CEVA HANDBOOK
OF POULTRY DISEASES

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DEFINITION

Chicken Anaemia is clinically and morphologically characterised by aplastic anaemia, severe immunosuppressive syndrome and frequent complications from secondary viral, bacterial and fungal infections.

HISTORY

Chicken Anaemia Virus (CAV), initially named Chicken Anaemia Agent (CAA) was isolated for the first time in Japan by Yuasa et al. (1979). Since that time, the virus has been detected serologically or through culturing in many countries in layer and broiler farms (Schat & Santen, 2008).
Some retrospective serological and clinical investigations, however, suggest that CAV was probably encountered before (Jakovski et al., 1970). The detection of Chicken Infectious Anaemia Virus (CIAV) in serum collections has shown that CIAV was present even before 1959 (Toro et al., 2006).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

CAV is a small virus without an envelope, from the family Circoviridae, genus Gyrovirus (Pringle, 1999). It is composed of DNA and proteins. The virion is icosahedral with a mean diameter of about 25 nm. The genome consists of single-stranded circular DNA with about 2,300 base pairs in its replicative form (Von Bulow and Schat, 1997). Viral proteins are VP1, a primary structure protein detected only in the capsid (51.6 kDa); VP2 – a skeleton-forming protein with protein phosphatase activity (24 kDa) and VP3, a nonstructural protein called also apoptin (13.6 kDa) due to its ability to induce apoptosis. VP1 and VP2 are the main targets of neutralising antibodies (Noteborn et al., 1992). The VP1 gene is the most variable (Schat, 2003), whereas VP3 was reported to be DNA-linked (Leliveld et al., 2003).
All known viruses currently identified worldwide obviously belong to the same serotypes. Due to the fact that, at present, only a few complete genome sequences for CAV strains from the USA, Europe, Asia and Australia are available, the emergence of new serotypes with a major impact on serodiagnostics and vaccination efficiency should not be excluded (Ducatez et al., 2006). In China, 25 CAV genomes were completely profiled (Eltahir et al, 2011).

Seroconversion is simultaneous with the onset of sexual maturity in SPF flocks, and the presence of viral DNA in male and female gonads and freshly laid fertilised eggs gave rise to the hypothesis that the genetic regulation of CAV was related to reproductive system regulation. The reproductive system has multiple genes, requiring space and time for expression.

The major part of this regulation occurs at the level of transcription with gene expression, regulated by promoter-specific sequences through a cell-specific process, and many of these genes are regulated by nuclear receptors from the steroid / thyroid family. These transcription factors regulate target genes via binding to specific DNA sequences or response elements and enhancer co-activators or corepressors (substances suppressing gene expression). Most estrogen-response genes contain an incomplete estrogen-response element involving up to 3 nucleotides, and spaced direct repeats in the DNA sequence responsible for controlling the various estrogen-regulated genes (Aumais et al., 1996; Krieg et al., 2001).

Viral DNA replication occurs via A double-stranded replicative process involving a rolling-circle mechanism. The most likely scenario is that virions enter the cells via conventional absorption and penetration (Schat & Van Santen, 2008). The main CAV transcript is unspliced polycistronic mRNA which codes for the three primary proteins (VP1, VP2, VP3). Although additional proteins may be coded by spliced CAV mRNAs, their biological importance is not yet clear (Kamada et al., 2006). Apoptin was shown to induce apoptosis selectively in transformed, but not in non-transformed cells (Oorschot et al., 1997; Maddika et al., 2006).

The presence of the apoptin gene in CAV confirms the importance of this protein in the life cycle of CAV, without providing any direct proof of a fundamental role in CAV replication. The first evidence about the need from apoptin in DNA replication and CAV viral particles production is reported by Prasetyo et al. (2009).

On the basis of the presence of three amino acid patterns related to rolling-circle replication of DNA, it was suggested that VP1 could be involved in DNA replication. The protein phosphatase activity of VP2 is important but not essential for CIAV replication. CIAV with mutation of the catalytic cysteine of VP2, which eliminates serine/threonine and tyrosine phosphatase activities, has a reduced replication potential

and cytopathogenicity (Schat & Van Santen, 2008). It has also been suggested that VP3 could affect the gene expression or DNA replication.
The replication of CIAV in young chickens occurs mainly in bone marrow haemocytoblasts and T-cell precursors of the thymus cortex. The viral replication in the thymus cortex has a fatal outcome due to VP3-mediated apoptosis (Jeurissen et al., 1992). In infected chickens 3–6 weeks of age, CIAV replication occurs in the thymus cortex and more rarely in the bone marrow (Smytb et al., 2006). Virus replication was also observed in other organs as well, but not always is association with lymphocytes.
No antigenic differences were identified among isolates from Europe, USA and Japan. It is assumed that all strains are of a single serotype. The existence of a second serotype CIAV -7 with similar but significantly less pronounced clinical and morphological signs than those induced by CIAV was suggested (Schat & Van Santen, 2008).
CIAV is exceptionally resistant to physical and chemical agents.
The virus survives for 1 h at 70°C and for 15 min at 80°C. It is completely inactivated after 15 min at 100°C. 24-hour fumigation with formaldehyde does not inactivate CIAV completely. The virus is also resistant to acid treatment at pH 3 for 3 hours and exposure to 90% acetone for 24 hours.

EPIDEMIOLOGY

CAV is spread both horizontally and vertically; in commercial and SPF chickens, the virus may be latent as well (Miller and Schat, 2004).
Vertical transmission was initially believed to be the most important factor in CAV spread (Hoop, 1992, 1993). Despite that, the horizontal spread of infection is a major factor in CAV infection of poultry flocks. Efforts to prevent CAV infection are primarily aimed at minimising horizontal spread, as chickens of all ages are susceptible to infection. The horizontal spread within a flock is attributed to the faecal-oral route of transmission, but this has not been consistently proved. The relatively limited available research on horizontal CAV spread in commercial flocks has yielded different results. Having attempted

to reproduce an experimental infection via oral exposure, some researchers concluded that this could not be a route of transmission in commercial flocks (Joiner et al., 2005; Van Santen et al., 2004). Opposite to this statement, others reported that adult birds could be orally infected with CAV (Smyth et al., 2006). It has also been suggested that intratracheal and oral mucosa could be routes for natural infections (Rosenberger & Cloud, 1989; Tan & Tannock, 2005). The marked resistance of CAV to extreme physical conditions (high temperatures, low pH etc.) explains the ubiquity of the virus among commercial flocks (Schat, 2003). The relatively wide spread and resistance are similar to those of another avian virus – Marek's disease virus (MDV).

In cases of MDV, the epithelium of feather follicles is the only site where the virus is reproduced in cell-free stable form, subsequently spreading through the dander and dust in poultry houses, inhaled through the mucous coating (Davidson and Borenshtain, 2003). Feather follicle epithelial cells are the targets for some members of the avian Circovirus genus, e. g. the beak and feather disease virus (BFDV) (Bassami et al., 1998; Pass and Perry, 1984; Niagro et al., 1998) and the agent of feather disease in Australian ravens (Corvus coronoides), (Stewart et al., 2006). In parallel to this data, it has been speculated and demonstrated that feather shafts of CAV -infected chickens contain a virus, which could be transmitted to and infect other chickens under experimental conditions (Davidson et al., 2008). To prove the significance of feather shafts as a source of CAV, a direct infection was attempted in chickens. This experiment demonstrated the penetration of the virus through the oral, nasal and ocular mucosa after experimental instillation. Thus, CAV transmission via a mechanism comparable to that of MDV was demonstrated (Davidson and Borenshtain, 2003). As feathers turned out to be a source of infective CAV, the transmission of the infection via the feathers could be an additional route of horizontal CAV transmission independent of the faecal-oral route. This presents us with certain specific challenges to avoid the horizontal spread of CAV infection. As MDV and CAV are widely prevalent, most commercial flocks carry both viruses at different viral loads (Davidson et al., 2008).

CAV is detected in many specific pathogen-free (SPF) chicken flocks. SPF eggs used for production of

vaccines may be infected with CAV and thus contaminate some of the vaccines.

Chickens are the only acknowledged host for CIAV. Antibodies against the virus have been reported in Japanese quails and ravens, but not in other bird species. Birds of all ages are susceptible, especially during the first 3 weeks of life (Schat & Van Santen, 2008).

CLINICAL SIGNS AND PATHOLOGY

Clinical infection

In general, clinical symptoms and death may be observed within 10-14 days following inoculation. The signs appear more rapidly after intramuscular injection as compared to oral inoculation. The CAV infection of chickens is manifested with a complex of clinical and pathological signs, some of them clinical, and others occult. Clinical anaemia is encountered in young chickens, while subclinical immunosuppression effects are observed when chickens older than 3 weeks of age are infected (Schat et al., 2003). In field conditions following vertical transmission, increased mortality is observed at 10–12 days with a peak at 17–24 days of age. Severely infected flocks may exhibit a second peak at 30–34 days of age, probably resulting from horizontal transmission (Schat & Van Santen, 2008).

Although the prevalence of CAV infection is high, the clinical disease is very rarely seen (McNeilly, 1991). Clinical infection was observed only in young chicks with vertically transmitted CAV from the parents. The symptoms include weakness, depression, anorexia and stunting. The standard death rate is 5–20%, but may reach 60%. The carcasses are typically pale, with marked anaemia of the skin, the comb and wattles, eyelids and legs. Blood haematocrit values in anaemic states vary between 6 and 27%. Reduced haematocrit, red blood cell, white blood cell and platelet counts are a common finding. Low haematocrit values are due to pancytopaenia secondary to infection of haemocytoblasts, manifested in a marked reduction of blood cell counts. Reduced blood clotting capacity is most likely a result of thrombocytopenia.

In chickens that survive the infection, recovery is slow, and usually takes 2–3 weeks. The slow remission and increased mortality rates are frequently associated with secondary bacterial or viral infections. Skin lesions are typical for clinical CAV infections, most commonly in the region of the wings, due to secondary bacterial infection (anaemia-dermatitis syndrome) (Hussein et al., 2002).

Subclinical infection

Horizontally transmitted infection in chickens older than 2 weeks of age is usually subclinical (McNulty et al., 1991). Although not apparent, it could influence the productive traits and be of economic significance. Breeder flocks involved in CAV transmission exhibit no signs of disease. Surveys in commercial breeder flocks transmitting CAV did not detect any alterations in egg yields, nor fertilisation and hatchability (McIlroy et al., 1992). Attempts for experimental transmission to hens did not result in changed live body weight or egg yields between infected and control groups of birds (Hoop, 1992).

Immunosuppression

Birds with subclinical and those remitted from clinical infection are immunosuppressed. Morphologically, the immunosuppression is manifested by a depletion of the lymphocytes in the spleen and the bursa of Fabricius and destruction of myeloid precursors of bone marrow cells between 2 and 3 weeks of age. Several investigations have demonstrated a transient immunosuppression and provided evidence for completely restored immune defence within 30 days of infection. Severe defects in T-cell mediator functions as well as reduction of macrophageal activity were identifed in CAV - infected chickens (Adair et al., 1991). The secondary bacterial or fungal infections are also signs of immunosuppression. A typical secondary bacterial infection is gangrenous dermatitis, starting with wing haemorrhages. Pulmonary aspergillosis has also been described (Randall et al., 1984; Goryo et al., 1987). In birds with seroconversion to CAV, failures in the immune response after vaccination against Marek´s disease or a weak response to NDV-vaccine (Otaki et al., 1987) were reported.

Gross lesions

Specific findings are the atrophy of central (thymus and bursa of Fabricius) and peripheral (bone marrow, spleen) immune organs. Atrophied bursa of Fabricius is less frequently associated with CAV infection. In a small minority birds its size is reduced, and the outer wall may become semi-transparent, revealing the mucosal folds. Sometimes, haemorrhages of muscles and theproventriculus mucosa are observed (Schat & Van Santen, 2008).

DIAGNOSIS

Tentative diagnosis is made on the basis of the clinical signs and macroscopic lesions. The flock history is a relatively simple method for determination of the breeders' role. When signs appear in the offspring, however, the parent flock should already have CAV antibodies (McNulty, 1991).

The diagnosis consists in virus isolation or detection of a virus-specific antigen or nucleic acid. Considering that CAV isolation in SPF chickens or cell cultures (MDCC-MSB1 cells) is a difficult and Laborious process, the preferred method is the detection of viral DNA through hybridisation or PCR techniques in tissue samples of infected birds (Yamaguchi et al., 2001). PCR is a rapid diagnostic tool for detection of viral genome in clinical samples –serum, urine (Arthur et al., 1989), faeces (Gouvea et al., 1990) and for DNA extraction from fresh (Arthur et al., 1989; Rogers et al., 1990) and formalin-fixed and paraffin-embedded tissues (Rogers et al., 1990). Naturally spread isolates belong to the same serotype and cannot be distinguished by serum neutralisation tests. The differentiation of CAV isolates is possible through immuno-fluorescent staining techniques with monoclonal antibodies (McNulty et al., 1990), restriction endonuclease analysis (Todd et al., 1992), and DNA sequence differentiation (Renshaw et al., 1996).

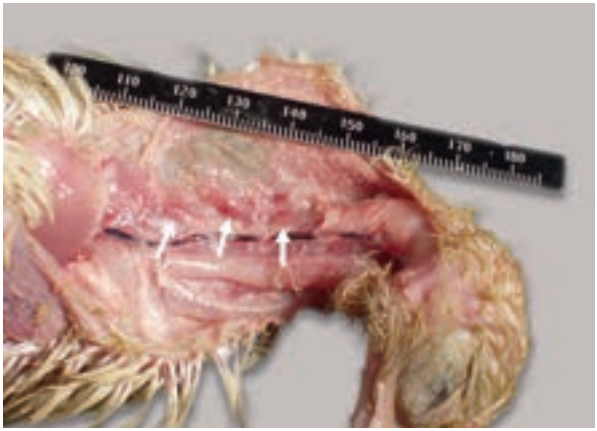

Fig.1

As a result of atrophy of the thymus the size of lobes of the gland is strongly reduced, and they may even disappear.

Fig.2

Bone marrow atrophy is the most specific lesion, most obvious in the femur. Affected bone marrow is fatty, yellowish or pale rose (left; right – normal appearance).

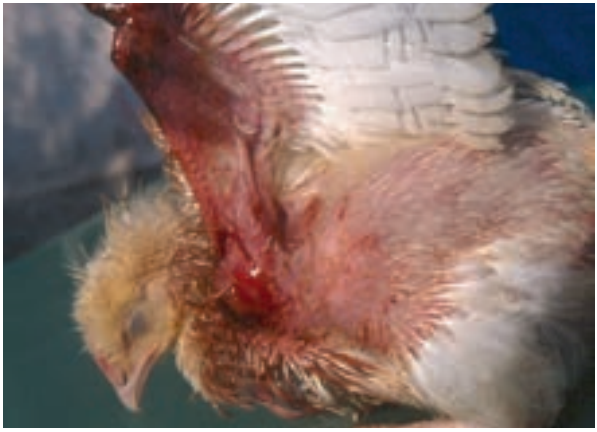

Fig.3

Gangrenous dermatitis findings are common after secondary clostridial or staphylococcal infection resulting from the severe immunosuppression.

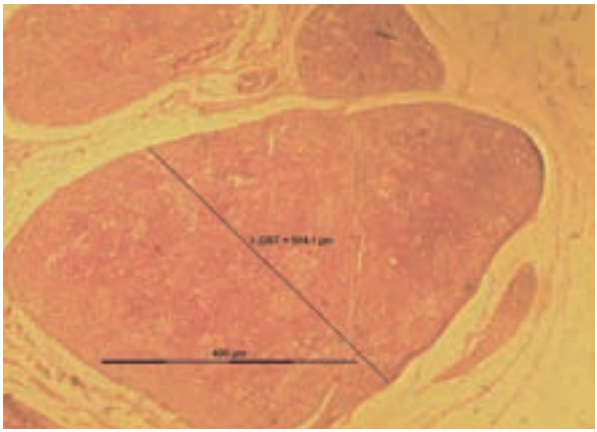

Fig.4

Routine histological examinations reveal simultaneous atrophy of cortical and medullary areas of the thymus as well as degeneration and necrosis of lymphoid cells.

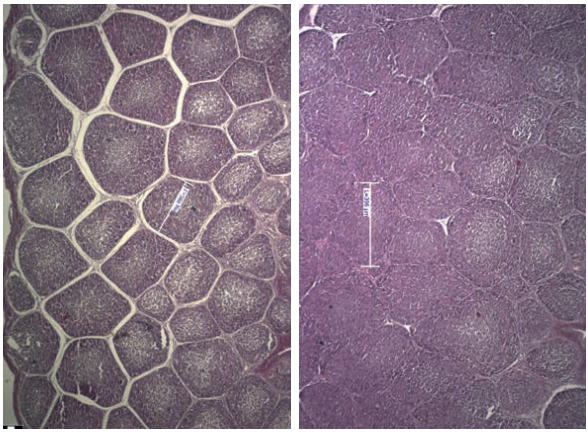

Fig.5

Left panel: atrophy of lymphoid follicles of B. Fabricii with central lymphoid tissue depletion ("empty" centres) and widened interstitial spaces. Right panel: control B. Fabricii.

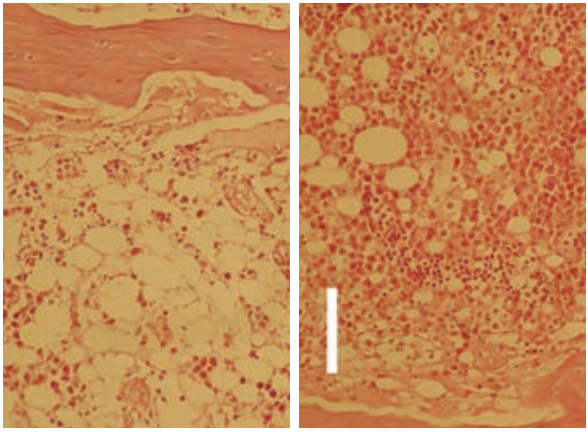

Fig.6

Left panel: severe bone marrow hypoplasia; complete lack of erythrocyte and granulocyte series, fatty substitution. Right panel: control bone marrow.

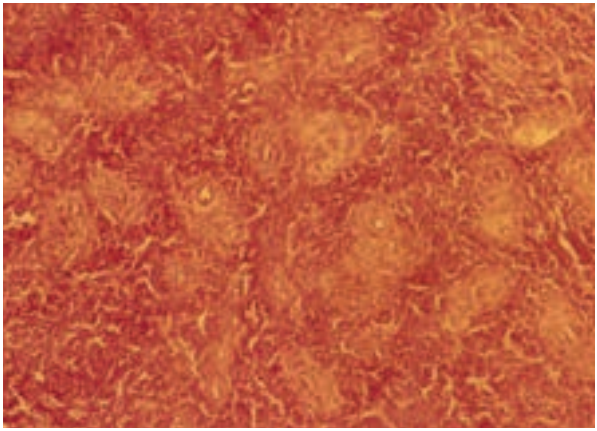

Fig.7

Spleen. Assembly of numerous periarteriolar centres in one observation field following tissue atrophy in the T-dependent zone.

DIFFERENTIAL DIAGNOSIS

CAV infection should be distinguished from states associated with atrophy of the central and peripheral immune organs and aplastic anaemia symptoms.
Such alterations may trigger viruses causing neoplastic lesions, such as the osteopetrosis virus, erythroblastosis viruses and MDV. The infectious bursitis virus induces lymphoid tissue atrophy, but the thymus is usually not affected. Adenoviral infections may result in aplastic anaemia, but generally speaking such infections are manifested simultaneously with inclusion body hepatitis. Intoxication with mycotoxins, e.g. aflatoxins, or sulfonamides could also provoke aplastic anaemia or haemorrhagic syndrome. In field conditions, however, chickens are rarely exposed to toxic doses of aflatoxin or sulfonamides (Schat & Van Santen, 2008).

PREVENTION AND CONTROL OF CHICKEN ANAEMIA

Biosecurity

Strict isolation and hygiene controls are needed to protect chickens from exposure to CAV. Although this is hardly possible for broilers, it can be achieved for breeders.
CIAV infection can be controlled by allowing for development of antibodies in breeder flocks before the period of laying. In such cases we rely upon natural immunity, and it should be remembered that in many regions, the egg-laying period of chicken flocks begins without previous exposure to CAV, most likely due to strict hygiene controls to ensure Salmonella-free flocks (McNulty, 1991). CAV eradication in field conditions is impossible, because of the high resistance of CAV and the possibility for vertical transmission of viral DNA.

Vaccination

To prevent the vertical CAV transmission from broiler breeders

to their progeny, since 1991 vaccination with a live vaccine is practiced. Many studies suggest that maternal antibodies could protect chickens against CIAV until the 3rd week of age (Goodwin et al., 1993; Otaki et al., 1992; Yuasa et al., 1980). The vaccination of breeders during the growing period protects the chicks from vertical transmission of the virus via provision of protective maternal antibodies. Commercial live vaccines are available for vaccination of breeder flocks. Immunisation can be performed after 8 weeks of age, but no later than 6 weeks before laying begins. The vaccinal virus may be shed in faeces. Biosecurity should be strict in order to prevent spread of the virus to younger chicks, flocks in egg production period or 6 weeks before laying begins. The use of such products in chickens younger than 3 weeks of age can trigger clinical infectious anaemia. Live vaccines may be administered by drinking water, by injections with an adjuvant or through the wing web application method using the enclosed 2-prong applicator. When broiler chickens are infected despite the presence of maternal antibodies, after MDA have waned vaccination of broilers may be necessary too. In the USA, a vaccine for application to one-day-old broiler chickens is licensed (Schat & Van Santen, 2008).

In SPF hens showing seroconversion, inactivated vaccines have also been tested. The titers of vaccinal antibodies in these cases were generally found to be low, and inactivated vaccines could thus prove to be ineffective.

In attempts to develop recombinant Vaccines against CAV and diagnostic systems for detection of the virus in chickens, two recombinant proteins – VP1Nd129 and VP2, were successfully expressed in E. coli, yielding 26.2 mg/L protein. The maximum yield of VP2 was 15.5 mg/L. Also, the antigenicity of VP1Nd129 and VP2 proteins expressed in E. coli with anti-CAV antibodies was considerable, suggesting that both proteins could be used as antigens for detection of anti- CAV antibodies in vaccinated and CAV -infected birds in breeder farms. Both recombinant proteins are able to induce specific neutralising antibodies against CAV, making them candidates for the development of recombinant CAV vaccines (Lee et al., 2008).

REFERENCES

- Adair, B.M., F. McNeilly, C.D.G. McConnell, D. Todd, R.T. Nelson, & M.S. McNulty, 1991. Effects of chicken anaemia agent on lymphokine production and lymphocyte transformation in experimentally infected chickens. Avian Dis. 35, 783-792.
- Arthur R.R., S. Dagostin & K.V. Shad, 1989. Detection of BK virus in urine and brain tissue by the polymerase chain reaction. J. Clin. Microb. 27, 1174-1179.
- Aumais, J.P., H.S. Lee, C. DeGannes, J. Horsford & J. H. White, 1996. Function of directly repeated half-sites as response elements for steroid hormone receptors. J. Biol. Chem. 271, 12568–12577.
- Bassami, M.R., D. Berryman, G.E. Wilcox, & S.R. Raidal, 1998. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circovirus and chicken anemia virus. Virology 249, 453–459.
- Danen - van Oorschot, A.A.A.M., D.F. Fischer, J.M. Grimbergen, B. Klein, S.-M. Zhuang, J.H.F. Falkenburg, C. Backendorf, P.H.A. Quax, A.J.v.d. Eb & M.H.M. Noteborn, 1997. Apoptin induces apoptosis in human transformed and malignant cells but not in normal cells. Proc. Natl. Acad. Sci. U.S.A. 94, 5843–5847.
- Davidson I., N. Artzi, I. Shkoda, A. Lublin, E. Loeb & K.A. Schat, 2008. The contribution of feathers in the spread of chicken anemia virus, Virus Research 132, 152–159
- Davidson, I. & R. Borenshtain, 2003. Novel applications of feather shafts extracts from MDV-infected chicks; diagnosis of commercial broilers, whole genome separation by PFGE and synchronic mucosal infection. FEMS Immunol. Med. Microbiol. 38, 199–203.
- Ducatez M.F., A.A. Owoade, J.O. Abiola & C.P. Muller, 2006. Molecular epidemiology of chicken anemia virus in Nigeria. Arch Virol, 151, 97-111.
- Eltahir Y.M., K. Qian, W. Jin, P. Wang & A. Qin, 2011. Molecular epidemiology of chicken anemia virus in commercial farms in China, Virology Journal, 8, 145.
- Goryo, M., Y. Shibata, T. Suwa, T. Umemura, & C. Itakura, 1987. Outbreak of anaemia associated with chicken anaemia agent in young chicks. Jpn. J. Vet. Sci. 49, 867-873.
- Gouvea V., R.I. Glass, P. Woods, K. Taniguchi, H.F. Clark, B. Forrester & Z. Fang, 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276-282.
- Hoop, R.K., 1992. Persistence and vertical transmission of chicken anemia agent in experimentally infected laying hens. Avian Pathol. 21, 493–501.
- Hoop, R.K., 1993. Transmission of chicken anemia virus with semen. Vet. Rec. 133, 551–552.
- Hussein, M.Z. Sabry, E.A. El Ibiary, M. El Safty & A.I. Abd El Hady, 2002. Chicken infectious anaemia virus in Egypt: Molecular diagnosis by PCR and isolation of the virus from infected flocks, Arab J. Biotech., Vol. 5, No.(2), 263-274.
- Jakowski, R.M., T.N. Fredrickson, T.W. Chomiak, & R.E. Luginbuhl. 1970. Hematopoietic destruction in Marek's disease. Avian Diseases, 14, 374-385.
- Jeurissen, S.H., F. Wagenaar, J.M. Pol, A.J. van der Eb & M.H. Notebom, 1992. Chicken anemia virus causes apoptosis ofthymocytes after in lliva infection and of cell lines after in vitro infection. Journal ofVirology, 66, 7383-7388.
- Joiner, K.S., S.J. Ewald, F.J. Hoerr, V.L. Van Santen, & H. Toro, 2005. Oral infection with chicken anemia virus in 4-wk broiler breeders: lack of effect of major histocompatibility B complex genotype. Avian Dis., 49, 482–487.
- Kamada, K., A. Kuroishi, T. Kamahora, P. Kabat, S. Yamaguchi, & S. Hino, 2006. Spliced mRNAs detected during the life cycle of chicken anemia virus. J. Gen. Virol. 87, 2227–2233.
- Krieg, S.A., A.J. Krieg & D.J. Shapiro. 2001. A unique downstream estrogen responsive unit mediates estrogen induction of proteinase inhibitor-9, a cellular inhibitor of IL-1beta-onverting enzyme (caspase 1). Mol. Endocrinol. 15, 1971–1982.
- Lee M.S., Y.Y. Lien, S.H. Feng, R.H. Huang, M.C. Ming-Cheng Tsai, W.T. H. Chang & H.J., si-Jien Chen, 2008. Production of chicken anemia virus (CAV) VP1 and VP2 protein expressed by recombinant Escherichia coli, Process Biochemistry, doi:10.1016/j.procbio.2008.11.016 Leliveld, S. R., R. T. Dame, M. A. Mommaas, H. K. Koerten,
- C. Wyman, A.A. Danen-van Oorschot, J.L. Rohn, M. H. Noteborn, & J.P. Abrahams, 2003. Apoptin protein multimers form distinct higher-order nucleoprotein complexes with DNA. Nucleic Acids Res., 31, 4805–4813.
- Maddika, S., F.J. Mendoza, K. Hauff, C.R. Zamzow, T. Paranjothy, & M. Los, 2006. Cancerselective therapy of the future: apoptin and its mechanism of action. Cancer Biol. Ther. 5, 10–19.

- McIlroy, S.G., M.S. McNulty, D.J. Bruce, J.A. Smyth, E.A. Goodall, & M.J. Alcorn, 1992. Economic effects of clinical chicken anaemia agent infection on profitable broiler production. Avian Dis. 36, 566-574.
- McNeilly, F., G.M. Allan, D.A. Moffat, & M.S. McNulty, 1991. Detection of chicken anaemia agent in chickens by immunofluorescence and immunoperoxidase staining. Avian Pathology, 20, 125-132.
- McNulty, M.S. 1991. Chicken anaemia agent: a review. Avian Pathol. 20, 187-203.
- Miller, M.M. & K.A. Schat, 2004. Chicken infectious anemia virus: an example of the ultimate host–parasite relationship. Avian Dis. 48, 734–745.
- Niagro, F.D., A.N. Forsthoefel, R.P. Lawther, L. Kamalanathan, B.W. Ritchie, K.S. Latimer & P.D. Lukert, 1998. Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. Arch. Virol. 143, 1723–1744.
- Noteborn M.H.M., C.A.J. Verschueren, D.J. van Roozelaar, S. Veldkamo, A.J. van der Eb & G.F. Boer, 1992. Detection of chicken anemia virus by DNA hybridization and polymerase chain reaction. Avian Pathol, 21, 107-118.
- Otaki, V., T. Nunoya, M. Tajima, H. Tamada, & Y. Nomura, 1987. Isolation of chicken anaemia agent and Marek´s disease virus from chickens vaccinated with turkey herpesvirus and lesions induced in chicks by inoculating both agents. Avian Pathol. 16, 291-308.
- Pass, D.A. & R.A. Perry, 1984. The pathology of psittacine beak and feather disease. Aust. Vet. J. 61, 69–74.
- Prasetyo A.A., T. Kamahora, A. Kuroishi, K. Murakami & S. Hino, 2009. Replication of chicken anemia virus (CAV) requires apoptin and is complemented by VP3 of human torque teno virus (TTV), Virology, 385, 85–92
- Pringle CR, 1999. Virus taxonomy at the XIth International Congress of Virology, Sydney, Australia. Arch Virol, 144, 2065-2069.
- Randall, C.J., W.G. Siller, A.S. Wallis & K.S. Kirkpatrick, 1984. Multiple infections in young broilers. Vet. Rec., 114, 270-271.
- Renshaw R.W., C. Soine, T. Weinkle, P.H. O'Connell, K. Ohashi, S. Watson, B. Lucio, S. Harrington & K.A. Schat, 1996. A hypervariable region in VP1 of chicken anemia virus mediates rate of spread and cell tropism in tissue culture. J. Virol. 70, 8872-8878.
- Rogers B.B., L.C. Alpert, E.A.S. Hine & G.J. Buffone, 1990. Analysis of DNA in fresh and fixed tissue by the polymerase chain reaction. Am. J. Pathol., 136, 541-548.
- Rosenberger, J.K. & S.S. Cloud, 1989. The effects of age, route of exposure and coinfection with infectious bursal disease virus on the pathogenicity and transmissibility of chicken anemia agent (CAA). Avian Dis., 33, 753–759.
- Schat K.A. & van V.L. Santen 2008. Chicken Infectious Anemia. In: Diseases of Poultry.12th Ed, Saif YM, Fadly AM, Glisson J.R., McDougald L.R., Nolan L.K. & Swayne D.E., eds. Iowa State University Press. Iowa, USA, pp: 211- 235.
- Schat, K.A., 2003. Chicken infectious anemia. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R., Swayne, D.E. (Eds.), Disease of Poultry, 11th ed. Iowa State University Press, Ames, Iowa, USA, pp. 182–202.
- Smytb, 1. A., D.A. Moffett, T.J. Connor & M.S. McNulty, 2006. Chicken anaemia virus inoculated by the oral route causes lymphocyte depletion in the thymus in 3-week-old and 6-week-old chickens. Avian Pathology 35, 254-259.
- Smyth, J.A., D.A. Moffett, T.J. Connor, & M.S. McNulty, 2006. Chicken anemia virus inoculated by the oral route causes lymphocyte depletion in the thymus in 3-week-old and 6-week-old chicks. Avian Pathol. 35, 254–259.
- Stewart, M.E., R. Perry, & S.R. Raidal, 2006. Identification of a novel circovirus in Australian ravens (Corvus coronoides) with feather disease. Avian Pathol. 35, 86–92.
- Tan, J. & G. Tannock, 2005. Role of viral load in the pathogenesis of chicken anemia virus. J. Gen. Virol., 86, 1327–1333.
- Todd D, K.A. Mawhinney & M.S. McNulty, 1992. Detection and differentiation of chicken anaemia virus isolates by using the polymerase chain reaction. J. Clin. Microbiol. 30, 1661-1666.
- Toro, H., S. Ewald & E.J. Hoerr, 2006. Serological evidence of chicken infectious anemia virus in the United States at least since 1959. Avian Diseases 50:124-126.
- Van Santen, V.L., K.S. Joiner, C. Murrey, N. Petrenko, F.J. Hoerr, & H. Toro, 2004. Pathogenesis of chicken anemia virus: comparison of the oral and intramuscular routes of infection. Avian Dis., 48, 494–504.
- Von Bulow, V. & K.A. Schat, 1997. Chicken Infectious Anemia. Pages 739–756 in: Diseases of Poultry. Chapter 30. Iowa State University Press; Ames, IA.
- Yamaguchi S., T. Imada, N. Kaji, M. Mase, K. Tsukamoto, N. Tanimura & N. Yuasa, 2001. Identification of a genetic determinant of pathogenicity in chicken anaemia virus. J Gen Virol, 82, 1233-1238.