tools for antibodies detection and virus molecular detection become an easy tool for confirmation **(McNulty, 1998; McNulty *et al.,* 1988).** Same investigation approach was adopt during this study, starting with clinical diagnosis "clinical signs and gross pathological findings of affected birds", also we made random detection of antibodies "sero-prevalence" and molecular detection of nucleic acid in tissues from diseased birds**.**

During our study, we have observed common clinical manifestations in all suspected cases such as anorexia, depression, ruffled feathers, pale comb and wattles, increase daily mortality, growth retardation, variance in live body weights, diarrhea and respiratory manifestations. These signs were common either CAV and/or ARV suspected cases similar to **(Yuasa *et al.,* 1987; Rekik and Silim, 1992).**

The main recorded postmortem lesions, in CAV suspected cases, were massive atrophy and congestion of thymus, generalized anemia (pallor), a reduction in the size of the spleen and the bursa of Fabricius, pale liver same as **(Hagood *et al* 2001; Schat, 2009).** But we didn't recorded cases for blue wing disease only hemorrhages on wing.

In general, the recorded variable mortality rates among all investigated suspected broiler flocks**;** have direct and indirect indication of immunosuppressant which increased suscepti-

bility to other diseases result in increased mortality and economic losses **(McNeilly *et al.* 1995; Toro *et al.,* 1997).**

ARV infection is the main cause of malabsorption syndrome. The main characteristics of this virus are growth retardation, large proventriculus, poorly-feathered birds, bloated intestines and diarrhea **(Rekik and Silim, 1992).** The morbidity is generally 5 to 15%, but may reach 40%. Mortality is observed especially during the first two weeks of life at levels of about 2 to 7% **(Rekik and Silim, 1992).** Macroscopic lesions of Reo virus suspected cases were enlarged livers with multiple white to yellow foci, splenomegaly with discoloration and hard consistency, hydropericardium, the intestines of the stunted chicks were pale and dilated with gaseous and watery contents. The intestinal contents of the stunted chicks showed poorly digested food materials also enlarged proventriculus and pancreas atrophy **(Songserm *et al.,* 2002 and Nili *et al.,* 2007)**.

A total 103 non vaccinated broiler flocks (3-8 weeks' ages) were monitored serologically for detection of antibody against CAV and ARV, using ELISA test. Serum samples were collected at 3-to-8 weeks of age to avoid false detection of maternal derived antibodies (MDA).

The overall Sero-prevalence rate of CAV during the 4 years of study period were 88% (84/96) farm which is confirm the naturally horizontal CAV infection **(Adair, 2000)**. However, sero-prevalence rates were differing from year-to-year 81% (25/31), 94% (34/36), 88% (15/17) and 83% (10/12) during 2015, 2016, 2017 and 2018 respectively (Table 1). These results are agreed with **Hegazy *et al.,* (2010)** and **Hegazy *et al.,* (2014)** who reported high sero-prevalence rate of CAV (87.8%) and (84.7%) in commercial broiler flocks in Sharkia province and agreed with **(Hussein *et al.* 2016)** who reported high sero-prevalence rate of CAV (85%) in fourty unvaccinated commercial broiler flocks in three Egyptian Provinces (El-Behera, Matrouh and Kafr El-Sheikh) during 2014-2015.

Furthermore, overall high sero-prevalence rate (88%) of ARV infection during the 4 years of study period. These results were also indicating infection of all positive flocks. Also the same year-to-year high sero-prevalence rates 83%, 91%, 93% and 87% during 2015, 2016, 2017 and 2018 respectively, as shown in (Table 2). The high sero-prevalence rate could be due to the natural high resistance of ARV which results in continuous challenge of the same flock in the field **(Meulemanns and Halen 1982).**

Furthermore, presence of specific CAV and/or ARV antibodies in broilers after 3 weeks of age are likely to reflect horizontally transmitted infection either from inter-bird contact or from contaminated environments **(Jorgensen, 1990)**.

The detection of CAV and ARV antibodies in the most districts of the Behera province confirmed the high prevalence rate in the province. In addition to the high sero-prevalence of both viruses, our study have recorded high co-infection rate (81%) of CAV and Reo virus antibody responses in the suspected examined flocks (Table 3). These results confirm the severity of the disease according to **McNeilly *et al.* (1995)** who infected one-day-old specific-pathogen-free white leghorn chicks with isolate of CAV and ARV strains by an oral route. Infected chicks had significantly lower weight gain and more severe tissue damage than chicks inoculated with either virus alone, but no increase in the severity of the disease signs.

Serological monitoring is potent tool for epidemiological study and screening of the flocks for CAV and ARV antibody but these does not indicate whether the chickens are currently infected or earlier infected **(Todd *et al.,* 1990).**

CAV virus and ARV virus were detected using PCR test in (10.7%) and (12.5%) farm respectively during the 4 years of study period . These agree with **Mansour *et al.* (2018)** who have confirm detection of ARV from Tenosynovitis and Malabsorption syndrome that affected broiler chickens in Sharkia Province, Egypt and **Hussein *et al.* (2016)** who have confirm detection of CAV using PCR testing affected broiler flocks in three Egyptian Provinces (El-Behera, Matrouh and Kafr El-Sheikh). In this study the positive farms using PCR test were differing in age from 1 to 32 days (Table 5) which indicates the presence of two routes of infection vertical and horizontal. However, the possibilities of vertical transmission incidence of CAV is probably restricted to a relatively short period after infection in parent flocks **(Yuasa and Yoshida, 1983)** but it will continue as one of possible risks for broiler chicken**.**

In conclusion, the recorded high sero-prevalence rates for CAV and ARV must grab our focus on the current vaccination regimes of the breeder flocks. Further, investigation is required to either improve the vaccination regimes or update the used vaccines to reduce the economic losses of CAV and ARV when chicks are infected during the first two weeks of life and this could be avoided when breeder hens are well vaccinated and enough maternal antibodies transfer to their progeny **(Claudio *et al.,* 2004 and Canal *et al.,* 2004).** Occurrence of serum negative breeder hens situation at any periods of life increase susceptible to infection and increase risk for CAV vertical transmition to their progeny resulting in disease **(Otaki *et al.,* 1992; Von Bulow and Schat, 1997)**.

Also **Eidson *et al* (1979)** showed that the progeny of vaccinated breeder hens were resistant to ARV challenge via the oral route at 1, 3, 10 and 17 days of age. However, due to the variety of pathogenic strains of ARV in the field, the efficacy of currently available vaccines may be limited to viruses that are not similar to the vaccine strain **(Eidson *et al* 1979).** Furthermore, maintaining an ARV-free flock is very difficult to be achieved, the principal methods to control ARV infection are strict biosecurity, good management practices, and vaccination. Because ARVs are resistant to disinfectants commonly used in poultry houses, effective cleaning and disinfection is required **(Meulemanns and Halen 1982).** However, the low PCR detection rate in comparing to serology results, these results may be due to early exposure and late timing of PCR test or improper compatibility of used PCR primers. However that we still recommend further studies to complete molecular characterization of circulated CAV and ARV to study their genetic diversity and relatedness to Egyptian viruses and also to be compared with the current used vaccines.

Finally, we are recommending the need for further detailed studies on CAV and ARV to study molecular characterization genetic diversity in Egypt. Revision the efficacy of vaccines and vaccination programs against CAV and ARV especially for breeder hens to avoid vertical transmission to produced chicks and transferring enough maternal antibodies to the progeny**.** Routinely check one day old chicks by PCR technique to confirm free from CAV and ARV vertical transmission. It is recommended to reduce risk of these immunosuppressive viruses by adopting biosecurity.

References

Abdel-Mawgod, S.; Adel, A.; Arafa, A.S. and Hussein, H.A. (2018). Full genome sequences of chicken anemia virus demonstrate mutations associated with pathogenicity in two different field isolates in Egypt. Virus Disease, 29(3), 333-341.

AboElkhair, M.; Abd El-Razak, A.G.; and Metwally, A.E.Y. (2014). Molecular characterization of chicken anemia virus circulating in chicken flocks in Egypt. Advances in virology.

Adair, B.M. (2000). Immunopathogenesis of chicken anemia virus infection. Develop. Compar. Immunol. 24, 247-255.

Aly, Mona M. (2001). Isolation of chicken infectious anemia virus from outbreaks in broilers chickens in Egypt. J. Egypt Vet. Med. Ass., 61(6): 137–147.

Bruhn, S.; Bruckner, L. and Ottiger, H.P. (2005). Application of rt-PCR for the detection of avian reovirus contamination in avian viral vaccines. Journal of virological methods. 123 : 179-186.

Canal, C.W.; D.J. Ferreira; M. Macagnan; L.C.B. Fallavena; H.L.S. Moraes and V.B. Wald (2004). Prevalence of antibodies against chicken anemia virus (CAV) in broiler breeders in Southern Brazil. Pesq. Vet. Bras. 24, 89-92.

Claudio, W.; D.J. Canal; Ferreia, M. Macagnan, L.C.B. Fallavena, Halmilton; L.S. Moraes and V.B. Wald (2004). Prevalence of antibodies against Chicken AnaemiaVirus (CAV) in broliler breeders in southern Brazil. Pesq. Vet. Bras, Riode Janeiro, 24.

Dhama, K.; Mahendran, M.; Somvanshi, R.; and Chawak, M.M. (2008). Chicken infectious anaemia virus: an immunosuppressive pathogen of poultry-A Review. Indian J. Vet. Pathol, 32(2), 158-167.

Eidson, C.S.; Page, R.K.; Fletcher, O.J. and Kleven, S.H. (1979). Vaccination of broiler breeders with a tenosynovitis virus vaccine. Poult. Sci; 58: 1490-1497.

El-Lethi, A.M. (1990). *Viral contamination of dressed poultry. MV Sc*. Diss. Thesis, Cairo University, Egypt.

Engström, B.E. (1999). Prevalence of antibody to chicken anaemia virus (CAV) in Swedish chicken breeding flocks correlated to outbreaks of blue wing disease (BWD) in their progeny. Acta Vet Scand 40: 97—107.

Erfan, A.M.; Selim, A.A. and Naguib, M.M. (2018). Characterization of full genome sequences of chicken anemia viruses circulating in Egypt reveals distinct genetic diversity and evidence of recombination. Virus research, 251, 78-85.

Fenner, F.J.; E.P.J. Gibb; F.A. Murphy; R. Rott; M.J. Studdert and D.O. White (1993). Veterinary Virology,2nd ed. Academic Press, San Diego, CA, p: 609.

Hagood, L.T.; Kelly, T.F.; Wright, J.C. and Hoerr, F.F. (2001). Evaluation of chickens infectious anemia virus and associated risk factors with diseases and production losses in broilers. Avian Diseases, v. 44, p. 803-808, 2001.

Hegazy, A.; Abdallah, F.; Abd-El Samie, L.K. and Nazim, A. (2014). Incidence of Chicken Anemia Virus in Sharkia governorate chicken flocks. Assiut Vet. Med. J. Vol. 60 No. 142.

Hegazy, A.M.; Abdallah, F.M.; Abd-El Samie, L.K. and Nazim, A.A. (2010). Chicken Infectious Anemia Virus (CIAV) in Broilers and Laying Hens in Sharkia Province, Egypt. Journal of American Science; 6 (9).

Hussein, E.; Arafa, A.E.; Anwar, N. and Khafaga, A. (2016). Molecular and pathological analysis of chicken anemia virus isolated from field infection in three Egyptian Provinces. Adv. Anim. Vet. Sci, 4(5), 218-229.

Hussein, H.A.; Sabry, M.Z.; El-Ibiary, E.A.; El-Safty, M. and El-Hady, A.A. (2002). Chicken infectious anaemia virus in Egypt: molecular diagnosis by PCR and isolation of the virus from infected flocks. Arab Journal of Biotechnology, 5(2), 263-274.

Jorgensen, P.H. (1990). A micro-scale serum neutralization test for the detection and titration of antibodies to chicken anaemia agent - prevalence of antibodies in Danish chickens. Avian Pathol., 19: 583-593.

Madbouly, H.M. and El-Sawah, A.A. (1999). Isolation of reovirus from naturally infected turkeys and turkey poults in Egypt. Beni-Suef, Vet. Med. J, Vol. 9, No. 3-B, 513-525.

Madbouly, H.M.; El-Sawah, A.A. and Tamam, S.M. (2001). An outbreak of avian reovirus in native breed broilers at El-Fayuom Governorate. Beni-Suef, Vet. Med. J, VolXI, No.(1)31-45.

Madbouly, H.M.; Hussein, A.S.; Zaki, T.K. and Ensaf, M.H. (2009). Preparation of oil adjuvant Inactivated avian reovirus vaccines. 3rd Sci. Conf., 29 Jan.-1 Feb. 2009 Benha & RasSudr, Egypt. Fac. Vet. Med.(Moshtohor), Benha Univ., 510-529.

Madbouly, H.M.; M.S. Saber; Amina, A.M. Nawar and Samah, H. Mohamed (1997). Studies on the avian reoviruses in Egypt I-.Isolation and identification of the virus. Beni -Suef, Vet.Med.Res.,Vol. VII, No.2, 29-45.

Mansour, S.M.; ElBakrey, R.M.; Orabi, A.; Ali, H. and Eid, A.A. (2018). Isolation and Detection of Avian Reovirus from Tenosynovitis and Malabsorption Affected Broiler Chickens with Involvement of Vertical Transmission. Journal of Virological Sciences, 4(1), 24-32.

Mathews, R.E.F. (1982). Classification andnomenclature of viruses. Intervirology. 17: 1-200.

McConnell, C.D.; B.M. Adair and M.S. McNulty (1993). Effects of Chicken Anemia Virus on Cell-Mediated Immune Function In Chickens Exposed to the Virus By A Natural Route. Avian Dis., 37: 366-74.

McNeilly, F.; Smyth, J.A.; Adair, B.M. and McNulty, M.S. (1995). Synergism between chicken anaemia vims (CAV) and avian reovirus. Avian Dis; 39, 532-537.

McNulty, M.S. (1993). Reovirus. In: McFerran J.B., McNulty M.S. (editors). Virus Infections of Birds. Elsevier Science Publishers, Amsterdam, London, New York, Tokyo, p. 181-198.

McNulty, M.S. (1998). Chicken anemia virus. A laboratory manual for the isolation and identification of avian pathogens. 4th Ed. Swayne DE, Glisson JR, Jackwood MW, Pearson JE, Reeds WM. Eds. Kenneth square: Am. Assoc. Avian Pathol. pp. 146-149.

McNulty, M.S. and J.B. McFerran (1996). "Diseases associated with the Picornaviridae." Poultry Diseases 187-198.

McNulty, M.S.; Connor, T.J.; McNeilly, F.; Kirkpatrick, K.S. and McFerran, J.B. (1988). A serological survey of domestic poultry in the United Kingdom for antibody to chicken anaemia agent. Avian Pathology, 17(2), 315-324.

McNeilly, F.; Smyth, J.A.; Adair, B.M. and McNulty, M.S. (1995). Synergism between chicken anaemia vims (CAV) and avian reovirus. Avian Dis; 39, 532-537.

Meulemanns, G. and Halen, P. (1982). Efficacy of some disinfectants against infectious bursal disease vims and avian reovirus. Vet. Rec;111: 412-413.

Mohamed, M.A. (2010). Chicken infectious anemia status in commercial broiler chickens flocks in assiut-upper Egypt: occurrence, molecular analysis using PCR-RFLP and apoptosis effect on affected tissues. International Journal of Poultry Science, 9(6), 591-598.

Nili, H.; Jahantigh, M. and Nazifi, S. (2007). Clinical observation, pathology, and serum biochemical changes in infectious stunting syndrome of broiler chickens. Comp. Clin Pathol. 16: 161–166.

Otaki, Y.; K. Saito; M. Tajima and Y. Nomura (1992). Persistence of maternal antibody to chicken anemia agent and its effect on the susceptibility of young chickens. Avian Pathol. 21,-147-151.

Rosenberger, J.K. and Cloud, S.S. (1998). Chicken anemia virus. Poult. Sci., 77: 1190-1192.

Rekik, M.R. and Silim, A. (1992). Comparison of a vaccine strain and field isolates of avian reovirus by T1-oligonucleotide mapping. Avian diseases, 237-246.

Rosenberger, J.K. (2003). Reovirus infections: introduction and viral arthritis. In: Saif Y.M, Barnes H.J., Glisson J.R., Fadly A.M., McDougald L.R., Swayne D.E. (editors). Diseases of Poultry. 11th ed., Iowa State Press, Ames, IA., p. 283-293.

Sabry, M.Z.; Khafagy, A.K.; Elsamadony, H.A. and Elmahgoub, K.M. (1998). A seroepidemiological survey of meat- and eggtype chickens in Egypt for antibody to chicken infectious anemia virus. Proc. of the 5th Sci. Conf., Egypt. Vet. Poult. Assoc., P: 77–98.

Schat, K.A. (2009). Chicken anemia virus. In TT Viruses (pp. 151-183). Springer, Berlin, Heidelberg.

Songserm, T.H.; Engel, B.; Van Roozelaar, D.J; Kok, G.L.; Pijpers, A.; Pol, J.M.A. and TerHuurne, A.A.H.M. (2002). Cellular immune response in the small intestine of two broiler chicken lines orally inoculated with malabsorption syndrome homogenates. Veterinary Immunology and Immunopathology. 85(1-2): 51-62.

Tantawi, H.H.; Amina, N.; Youssef, Y.I.; Fawzia, M.; Al-Abdulla, J.M.; El-Batrawi, A.; ElGhawas, A.; Nasser, A.A. and Reda, I.M. (1984). Infectious tenosynovitis in broilers and broiler breeders in Egypt. Vet Res Commun 8: 229-235.

Todd, D.; Mackie, D.P.; Mawhinney, K.A.; Connor, T.J.; McNeilly, F. and McNulty, M.S. (1990). Development of an enzyme-linked immunosorbent assay to detect serum antibody to chicken anemia agent. Avian diseases, 359-363.

Toro, H.; A.M. Ramirez and J. Larenas (1997). Pathogenesity of Chicken Anemia Virus (isolate 10343) for Young and Older Chickens. Avian Pathol., 26: 485-499.

Von Bulow, V. and K.A. Schat (1997). Chicken infectious anemia. In: Diseases of Poultry.)Calnek, B. W., Ed.) Ames, Iowa, USA: Iowa State University Press, 739-756.

Yuasa, N. and Yoshida, I. (1983). Experimental egg transmission of chicken anemia agent. Natl. Inst. Anim. Health Q., 23: 99 100.

Yuasa, N.; Taniguchi, T. and Yoshida, I. (1979). Isolation and some characteristics of an agent inducing anemia in chicks. Avian Diseases, 366-385.

Yuasa, N.; Imai, K.; Watanabe, K.; Saito, F.; Abe, M. and Komi, K. (1987). Aetiological examination of an outbreak of haemorrhagic syndrome in a broiler flock in Japan. Avian Pathology, 16(3), 521-526.

Zaki, A.A. and El-Sanousi, A.A. (1994). Chicken anemia agent in Egypt: A serological survey of antibody against chicken anemia agent I some commercial chicken flocks using indirect immunofluorescent technique. Vet. Med. J. Giza, 24(3): 53–58.