IMMUNOREGULATION OF CHICKENS' B LYMPHOCYTES AND THREE-DIMENSIONAL LYMPHOID TISSUE CULTURE INFECTED WITH NEWCASTLE DISEASE VIRUS AND INFECTIOUS BURSAL DISEASE VIRUS

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By

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February 2014

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Among the common poultry disease worldwide, Newcastle Disease (ND) and Infectious Bursal Disease (IBD) are contagious and pose a major threat in devastating the poultry industry. Numerous studies were carried out to evaluate host response of avian lymphocytes against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) infection, but none has reported on the effect of these highly pathogenic viruses on pure B cells population. As B cell lineage lymphocytes are responsible for the production of antibodies, which play a role in preventing viral infection, this study investigated the responses of enriched B lymphocytes following infection of highly pathogenic NDV and very virulent IBDV strains. Cell viability and proliferation rate of *in-vitro* cultured B lymphocytes were assessed upon NDV and IBDV infection and results showed that other than the virus infection dosage, time course infection of the virus also affected the viability and inhibited the proliferation of B lymphocytes population in the culture. In the *in-vivo* study, chickens' spleen and bursa of Fabricius were investigated on their cell population changes and oxidative stress in relationship with the B lymphocytes response towards the infection of different genotypes of NDV and IBDV. NDV caused increment of macrophage in the organ which led to the elevation of nitric oxide content and NDV genotype VIII induced greater chronic impairment in chickens' spleen and bursa B cells with lower viral load detected compared to the infection by NDV Genotype VII. *In-vivo* study with IBDV infection revealed that the virus caused more severe damage in chicken bursa of Fabricius compared to the spleen. Further details showed that the depletion of B lymphocytes in chicken spleen is more relevant to the oxidative stress caused by the virus infection rather than the amount of virus residue in the cells. Meanwhile, the cell death event in B lymphocytes from bursa was in an increasing manner considerably with time of infection and viral load detected in the cells.

The second part of the study demonstrated the establishment of an *in-vitro* culture of chicken lymphoid tissue, simulating chicken embryonic bursa of Fabricius to study the interaction of chicken embryonic B cells upon infection with NDV and IBDV. Following infection with the viruses, cell population changes, viability, apoptosis and viral load were investigated. Results showed that IBDV caused drastic depletion in the matured (IgM+) and immature B cell (Bu-1a) populations while NDV infection induced the production of IgM+ cells, maybe as an effort to combat the virus infection. Cell death by apoptosis was assayed and the result showed that the *in-vitro* culture of chicken lymphoid tissue is susceptible to NDV and IBDV infection as higher titer of virus infection caused higher frequency of apoptosis as well as higher amount of viral load detected in the culture. The findings showed that the *in-vitro* model of chicken lymphoid tissue can be used to study the virus and host cells interaction. Moreover, the phenotypic and cell viability changes in the established mini organ upon virus infection are similar to previous reports by other researchers in their *in-vitro* and *in-vivo* approaches. In conclusion, B lymphocytes from chicken spleen and bursa of Fabricius at different age reacted unalike when infected with different strains of pathogenic NDV and IBDV. The depletion of the B lymphocytes population may be caused by population changes, oxidative stress or amount of virus resides in the cells.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

RESPON REGULASI-IMUN B LIMFOSIT DAN TISU LIMFOID AYAM TIGA DIMENSI TERHADAP JANGKITAN VIRUS NEWCASTLE DAN BURSA BERJANGKIT

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Antara penyakit ayam yang lazim berlaku di seluruh dunia adalah penyakit Newcastle (ND) dan penyakit berjangkit Bursal (IBD) yang menjadi ancaman utama dalam industri penternakan ayam. Banyak kajian telah dijalankan untuk menilai respons limfosit burung terhadap Virus Penyakit Newcastle (NDV) dan Virus Penyakit Jangkitan Bursa (IBDV), tetapi tiada laporan mengenai kesan terhadap populasi limfosit B tulen. Oleh kerana limfosit B bertanggungjawab untuk penghasilan antibodi yang memainkan peranan penting dalam mencegah jangkitan virus, kajian ini bertujuan untuk mengkaji respon limfosit B yang telah diperkaya selepas jangkitan NDV dan IBDV. Akibat jangkitan NDV dan IBDV, kebolehhidupan dan kadar proliferasi limfosit B telah dikaji secara *in-vitro* dan keputusan menunjukkan bahawa selain daripada dos jangkitan, masa jangkitan turut menjejaskan kebolehhidupan and menghalang proliferasi limfosit B. Dalam kajian *in-vivo*, siasatan terhadap perubahan populasi sel dan kesan tekanan oksidatif kepada limpa dan bursa Fabricius ke atas respon limfosit B telah dijalankan selepas jangkitan NDV dan IBDV yang berlainan genotip. Keputusan telah menunjukkan bahawa NDV menyebabkan peningkatan frekuensi makrofaj dalam kedua-dua organ dan seterusnya menyebabkan peningkatan kandungan oksida nitrik. NDV genotip VIII menyebabkan kemerosotan kronik yang lebih teruk ke atas sel limpa ayam dan bursa dengan kuantiti virus yang lebih rendah berbanding dengan jangkitan oleh NDV genotip VII. Kajian *in vivo* dengan jangkitan IBDV mendedahkan bahawa virus tersebut menyebabkan kerosakan yang lebik teruk pada bursa ayam berbanding limpa. Maklumat selanjutnya menunjukkan bahawa penyusutan limfosit B dalam limpa ayam adalah kerana tekanan oksidatif yang disebabkan oleh jangkitan virus. Sementara itu, kematian sel limfosit B dalam Bursa didapati meningkat sejajar dengan masa jangkitan dan peningkatan jumlah virus yang dikesan di dalam sel.

Bahagian kedua kajian ini mendemostrasikan pembentukan kultur tisu limfoid ayam tiga dimensi secara *in vitro*, yang mensimulasi bursa Fabricius embrio ayam untuk mengkaji interaksi antara sel antibodi embrio terhadap jangkitan NDV dan IBDV. Selepas jangkitan virus, perubahan populasi, kebolehidupan, apoptosis dan kuantiti virus telah dikaji. Keputusan menunjukkan IBDV menyebabkan penyusutan drastik dalam populasi antibodi IgM+ matang dan sel B kurang matang sementara itu jangkitan NDV pula mendorong penghasilan sel IgM+ yang mungkin disebabkan oleh tindak balas sel untuk mencegah jangkitan virus tersebut. Ujian telah dijalankan untuk melihat kematian sel secara apoptosis dan hasil ujian menunjukkan bahawa kultur in vitro tersebut boleh dijangkiti oleh IBDV dan NDV kerana kepekatan virus yang tinggi menyebabkan frekuensi apoptosis dan kuantiti virus di dalam kultur tersebut meningkat. Penemuan ini menunjukkan bahawa model *in vitro* sel kultur limfoid ayam boleh digunakan untuk mengkaji interaksi antara virus dan sel perumah. Tambahan pula, keputusan perubahan populasi sel dan kebolehhidupan sel di dalam organ mini semasa jangkitan virus adalah seiring dengan keputusan yang dilaporkan oleh penyelidik yang lain dalam pendekatan *in vitro* dan *in vivo* mereka. Kesimpulannya, sel B daripada limpa dan bursa ayam mempunyai tindak balas yang berbeza mengikut masa jangkitan apabila dijangkiti oleh IBDV dan NDV yang berbeza. Penyusutan populasi sel limfosit B mungkin disebabkan oleh perubahan populasi, tekanan oksidatif atau kuantiti virus yang menjangkiti sel.

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

2D	2 Dimension
3D	3 Dimension
7-AAD	7-aminiactinomycin A
AGID	Agar gel immunodiffusion test
ANOVA	Analysis of Variance
AO/PI	Acridine Orange/ Propidium Iodide
BrdU	Bromodeoxyuridine
BSA	Bovine Serum Albumin
CD	Cluster of differentiation
CDNA	Complementary deoxyribonucleic acid
CFSE	Carboxyfluorescein succinimidyl ester
CO_2	Carbon dioxide
Cq	Quantification cycle
Ct	Threshold cycle
DDA	Dimethyl dioctadecyl ammonium bromide
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
F	Fusion protein
FACS	Fluorescence-activated cell sorter
FITC	Fluorescein isothiocyanate
FSC	Forward Scatter

HA	Haemagglutination
HI	Haemagglutination inhibition
HN	Hemagglutinin-neuraminidase
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IBS	Institute of Bioscience
ICPI	Intracerebral pathogenicity index
IgG	Immunoglobulin G
IgM	Immunoglobulin M
iNoS	Inducible nitric oxide synthase
M	Matrix protein
MDA	Malondialdehyde
MDA	Malondialdehyde
MgCl2	Magnesium Chloride
MHC	Major histocompatibility complex
mm	Millimeter
mM	Millimolar
MOI	Multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MyCC	Malaysia Competition Commission
Na_2HPO_4	Disodium hydrogen phosphate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
ND	Newcastle disease
NDV	Newcastle disease virus

NO	Nitric Oxide
NP	Nucleoprotein
NTC	Non template control
OD	Optical density
OIE	Office International des Epizooties
ORF	Open reading frames
P	Phosphoprotein
PALS	Peri-arteriolar lymphoid sheath
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PE	Phycoeryhtrin
PerCP	Peridinin chlorophyll
PI	Propidium iodide
PS	Phosphatidylserine
PWP	Peripheral white pulp
RNA	Ribonucleic acid
rpm	Rotation per minute
RT-PCR	Reverse transcription-polymerase chain reaction
S.E.M	Standard Error Mean
SSC	Side Scatter
UPM	Universiti Putra Malaysia
vvIBDV	very virulent Infectious Bursal Disease Virus

CHAPTER 1

INTRODUCTION

Poultry industry is considered one of the tremendously growing sectors in Malaysia. Among all livestock in this country, chicken meat is consumed the most due to cultural and religious reasons. According to the review of domestic broiler market report issued by Malaysia Competition Commission (MyCC), (2012), annual consumption of broiler meat have been increasing steadily from about 31 kg per capita in year 2000 to 37 kg per capita in year 2010. In term of supply side, the 546,000 day-old-chicks produced in 2010 was about 56% higher than the 364,000 chicks produced in 2000. The total quantity supplied of 524,000 broilers in 2010 was more than 63% higher than the 364,300 broilers supplied in 2000. To date, Malaysia has in continued with its self-sufficiency of poultry meat supply that was first achieved in 1990. Thus, this industry is regarded as one of the most important livestock sector in Malaysia. Due to its economic significance, it is crucial to monitor and have control measures taken to prevent losses and devastation caused by the poultry disease (Malaysia Competition Commision, 2012).

Among the major threats that cause heavy losses in the poultry industry throughout the world are the Newcastle Disease (ND) caused by Newcastle Disease Virus (NDV) and Infectious bursal Disease (IBD), which is an acute contagious virus disease of young birds caused by Infectious bursal Disease Virus (IBDV).

Newcastle Disease Virus has been classified into the order of *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae* and genus *Rubulavirus* (Alexander, 1988a). The virus primarily infects poultry and can be categorized into three pathotypes; 8which produces respiratory and nervous signs with moderate mortality and the viscerotropic or neurotropic velogenic strain which causes severe intestinal lesions or neurological disease resulting in high mortality (Alexander, 1988b; Aini, 1990).

The first outbreak of IBD was reported in Gumboro Delaware, USA (Cosgrove, 1962), caused by the infectious bursal disease virus (IBDV), an avian *Birnavirus.* The serotype 1 IBDV can be classified into different group based on their virulence: mild, classical virulent, very virulent and variant strains (Cao et al., 1998). The IBDV targets for cells of the bursa of Fabricius in chickens and infects the actively dividing and differentiating B cell lineage lymphocytes (Burkhardt and Muller., 1987). This virus causes severe acute disease on 3-6 weeks old birds with high mortality rate. However, when infecting chicks younger than 3 weeks old, the virus causes less acute disease (OIE, 2008).

Against the infection of virus, adaptive immunity consisting of antibodies and lymphocytes, known as the humoral and cell mediated responses are important (Abbas et al., 2007). The antibody response is crucial in preventing viral infections and may contribute to the resolution of the viral infection. Upon infection with virus, antibodies are produced against epitopes on viral protein and subsequently block the virus infection via virus neutralization (Klimpel, 1996). Antibodies work in various ways to neutralize viral infectivity such as interfering the virion binding to receptors, block uptake into cells, prevent uncoating of the genomes in endosomes, or cause aggregation of virus particles (Klimpel, 1996). The B cell lineage lymphocytes are the one responsible for the production of antibodies against the virus infection. It was reported that certain NDV strains caused decreased in B cell population (Russell et al., 1997) and resulted in a compromised immune response in the chickens. As for IBDV, the virus was also shown to target IgM bearing B lymphocytes (Ogawa et al., 1998). To date, no study has been reported on the effect of very virulent strains of NDV and IBDV on pure B cells population. Therefore in the initial part of the study, the interaction between enriched B cells population in chicken lymphoid tissues and the virus infection *in vitro* and *in vivo* was examined.

The second part of this study focused on the *in vitro* simulation of chicken lymphoid tissues against the infection of very virulent NDV and IBDV. A better defined geometry 3 dimensional (3D) culture is important to directly relate structure to function analysis. Today, there is increasing awareness of the drawbacks of 2D cell culture and the related effect on the value of the research being performed. Not surprisingly, scientists are shifting their focus to cells cultured in 3D. For example, human breast epithelial cells (Lee et al., 2007) and cardiac stem cells (Hosseinkhani et al., 2010) as well as analogues of mammalian B cell development 3D models (Henderson and Dorshkin, 1990; Nunzia et al., 2011) were developed for *in vitro* studies. However, comparable avian systems to mimic and evaluate B cell maturation are still lacking even though B cells were originally described in chick studies before extrapolation to mammals. Therefore in our study, we propose the establishment of an *in vitro* system of chicken lymphoid tissue which mimics the chicken bursa of Fabricius and offers us the advantage for manipulation under controlled condition and eliminates the need of sacrificing large amounts of animals. Such *in vitro* system should be able to support the replication of NDV and IBDV so that the interaction between the *in vitro* model and virus infection can be investigated.

Thus, the objectives of this study were:

1) To investigate and compare the response of chicken enriched B lymphocytes population upon infection with different strains of NDV and IBDV.
2) To establish an *in vitro* system of chicken lymphoid tissues mimicking chicken bursa of Fabricius upon infection of different strains of NDV and IBDV.

REFERENCES

Abbas A.K., Lichtman A.H., Pillai S., 2007. Properties and overview of immune responses. In *Cellular and Molecular Immunology,* ed D.L. Baker and A. Baker pp 1-18. Elsevier Saunders Publisher, USA.

Agrawal P.K., Reynolds D.L., 1991. Evaluation of the cell-mediated immune response of chickens vaccinated with Newcastle disease virus as determined by the under-agarose leukocyte-migration-inhibition technique. *Avian Diseases,* 35: 360-364.

Ahmed K.A., Saxena V.K., Ara A., Singh K.B., Sundaresan N.R., Saxena M., Rasool T.J., 2007. Immune response to Newcastle disease virus in chicken lines divergently selected for cutaneous hypersensitivity. *International Journal of Immunogenetics,* 34: 445-455.

Aini, I., 1990. Control of Newcastle disease. A review. *Jurnal Veterinar Malaysia,* 2(1): 1-3.

Akaike T., 2001. Role of free radicals in viral pathogenesis and mutation. *Reviews in Medical Virology,* 11:87-101.

Aldous E.W., Collins M.S., McGoldrick A., Alexander D.J., 2001. Rapid pathotyping of Newcastle disease virus (NDV) using fluorogenic probes in PCR assay. *Veterinary Microbiology,* 80: 202-212.

Alexander D. J., 1991. Newcastle disease and other paramyxovirus infections. In B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. (ed.), Diseases of poultry 9th edn. Iowa State University Press, Ames, pp 469-519.

Alexander D.J., 1988a. Newcastle disease virus – an avian paramyxovirus. In *Newcastle Disease*, ed. D.J. Alexander pp 11-12. Kulwer Academic Publishers, USA.

Alexander D.J., 1988b. Newcastle disease diagnosis. In *Newcastle Disease*, ed. D.J. Alexander, pp 147-160. Kulwer Academic Publishers, USA.

Alexander D.J., 1988c. Newcastle Disease: Methods of Spread. In *Newcastle Disease,*ed. D.J. Alexander, Springer, USA, PP256-272.

Alexander D.J., 1997. Newcastle disease and other *Paramyxoviridae* infections. In B.W. Calneck (Ed.), Diseases of Poutlry 10th edn. Ames, IA: Iowa State University Press, pp 541-569.

Alexander D.J., 2003. Newcastle disease, other avian paramyxoviruses and pneumovirus infections. In: *Disease of poultry,* ed. Y.M. Saif, 11th edn. Ames: Iowa State Press, pp 63-92.

Alitheen N., McClure S., McCullagh P., 2001. Segregation of B lymphocytes into stationary apoptotic and migratory proliferating subpopulations in agglomerate cultures with ileal epithelium. *European Journal of Immunology,* 31: 2558-2565.

Azad A.A., Jagadish M.N., Brown M.A., Hudson P.J., 1987. Deletion mapping and expression in Escherichia coli of the large genomic segment of *birnavirus*. *Virology*¸161: 145-152.

Bakshi H., Sam S., Rozati R., Sultan P., Islam T., Rathore B., Lone Z., Sharma M., Triphati J., Saxena R.C., 2010. DNA fragmentation and cell cycle arrest: a hallmark of apoptosis induced by crocin fro kashmiri saffron in a human pancreatic cancer cell line. *Asian Pacific Journal of Cancer Prevention,* 11(3): 675-679.

Balamurugan V., Kataria J.M., 2006. Economically important non-oncogenic immunosuppressive viral diseases of chicken current status. *Veterinary Research Communications,* 30(5): 541-566.

Bank H.L., 1988. Rapid assessment of islet viability with acridine orange and propidium iodide. *In vitro Cellular & Development Biology-Plant,* 24: 266-273.

Basavaraj B., Hari-Babu Y., Sreekumar E., 2009. Immunomodulatory effect of recombinant chicken interferon-gamma on specific and non-specific immune responses in chicken vaccinated against NDV. *International Journal of Poultry Science*¸ 8(2): 122-127.

Becht H., Muller H., 1991. Infectious bursal disease – B cell dependent immunodeficiency syndrome in chickens. *Behring Institute Mitteilungen*, 2: 17-25.

Beckman J.S., Koppenol W.H., 1996. Nitric oxide, superoxide and peroxynitrite: the good, the bad and the ugly. *American Journal of Physiology*, 271: 1424-1437.

Benatar T., Iacampo S., Tkalec L., Ratcliffe M.J., 1991. Expression of immunoglobulin genes in the avian embryo bone marrow revealed by retroviral transformation. *European Journal of Immunology*, 21: 2529-2536.

Bergmann M., Garcia-Sastre A., Carnero E., et al., 2000. Influenza virus NS1 protein counteracts PKR-mediated inhibition of replication. *Journal of Virology,* 74: 6203-6206.

Biedermann K., Dandachi N., Trattner M., Vogl G., Doppelmayr H., More E., Staudach A., Dietze O., Hauser-Kronberger C., 2004. Comparison of real-time PCR signal-amplified in situ hybridization and conventional PCR for detection and quantification of human papillomavirus in archival cervical cancer tissue. *Journal of Clinical Microbiology,* 42(8): 3758-3765.

Birgersdotter A., Sanberg R., Ernberg I., 2005. Gene expression perturbation in vitro – a growing case for three dimensional culture system. *Seminars in Cancer Biology,* 15: 405-412.

Birghan C., Mundt E., Gorbalenya A., 2000. A non-canonical ion proteinase lacking the ATPase domain employs the Ser-Lys catalytic dyad to exercise broad control over the life cycle of a double stranded RNA virus. *EMBO Journal,* 19: 114-123.

Bisell M.J., 1981. The differentiated state of normal and malignant cells or how to define a normal cell culture. *International Review of Cytology,* 70:27-100

Bitzer M., Prinz F., Bauer M., Spiegel M., Neubert W.J., et al., 1999. Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32). *Journal of Virology,* 73:702-708.

Brown C., King D.J., Seal B.S, 1999. Pathogenesis of Newcastle disease in chickens experimentally infected with viruses of different virulence. *Veterinary Pathology,* 36: 125-132.

Burkhardt E., and Muller H., 1987. Susceptibility of chicken blood lymphoblasts and monocytes to infectious bursal disease virus (IBDV). *Archives of Virology*, 94: 297-303.

Cantin C., Holguera, J., Ferreira L., Villar E., Munoz-Barroso I., 2007. Newcastle disease virus may enter cells by caveolae-mediated endocytosis. *Journal of General Virology*, 88: 559-569.

Cao Y.C., Yeung W.S., Law M., Bi Y.Z., Leung F.C., Lim B.L., 1998. Molecular characterization of seven Chinese isolates of infectious bursal disease virus: classical, very virulent and variant strains. *Avian Disease*, 42(2): 340-351.

Chambers P., Millar N.S., Emmerson P.T., 1986. Nucleotide sequence of the gene encoding the fusion glycoprotein of Newcastle disease virus. *Journal of General Virology*, 67: 2685-2694.

Chen W., Calvo P.A., Malide D., et al., 2001. A novel influenza A virus mitochondrial protein that induces cell death. *Nature Medicine,* 7: 1306-1312.

Ciriaco, E., Pinera P.P., Diaz-Esnal B., Laura R., 2003. Age-related changes in the avian primary lymphoid organs (thymus and bursa of Fabricius). *Microscopy Reseacrh and Technique*, 62-482-487.

Collins J.A., Schandl C.A., Young K.K., Vesely J., Willingham M.C., 1997. Major DNA fragmentation is a late event in apoptosis. *The Journal of Histochemistry and Cytochemistry*, 45: 923-934.

Collins M.S., Govey S.J., Alexander D.J., 2003. Rapid in vitro assessment of the virulence of Newcastle disease virus isolates using the ligase chain reaction. *Archives of Virology*, 148: 1851-1862.

Cosgrove A.S., 1962. An apparently new disease of chickens – avian nephrosis. *Avian Disease*, 6: 385-389.

Craft D.W., Brown J., Luert P.D., 1990. Effects of standard and variant strains of infectious bursal disease virus on infections of chickens. *American Journal of Veterinary Research,* 51: 1192-1197.

Cui S.J., Fung Y.W.W., Lau L.T., Liu W.B., Wng Y.F., Tong G.Z., Chen J., Cheung A.H.Y., 2007. Detection of Newcastle disease virus using nucleic acid sequence-based amplification. *Biologicals*, 35: 13-18.

Djeraba A., Musset E., Lowenthal J.W., Boyle D.B., Chaussé A.M.., Péloille M., Quéré P., 2002. Protective effect of aivian myelomonocytic growth factor in infection with Marek's disease virus. *Journal of Virology*, 76: 1062 – 1070.

Dohmns J.E., Jaeger J.S., 1988. The effect of infectious bursal disease virus infection on local and systemic antibody responses following infection of 3-week-old broiler chickens. *Avian Diseases,* 32(4): 632-640.

Eagle H., 1955. Nutrition needs of mammalian cells in tissue culture. *Science*, 122(3168): 501-504.

Ecco R., Brown C., Susta L., Cagle C., Cornax I., Jackwood M.P., Miller P.J., Afonso C.L., 2011. In vivo transcriptional cytokine responses and association with clinical and pathological outcomes in chickens infected with different Newcastle disease virus isolates using formalin-fixed paraffin-embedded samples. *Veterinary Immunology and Immunopathology,* 141: 221-229.

Erf G.F., 2004. Cell-mediated immunity in poultry. *Poultry Science,* 83: 580-590.

Estevez A.G., Crow J.P., Sampson J.B., Reiter C., Zhuang Y., Richardson G.J., Tarpey M.M., Barbeito L., Beckman J.S., 1999. Induction of nitric oxide- dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. *Science,* 286: 2498-2500.

Etteradosis N., Arnauld C., Toquin D., Rivallan G., 1998. Critical amino acid changes in VP2 variable domain are associated with typical and atypical antigenenicity in very virulent infectious bursal disease viruses. *Archives of Virology*, 143: 1627-1636.

Etteradossi N., Picault J.P., Drouin P., Guitter M., L'Hospitalier R., Bennejean G., 1992. Pathogenicity and preliminary antigenic characterization of six infectious bursal disease virus strains isolated in France from acute outbreaks. *Journal of Veterinary Medical,* 39: 683-691.

Fahey K.J., Erny K, Crooks J., 1989. A conformational immunogen on VP2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. *Journal of General Virology*, 70: 1473-1481.

Fahey K.J., McWaters P., Brown M.A., Erny K., Murphy V.J., Hewish D.R., 1991. Virus neutralizing and passively protective monoclonal antibodies to infectious bursal disease virus of chickens. *Avian Diseases,* 35:365-373.

FAO Corporate Document Repository. Retrieved 25 September 2014 from http://www.fao.org/docrep/005/ac802e/ac802e0o.htm

Fernandes G., Chandra S.B., Luan X., Troyer D.A., 1996. Modulation of antioxidant enzymes and programmed cell death by n-3 fatty acids. *Lipids*, 31:591-596.

Fernandes-Arias, A., Martinez S., Rodriguez J.F., 1998. The major antigenic protein of infectious bursal disease virus, VP2, is an apoptosis inducer, *Journal of Virology*, 71: 8014-9018.

Ficken M.D., Edwards J.F., Lay J.C., 1987. Effects of Newcastle disease virus infection on the binding, phagocytic and bactericidal activities of respiratory macrophages of the turkey. *Avian Diseases*, 31: 888-894.

Fink S.L., Cookson B.T., 2005. Apoptosis, pyroptosis and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infection and Immunity*, 73(4): 1907-1916.

Freshney I.R., 2005. *Culture of animal Cells. A manual of Basic Technique,* 5th edn. John Wiley & Sons, Hoboken, NJ.

Frederick S.B.K., 2011. Avibirnavirus. In *The Springer Index of Viruses*, ed. Christian T. and Gholamreza D., 2, 10.1007/978-0-387-95919-1_20.

Funk P.E., Thompson C.B., 1996. Current concepts in chicken B cell development. *Current Topics in Microbiology and Immunology*, 212: 17-28.

Glick B., 1955. The bursa of Fabricius and antibody production. PhD dissertation. Columbus, Ohio: State University, Columbus, 1-102.

Hang C., Kong Y., Zhang H., 2012. Oxidative stress, mitochondrial dysfunction and aging. *Journal of Signal Transduction*, Article ID 646354, 13 pages, 2012. doi:10.1155/2012/646354.

Harrison L., Brown C., Afonso C., Zhang J., Susta L., 2011. Early occurance of apoptosis in lymphoid tissues from chickens infected with strains of Newcastle disease virus of varying virulence. *Journal of Comparative Pathology,* 145(4): 327-335.

Heller E.D., Levy A.M., Vaiman R., Schwartburd B., 1997. Chicken-embryo fibroblast produce two types of interferon upon stimulation with Newcastle disease virus. *Vetertinary Immunology and Immunopathology*, 57: 289-303.

Henderson A.J, Dorshkin K., 1990. In- vitro models of B lymphocyte development. *Seminar in Immunolog,* 2: 181-187.

Henderson A.J., Dorshkin K., 1990. In vitro models of B lymphocytes development. *Seminars in Immunology,* 2: 181-187.

Hitchner S.B., 1970. Infectivity of infectious bursal disease virus for embryonating eggs. *Poultry Science*, 49: 511-516.

Hortelano S., Alvarez A.M., Bosca L., 1999. Nitric oxide induces tyrosine nitration and release cytochrome c preceding an increase of mitochrondrial transmembrane potential in macrophages. *FASEB Journal,* 13: 2311-2317.

Hosseinkhani H., Hosseinkhani M., Hattori S., Matsuoka R., Kawaguchi N., 2010. Micro and nano-scale in vitro 3D culture system for cardiac stem cells. *Journal of Biomedical Materials Research Part A,* 94(1): 1-8.

Hosseinkhani H., Hosseinkhani M., Hattori S., Matsuoka R., Kawaguchi N., 2010. Micro and nano-scale in vitro 3D culture system for cardiac stem cells. *Journal of Biomedical Material Research. Part A,* 94(1): 1-8.

Houssaint E., Mansikka A., Vainio O., 1991. Early separation of B and T lymphocyte precursor in chick embryo. *Journal of Experimental Medicine,* 174: 376-406.

Hu B., Huang Y., He Y., Xu C., Lu X., Zhang W., Meng B., Yan S., Zhang X., 2010. Avian influenza virus and Newcastle disease virus (NDV) surveillance in commercial breeding farm in China and the characterization of Class 1 NDV isolates. *Veterinary Microbiology*, 144(1-2): 82-86.

Hudson P.J., McKern N.M., Power B.E., Azad A.A., 1986. Genomic structure of the large RNA segment of Infectious bursal disease virus. *Nucleic Acids Research*, 14: 5001-5012.

Hugh-Jones M., Allan W.H., Dark F.A., Harper G.J., 1973. The evidence for the airborne spread of Newcastle disease. *Journal of Hygiene*, 71: 325-339.

Ian T., 1979. Avian immune responses: A brief review. *Avian Diseases,* 23(2): 290-298.

Imre O., Lonneke V., 2011. *Structure of the Avian Lymphoid System*. Fred Davison, Bernd Kaspers, Karel A. Schat, Pete Kaiser, eds, Academic Press, pp13-50.

Islam M.R., Zierenberg K., Etteradossi N., Toquin U., Rivallan G.,Muller H., 2001. Molecular and antigenic characterization of Bangladeshi isolates of infectious bursal disease virus demonstrate their similarities with recent European, Asian and African very virulent strains. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health,* 48: 757-759.

Ismail N.M., Saif Y.M., Wigle W.L., Havenstein G.B., Jackson C., 1990. Infectious bursal disease virus variant from commercial Leghorn pullets. *Avian Diseases*, 34: 141-145.

Ivanyi J., Morris R., 1976. Immunodeficiency in the chicken. Part IV: An immunological study of infectious bursal disease. *Clinical and Experimental Immunology,* 23: 154-165

Jackwood D.H., and Saif Y.M., 1987. Antigenic diversity of infectious bursal disease virus. *Avian Diseases*, 31: 770-776.

Jackwood D.J., Saif Y.M., Hughes J.H., 1982. Characteristic and serologic studies of two serotypes of infectious bursa disease virus in turkeys. *Avian Diseases*, 26: 871-882.

Jackwood D.J., Sommer S.E., 1997. Restriction fragment length polymorphism in the VP2 gene of infectious bursal disease viruses. *Avian Diseases,* 41: 627-637.

Janeway C.A.Jr., Travers P., Walport M., et al., 2001. B-cell activationby armed helper T cells, *Immunobiology: The Immune System in Health and Disease.* 5th ed. New York: Garland Science.

Jeurissen S.H.M , 1993. The role of various compartments in the chicken spleen during an antigen specific humoral response. *Immunology*, 80: 29-33.

Jeurissen S.H.M., Janse E.M., Ekino S., Nieuwenhuis P., Koch G., De Boer G.F., 1988. Monoclonal antibodies as probes for defining cellular subsets in the bone marrow, thymus, bursa of Fabricius and spleen of the chicken. *Veterinary Immunology and Immunopathology*, 19: 225-238.

Jungmann A., Nieper H., Muller H., 2001. Apoptosis is induced by infectious bursal disease virus replication in productively infected cells as well as in antigen-negative cells in their vicinity. *Journal of General Virology,* 82: 1107-1115.

Kadiam C., Subbaiah V., Wudayagiri R., Valluru L., 2013. Newcastle disease virus (NDV) modulates pro/antioxidant status in different brain regions of chicken. *Free Radical and Antioxidants*, 3: 81-86.

Kaiser P., Rothwell L., Galyov E.E., Barrow P.A., Burnside j., Wigley P., 2000. Differential cytokine expression in avian cells in response to invasion by Salmonella typhimirium, Salmonella enteritidis and Salmonella gallinarum. *Microbiology,* 146: 3217- 3226

Kapuscinski J., 1990. Interactions of nucleic acids with fluorescent dyes: Spectral properties of condensed complexes. *The Journal of Histochemistry and Cytochemistry,* 38: 1323-1329.

Katchanov J., Harms C., Gertz K., Hauck L., Waeber C., Hirt L., Priller J., von Harsdorf R., Bruck W., Hortnagl H., Dirnagl U., Bhide P.G., Endres M., 2001. Mild celebral ischemia induces loss of cyclin-dependent kinase inhibitors and activation of cell cycle machinery before delayed neuronal cell death. *Journal of Neuroscience,* 21: 5045-5053.

Katz D., Inbar I, Samina I., Peleq B.A., Heller D.E., 1993. Comparison of dimethyl dioctadecyl ammonium bromide, Freund's complete adjuvant and mineral oil for induction of humoral antibodies, cellular immunity and resistance to Newcastle disease virus in chickens.. *Immunology in Medical Microbiology,* 7(4): 303-313.

Kaufer I., Weiss E., 1980. Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. *Infection and Immunity,* 27(2): 364-367.

Ke M.G., Liu J.H., Lin Y.M.H., Chen J., Tsai S.S., Chang C.P., Molecular characterization of Newcastle disease viruses isolated from recent outbreaks in Taiwan. *Journal of Virological Methods,* 97: 1-11.

Kerr J.F.R., Wyllie A.H., Currie A.R., 1972. Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. *British Journal of Cancer,* 26: 239-257.

Khatri M., Palmquist J.M., Cha R.M., Sharma J.M., 2005. Infection and activation of bursal macrophages by virulent infectious bursal disease virus. *Virus Research,* 113:44-50.

Kibenge F.S.B., Qian B., Cleghorn F.R., Martin C.K., 1997. Infectious bursal disease virus polyprotein processing does not involve cellular proteases. *Archives of Virology*, 142: 2401-2419.

Kim I.J., Karaca, K., Pertile T.L., Erickson S.A., Sharma J.M., 1998. Enhanced expression of cytokine genes in spleen macrophages during acute infection with infectious bursal disease virus in chickens. *Veterinary Immunology and Immunopathology*, 61(2-4): 331-341.

Kim I.J., You S.K., Kim H., Yeh H.Y., Rautenschlein S., Sharma J.M., 2000. Chracteristics of bursal T lymphocytes induced by infectious bursal disease virus. *Journal of Virology,* 74(19): 8884-8892.

Klimpel G.R., 1996. Immune Defenses, In *Medical Microbiology,* 4th ed. Baron S., Galveston (TX); University of Texas Medical Branch at Galveston, Chapter 50.

Kommers G.D., King D.J., Seal B.S, 2003. Virulence of six heterogenous-origin Newcastle disease virus isolates before and after sequential passages in domestic chickens. *Avian Pathology*, 32(1): 81-93.

Kommers G.D., King D.J., Seal B.S., Brown C.C., 2002. Pathogenesis of six pigeon-origin isolates of Newcastle disease virus for domestic chickens. *Veterinary Pathology,* 39(3): 353-362.

Krishnamurthy S., Samal S.K., 1998. Nucleotide sequences of the trailer, nucleocaspid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. *Journal of General Virology*, 79: 2419-2424.

Lam K.M., 1995. Apoptosis in chicken embryo fibroblast caused by Newcastle disease virus. *Veterinary Microbiology,* 47(3-4): 357-363.

Lam K.M., 1996. Newcastle disease virus-induced apoptosis in the peripheral blood mononuclear cells of chickens. *The Journal of Comparative Pathology*, 114:63-71.

Lam K.M., 1997. Morphological evidence of apoptosis in chickens infected with infectious bursal disease virus. *Journal of Comparative Pathology,* 116: 367-377.

Lam K.M., Hao Q., 1987. Induction of lymphocyte agglutination and lysis by Newcastle disease virus. *Veterinary Microbiology*, 15: 49-56.

Lam K.M., Vasconcelos A.C., 1994. Newcastle disease virus induced apoptosis in chicken peripheral blood lymphocytes. *Veterinary Immunology and Immunopathology,* 44: 45-56.

Lamb R.A., Kolakofsky D., 1996. *Paramyxoviridae*: the viruses and their replication. In B.N. Fields, D.M.Knipe and P.M Howley (Eds.). *Field Virology* 3rd edn, Vol 1, Philadelphia, PA: Lippincott- Raven, pp 1177-1203.

Lamb R.A., Park G.D., 2007. Paramyxiviridae: The viruses and their replication. In: *Field Virology*, D.M. Knipe, P.M. Howley, ed., 5th edn, Wolters Kluwer/ Lippincott Williams & Wilkins, Philadelphia, PA, pp 1449-1496.

Lancaster J.E., 1981. Newcastle Disease – Pathogenesis and Diagnosis. *World Poultry Science Journal*, 37: 26-33.

Laurettia F., Lucas de Meloa F., Benatia F.J., Volotaob Eduardo de Mello, Santos N., Linharesa R.E.C., Nozawa C., 2003. Use of acridine orange staining for the detection of rotavirus RNA in polyacrylamide gel. *Journal of Virological Methods*, 114: 29-35.

Lee G.Y., Kenny P.A., Lee E.H., Bissell M.J., 2007. Three-dimensional culture models of normal and malignant breast epithelial cells. *Nature Methods*, 4(4): 359-365

Lee G.Y., Kenny P.A., Lee E.H., Bissell M.J., 2007. Three-dimensional culture models of normal and malignant breast epithelial cells. *Nature Methods,* 4(4): 359-365.

Leene W., Duyzings M.J.M., van Steeg C., 1973. Lymphoid stem cell identification in the developing thymus and bursa of fabricius of the chick. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 136(4): 521-533.

Lehner B., Sandner B., Marschallinger J., Lehner C., Furtner T., Couillard-Depres S., Rivera F.J., Brockhoff G., Bauer H.C., Weidner N., Aigner L., 2011. The dark side of BrdU in neural stem cell biology: detrimental effects on cell cycle, differentiation and survival. *Cell and tissue research*, 345(3): 313-328.

Leslie G.A., Clem W.L., 1969. Phylogeny of immunoglobulin structure and function. 3. Immunoglobulins of the chicken. *Journal of Experimental Medicine*, 130: 1337-1352.

Lin R.Z., Chang H.Y., 2008. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnology Journal,* 3: 1172-1184.

Lombardo E., Maraver A., Espinosa I., Fernanderz-Arias A., Rodriguez J.F., 2000. VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. *Virology,* 277:345-357.

Lombardo E., Maraver A., Casten J.R., Rivera J., Fernandes-Arias A., Serrano A., Carrascosa J.L., Rodriguez J.F., 1999. VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the caspid protein VP3, leading to efficient encapsidation into virus-like particles. *Journal of Virology,* 73: 6973-6983.

Lukert P.D., Saif Y.M., 1997. Infectious bursal disease. In: *Disease of Poultry,* B.W. Calnek ed., 10th edn, Iowa State University Press, Ames, Iowa, USA, pp 721-738.

Lukert P.D., Saif Y.M., 2003. Infectious bursal disease. In: *Disease of Poultry*, Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, D.E. Swayne eds. 11th edn. Iowa State University Press, Ames, Iowa, pp 161-180.

MacPherson L.W., 1956. Some observation on the epizootiology of Newcastle disease. *Canadian Journal of Comparative Medicine*, 20: 155-168

Malaysia Competition Commision, Review of Domestic Broiler Market: Issues Paper, MyCC, Malaysia, 2012

Marino O.C., Hanson R.P., 1987. Cellular and humoral response of in ovo-bursectomized chickens to experimental challenge with velogenic Newcastle disease virus. *Avian Diseases,* 31: 293-301.

Marnett L.J., 2002. Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, 181-182: 219-222.

Marquardt W.W., Johnson R.B., Odenwald W.F., Schlotthober B.A., 1980. An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Avian Diseases,* 24: 375-385.

Martinez-Torrecuadrada J.L., Caston J.R., Caston M., Carrascosa J.L., Rodriguez J.F., Casal J.I., 2000. Different architectures in the assembly of infectious bursal disease virus caspid proteins expressed in insect cells. *Virology*, 278(2): 322-331.

Masaji M., Imai K., Sanada Y., Sanada N., Yuasa N., Imada T., Tsukamoto K., Yamaguchi S., 2002. Phylogenetic Analysis of Newcastle Disease Virus Genotypes Ioslated in Japan. *Journal of Clinical Microbiology*, 40(10): 3826-3830.

Mascotti K., McCullough J., Burger S.R., 2000. HPA viability measurement: trypan blue versus acridine orange and propidium iodide. *Transfusion,* 40: 693-696.

Mayo M.A., 2002. A summary of taxanomic changes recently approved by ICTV. *Archives of Virology*, 147: 1655-1656.

McCormack W.T., Tjoelker L.W., Barth C.F., Carlson L.M., Petryniak B., Humphries E.H., 1989. Selection for B cells with productive IgL gene rearrangements occurs in the bursa of Fabricius during chicken embryonic development. *Genes & Development,* 3:838–847.

McCormack W.T., Tjoulker L.W., Thompson C.B., 1991. Avian B cell development: generation of an immunoglobulin repertoire by gene conversion. *Annual Reviews of Immunology,* 9: 219-241.

Mcferran J.B., Mcnulty M.S., Mckillop F.R, Cornor T.J., Mccraken R.M., Collins D.S., Allan G.M., 1980. Isolation and Serological Studies with Infectious Bursal Disease Viruses from Fowl, Turkeys and Ducks – Demonstration of a 2nd Serotype. *Avian Pathology,* 9(3) : 395-404.

Meers P., Mealy T., 1993. Calcium dependent Annexin V binding to phospholipids: stoichiometry, specificity and the role of negative charge. *Biochemistry,* 23: 11711-11712.

Momayez R., Gharahkhani P., Pourbakhsh S.A., Toroghi R., Shoushtari A.H., Banai M., 2007. Isolation and pathogencity identification of avian paramyxovirus serotype 1 (Newcastle disease) virus from a Japanses quail flock in Iran. *Archives of Razi Institute,* 62(1): 39-44.

Moore M.A., Owen J.J., 1966. Experimental studies on the development of the bursa of Fabricius. *Developmental Biology,* 14: 40-51.

Moore M.A.S., Owen J.J.T., 1965. Chromosome marker studies on the development of the hemopoietics system in the chick embryo. *Nature,* 208-956.

Mosmann T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65: 55-63.

Muller H., Nitschke R., 1987. Molecular weight determination of the two segments of double stranded RNA of infectious bursal disease virus, a member of the birnavirus group. *Medical Microbiology and Immunology*, 176(2): 113-121.

Muller H., Scholtissek C., Becht H., 1979. The genome of infectious bursal disease virus consists of two segments of double-stranded RNA. *Journal of Virology*, 31: 584-589.

Nagai Y., Klank H.D., Rott R., 1976. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. *Virology*, 72: 494-508.

Nakai T., Hirai K., 1981. In vitro infection of fractionated chicken lymphocytes by infectious bursal disease virus. *Avian Diseases,* 25(4): 831-838.

Nicoletti I., Migliorati G., Pagliacci M.C., Grignani F., Riccardi C., 1991. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *Journal of Immunological Methods,* 139: 271-280.

Nunoya T., Otaki Y., Tajima M., Hiraga M., Saito T., 1992. Occurance of acute infectious bursa disease with high mortality in Japan and pathogenicity of field isolates in SPF chickens. *Avian Diseases,* 36: 597-609.

Nunzia D.M., Piccinini E., Jaworski M., Trumpp A., Wendt D.J., Martin I., 2011. Toward modeling the bone marrow niche using scaffold-based 3D culture system. *Biomaterials*, 32: 321-329.

Ogawa M., Yamaguchi T., Setiyono A., Ho T., Matsuda H., Furusawa S., Fukushi H., Hirai K., 1998. Some characteristic of a cellular receptor for virulent infectious bursal disease virus by using flow cytomtery. *Archives of Virology*, 143: 2327-2341.

OIE, 2008. Terrestrial Manual – Infectious bursal disease (Gumboro disease), Biological Standard Commission, Paris: World Organization for Animal Health, pp 549-565.

Oldoni I., Brown C.C., Daniel J.K., Siba S., Seal B.S., 2005. The use of in situ hybridization and immunohistochemistry to study the pathogenesis of various Newcastle disease virus strains and recombinants in embryonated chicken eggs. *Microbial Pathogenesis*, 39(3): 69-75.

Palmieri B., Sblendorio V., 2007. Oxidative stress: overview on reliability and use (Part 1). *European Review for Medical and Pharmacological Sciences,* 11: 309-342.

Patcher P., Beckman J.S., Liaudet L., 2007. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Reviews,* 87(1): 315-424.

Payne L.N., 1971. The lymphoid system. *In Physiology and Biochemistry of the Domestic Fowl* eds D.J. Bell and B.M. Freeman, Academic Press, London, pp. 985-1037.

Peeters B.P.H, De Leeuw O.S., Koch G., Gielkens A.L.J., 1999. Rescue of Newcastle disease virus from clones cDNA: evidence that cleavability of the fusion protein is a major determinant for virulence. *Journal of Virology*, 73: 5001-5009.

Peterson O.W., R'onnov-Jessen L., Howlett A.R., Bisell M.J., 1992. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and maglinant human breast epithelial cells. *Proceedings of the National Academy of Sciences,* 89:9064-9068.

Pham H.M., Nakajima C., Ohashi K., Onuma M., 2005. Loop-mediated isothermal amplification for rapid detection of Newcastle disease virus. *Journal of Clinical Micorbiology*, 43 : 1646-1650.

Pike K.A., Ratcliffe M.J.H, 2002. Cell surface immunoglobulin receptors in B cell development. *Seminars in Immunology*, 14: 351-358.

Poch O., Blumberg B.M., Bougueleret L., Tordo N., 1990. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. *Journal of General Virology*, 7(5): 1153-1162.

Qureshi M.A., Hussain I, Heggen C.L., 1998. Understanding immunology in disease development and control. *Poultry Science*, 77: 1126-1129.

Rasoli M., Yeap S.K., Tan S.W., Moeini H., Aini I., Hair-Bejo M., Alitheen N.B., Kaiser P., Omar A.R., 2014. Alteration in lymphocytes responses, cytokine and chemokine profiles in chickens infected with genotype VII and VIII velogenic Newcastle disease virus. *Comparative Immunology, Microbiology and Infectious Diseases,* 37(1): 11-21.

Ratcliffe M.J., 2006. Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development, *Developmental & Comparative Immunology*, 30: 101-118.

Ratcliffe M.J.H., 1997. Chicken immunoglobulin isotypes and allotypes. In: *Handbook of Experimental Immunology,* 5th ed. Weir D.W., Herzenberg L.A & Herzenberg L.A., Oxford, Blackwell, pp 24.1-24.15.

Rautenschlein S., Yeh H.Y., Njenga M.K., Sharma J.M., 2002. Role of intrabursal T cells in infectious bursal disease virus (IBDV) infection: T cells promote viral clearance but delay follicular recovery. *Archives of Virology*, 147(2): 285-304.

Rauw, F., Gardin Y., Palya V., van Borm S., Gonze M., Lemaire S., van der Berg T., Lambrecht B., 2009. Humoral, cell-mediated and mucosal immunity induced by oculo-nasal vaccination of one-day-old SPF and conventional layer chicks with two different love Newcastle disease vaccines. *Vaccine,* 27:3631-3642.

Ravindra P.V., Tiwari A.K., Ratta B., Chaturvedi U., Palia S.K., Chauhan R.S., 2009. Newcastle disease virus-induced cytopathic effect in infected cells is caused by apoptosis. *Virus Research,* 141(1): 13-20.

Reed L.J. and Muench H., 1938. A simple method of estimating fifty per cent endpoints. *The American Journal of Hygiene*, 27 (3): 493 – 497.

Reynolds D.L., Maraga A.D., 2000a. Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. *Avian Diseases,* 44: 138-144.

Reynolds D.L., Maraga A.D., 2000b. Protective immunity against Newcastle disease: the role of cell mediated immunity. *Avian Diseases,* 44: 145-154.

Rodenberg J., Sharma J.M., Belzer S.W., Nodgren R.M., Naqi S., 1994. Flow cytometric analysis of B cell and T cell subpopulation in specific-pathogen-free chickens infected with infectious bursal disease virus. *Avian Diseases,* 38(1): 16-21.

Rodriguez-Lecompte J.C., Nino-Fong R., Lopez A., Markham R.J.F., Kibenge F.S.B., 2005. Infectious bursal disease (IBDV) induces apoptosis in chicken B cells. *Comparative Immunology, Microbiology & Infectious Diseases,* 28: 321-337.

Rose M.E, Orlans E., Buttress N., 1974. Immunoglobulin classes in the hen's egg: Their segregation in yolk and white. *European Journal of Immunology*, 4: 521-523.

Roulston A., Marcellus R.C., Branton P.E., 1999. Viruses and Apoptosis. *Annual Review of Microbiology*, 53: 577-628.

Rubbo H., Radi R., Trujillo M., Telleri R., Kalyanaraman B., Barnes S., Kirk M., Freeman B.A., 1994. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation – formation of novel nitrogen-containing oxidized lipid derivatives. *Journal of Biological Chemistry,* 269: 26066-26075.

Rusell P.H., Ezeifeka G.O., 1995. The Hitchner B1 strain of Newcastle disease virus induces high levels of IgA, IgG and IgM in newly hatched chicks. *Vaccine,* 13(1): 61-66.

Rusell, P.H., Koch G., 1993. Local antibody forming cell responses to the Hitchner B1 and Ulster strains of Newcastle disease virus. *Veterinary Immunology and Immunopathology,* 37(2): 165-180.

Russell P.H., Dwivedi P.N., Davison T.F., 1997. The effects of cyclosporin A and cyclophosphamide on the populations of B and T cells and virus in the Harderian gland of chickens vaccinated with the Hitchner B1 strain of Newcastle disease virus. *Verterinary Immunology and Immunopathology*, 60: 171-185

Sanford K.E., Earle W.R., Likely G.D., 1948. The growth in vitro of single isolated tissue cells. *Journal of National Cancer Institute,* 9(3): 229-246.

Sawant, P.M., Verma P.C., Subudhi P.K., Chaturvedi U., Singh M., Rajeev Kumar, Tiwari A.K., 2011. Immunomodulation of bivalent Newcastle disease DNA vaccine induced immune response by co-delivery of the chicken IFN-g and IL-4 genes. *Veterinary Immunology and Immunopathology,* 144 (1-2): 36-44.

Sayegh C.E., Denmaries S.L., Pike K.A., Friedman J.E., Ratcliffe M.J.H, 2000. The chicken B-cell receptor complex and its role in avian B-cell development. *Immunological Reviews*, 175: 187-200.

Schranner, I., Losch U., 1986. Immunological identification of avian monomeric and polymeric immunoglobulin M and immunoglobulin A after fractionation on sodium dodecylsulfate pore gradient polyacrylamide gels. *Poultry Sciences,* 65: 360-368.

Schwarz K.B., 1996. Oxidative stress during viral infection: a review. *Free Radical Biology & Medicine,* 21: 641-649.

Seal, B.S., Crawford J.M., Sellers H.S., Locke D.P., Kind D.J., 2002. Nucleotide sequence analysis of Newcastle disease virus nucleocaspid protein gene and phylogenetic relationship among the *Paramyxoviridae*. *Virus Research*, 83: 119-129.

Seto F., 1988. Dissociation of the RES and immune components in the transient splenic response of embryos and neonatal chicks to immunization. *Developmental and Comparative Immunology*, 12: 843-854.

Shapiro G.I., Harper J.W., 1999. Anticancer drug targets : cell cycle and checkpoint control. *Journal of Clinical Investigation,* 104(12): 1645-1653.

Sharma J.M., 1997. The structure and function of the avian immune system. *Acta Veterinaria Hungarica*, 43(3): 229-238.

Sick C., Schneider K., Staeheli P., Weining K.C., 2000. Novel chicken CXC and CC chemokines. *Cytokine*, 12: 181-186.

Sijtsma S. R., Rombout J. H. W. M., Kiepurski A. K., West C. E., van der Zijpp A. J., 1991. Changes in lymphoid organs and blood lymphocytes induced by vitamin A deficiency and Newcastle disease virus infection in chickens. *Developmental and Comparative Immunology,* 15:349-356.

Spies U., Muller H., 1990. Demonstration of enzyme activities required for cap structure formation in infectious bursal disease virus, a member of the birnavirus group. *Journal of General Virology,* 71: 977-981.

Squier M.K.T, Sehnert A.J., Cohen J.J., 1995. Apoptosis in leukocytes. *Journal of Leukocyte Biology,* 57: 2-10.

St. Hill C.A., Sharma J.M., 2000. Viral pathogenesis in chicken embryos and tumor induction in chickens after in ovo exposure to serotype 1 Marek's disease virus. *Avian Diseases,* 44: 842–852.

Steinberg D., 2009. The LDL modification hypothesis of atherogenesis: an update. *Journal of Lipid Research,* 50: 5376-5381.

Steward M., Vipond I.B., Millar N.S., Emmerson P.T., 1993. RNA editing in Newcastle disease virus. *Journal of General Virology*, 74: 2539-2547.

Susanna P., Timo Y., Eeva O., Heimo S., Markku P.H., Pentti T., 1998. Progesterone receptor in chicken bursa of Fabricius and thymus: evidence for expression in B-lymphocytes. *Molecular and Cellular Endocrinology*, 141: 119-128.

Tan S.W., Aini I., Omar A.R., Yusoff K., Hair-Bejo M., 2009. Detection and differentiation of velogenic and lentogenic Newcastle disease viruses using SYBR Green I real-time PCR with nuclecaspid gene-specific primers. *Journal of Virological Methods*, 160 (1-2): 149-156.

Tan S.W., Omar A.R., Aini I., Yusoff K., Tan W.S., 2004. Detection of Newcastle disease virus (NDV) using a SYBR Green I real-time polymerase chain reaction. *Acta Virologica,* 48: 23-28.

Tanimura N., Tsukamoto K., Nakamura K., Narita M., Maeda M., 1995. Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. *Avian Diseases*, 39: 9-20.

Telford W.G., King L.E., Fraker P.J., 1994. Rapid quantitation of apoptosis in pure and heterogenous cell populations using flow cytometry. *Journal of Immunological Methods,* 172:1-16.

Tham K.M., Moon C.D., 1996. Apoptosis in cell cultures by infectious bursal disease virus following in vitro infection. *Avian Diseases*, 40(1): 109-113.

Thong C.L., Yusoff K., Nathan S., Tan W.S., 2006. Detection of virulent Newcastle disease virus using a phage-capturing dot blot assay. *Journal of Virological Methods*, 136: 224-229.

Thornton B.P., Vetvicka V., Ross G.D., 1994. Natural antibody and complement-mediated antigen processing and presentation by B lymphopcytes. *Journal of Immunology*, 152: 1727-1737.

Tsukamoto K., Tanimura N., Hihira H., Shirai J., Imai K., Nakamura K., Maeda M., 1992. Isolation of very virulent infectious bursal disease virus from filed outbreaks with high mortality in Japan. *Journal of Veterinary Medical Science.* 54: 153-155.

Uchida K., 2003. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Progress in Lipid Research*, 42: 318-343.

Umansky V., Shatrov V.A., Lehmann V., Schirrmacher V., 1996. Induction of NO synthesis in macrophages by Newcastle disease virus is associated with activation of nuclear factor-kB. *International Immunology,* 8(4): 491-498.

Van der Berg T.P., 2000. Acute infectious bursal disease virus in poultry: a review. *Avian Pathology*, 29: 175-194.

Vasconcelos A.C., Lam K.M., 1995. Apoptosis in chicken embryos induced by the infectious bursal disease virus. *Journal of Comparative Pathology,* 112(4): 327-38.

Vermes I., Haanan C., Steffens-Nakken H., Reutelingsperger C., 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. *Journal of Immunological Methods,* 184(1): 39-51.

Vindelov L.L., Christensen I.J., Nissen N.I., 1983. Adetergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. *Cytometry*, 3:323-327.

Wakamatsu N., King D.J., Seal B.S., Samal S.K., Brown C.C., 2006. The pathogenesis of Newcastle disease: A comparison of selected Newcastle disease virus wild-type strains and their infectious clones. *Virology*, 353: 333-343.

Waning D.L., Schmitt A.P., Leser G.P., Lamb R.A., 2002. Roles for the cytoplasmic tails of the fusion and hemagglutinin-neuramidase proteins in budding of the paramyxovirus simian virus. *Journal of Virology,* 76: 9284-9297.

Wang X., Sun L., Maffini M.V., Soto A., Sonnenschein C., Kaplan D.L., 2010. A complex 3D human tissue culture system based on mammary stromal cells and silk scaffolds for modeling breast morphogenesis and function. *Biomaterials*, 31(14): 3920-3929.

Watzinger F., Ebner K., Lion T., 2006. Review: Detection and monitoring of virus infections by real time PCR. *Molecular Aspects of Medicine,* 27:254-298.

Watzinger F., Suda M., Preuner S., Baumgartinger R., Ebner K., Baskova L., Niester H.G., Lawitschka A., Lion T., 2004. Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. *Journal of Clinical Microbiology,* 42(11): 5189-5198.

Wilkins R.C., Kutzner B.C., Truong M., Sanchez-Dardon J., McLean J.R.N., 2002. Analysis of radiation-induced apoptosis in human lymphocytes: flow cytometry using Annexin V and propidium iodide versus the neutral comet assay. *Cytometry,* 48:14-19.

Wise M.G., Suarez D.L., Seal B.S., Pederson J.C., Senne D.A., King D.J., Kapczynski D.R., Spackman E., 2004. Development of a real time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. *Journal of Clinical Microbiology*, 42: 329-338.

Yusoff K., Tan W.S., 2001. Newcastle disease virus: macromolecules and opportunities. *Avian Pathology*, 30:439-455.

Zhang Z.W., Zhang J.L., Zhang Y.H., Wang Q.H., Li S., Wang X.L., Xu S.W., 2013. Effect of oxygen free radicals and nitric oxide on apoptosis of immune organ induced by selenium deficiency in chickens. *Biometals*, 26(2): 355-365.

LIST OF PUBLICATIONS

Alitheen N.B., McClure S.J., Yeap S.K., Kristeen-Teo Y.W., Tan S.W., McCullagh P., 2012. Establishment of an *in vitro* system representing the chicken gut-associated lymphoid tissue. *PLOSONE* 7(11): e49188

Kristeen-Teo Y.W, Omar A.R., Yeap S.K., Tan S.W., Tan S.G., Alitheen N.B. Responses of enriched chicken B lymphocytes population towards infection of different genotypes of velogenic Newcastle disease virus. In Proceeding of WPSA (Malaysia Branch) adnd WVPA (Malaysia Branch) Scientific Conference 2013, 30 November – 1 December 2013, Faculty of Veterinary Medicine, Universiti Putra Malaysia

Kristeen-Teo Y.W., Yeap S.K., Tan S.W., Kiarash R., Omar A.R., Tan S.G., Alitheen N.B., 2014. Genotype VII and VIII of velogenic Newcastle Disease virus induced differential macrophage infiltration and IFN-γ expression in bursa of infected SPF chicken. *Research in Veterinary Science.* Submitted

Kristeen-Teo Y.W., Yeap S.K., Tan S.W., Omar A.R., Aini I., Tan S.G., Alitheen N.B., 2014. Responses of enriched chicken B lymphocytes population towards infection of different genotypes of velogenic Newcastle Disease Virus. *Veterinary Immunology and Immunopathology*. Submitted.

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