JWPR
Journal of World's Poultry Research

J. World's Poult. Res. 3(3): 83-88, 2013

Original Article

Comparison between Haemagglutination Inhibition Test and Enzyme Linked Immune Sorbent Assay in Evaluation of Newcastle disease Antibodies in Japanese Quails

Abdel Rhman S.S.,*[1&3], Al Jassem A.H[1], Hussein G. M[2&3] and M.H. Al-Blowi.[1]

[1]*Animal Resources Administration, Ministry of Agriculture, Riyadh, 11195 Kingdom of Saudi Arabia*
[2]*Veterinary Vaccine Production Center, Ministry of Agriculture Riyadh 11454, Saudi Arabia*
[3]*Veterinary Serum and Vaccine Research Institute, Abbassia 11381, Cairo., Egypt*

*Corresponding author: Dr. Salah Shaban Abdel Rhman; Senior researcher at Veterinary Serum and Vaccine Research Institute, Abbassia 11381, Cairo., Egypt. Email: salahvet@ymail.com

ABSTRACT

Comparison between Haemagglutination Inhibition (HI) test and Enzyme Linked Immune Sorbent Assay (ELISA) in evaluation of humoral immune response to Newcastle disease (ND) in vaccinated quails was investigated. The obtained results showed higher NDV antibody titer (2, 5 and 8) $\log_2$ at zero, 14 and 28 day post vaccination in serum of La Sota vaccinated quails when the antigen used in HI test was homologous (La Sota), whereas lower antibody response (2, 4 and 6) $\log_2$ were obtained when the antigen used was heterologous (Hitchner B1). However, antibody titers in serum sample from Hitchner B1 vaccinated quails were (2, 4 and 6) $\log_2$ at zero, 14 and 28 day post vaccination against both homologous (Hitchner B1) and heterologous (La Sota) antigens. Non treated sera from control non vaccinated quails gave false positive results (6 $\log_2$) in HI test. Treatment of serum by heat (56$^{\circ C}$ /30 minutes) or by washing with 10% Chicken red blood overcome false positive results obtained by non treated serum in HI test. No difference between chicken and quail red blood cells were observed when used as red blood cells indicators in HI test to detect Newcastle disease virus antibodies of vaccinated quails. Two commercial ELISA kits used in this study failed to detect any positive sera (0.0 %) for ND antibodies in both vaccinated and non vaccinated quails at different time periods.

Key words: ELISA, HI, Newcastle disease, Quail

INTRODUCTION

Newcastle disease virus (NDV), the cause of Newcastle disease (ND) in poultry is classified in the genus Avulavirus, family Paramyxoviridae and order Mononegavirales (Mayo, 2002). Several outbreaks of Newcastle disease in flocks of Japanese quail (*Coturnix Coturnix Japonica*) have been reported (Chandrasekaran and Aziz, 1989; Islam *et al*., 1994; Merino *et al.*, 2009). Quails experimentally infected with Newcastle disease virus strain VN91 at 20 day old showed mortality rate of 100, 60 and 20% after intra-muscular, intra-buccal and contact infection respectively, and when infection occurred at 40 day old quails, the corresponding figures were 60, 50 and 20% (Nguyen *et al.*, 2000). Newcastle disease virus isolation from natural outbreak of ND in Japanese quail manifested clinically by central nervous system dysfunction with 100% morbidity and mortality was reported by (Czirják *et al.*, 2007). Momayez *et al.* (2007) isolated velogenic strain of NDV with ICPI of 1.62 from two out of five sick quails. Haemagglutination Inhibition (HI) test is still the most widely used serological method for measuring NDV antibodies in chicken sera and considered the gold standard test for this disease (OIE, 2012). However**,** sera from species other than chickens tend to give false positive results. Previous work by (Sakai *et al.*, 2006) indicated that ostrich sera are highly cross reactive when tested with HI test for the presence of non specific inhibitors of haemagglutination with the consequence of high number of false positives. Silva *et al.* (2010) verified that, the use of different antigens had influenced the HI test results for both infected and vaccinated pigeons. Scientific articles dealing with serological assays (HI and ELISA) in quail sera are rare and not studied their performance and reliability in evaluation of NDV antibodies in vaccinated quail.

To cite this paper: Abdel Rhman S.S., Al Jassem A.H., Hussein G.M. and M.H. Al-Blowi. 2013. Comparison between Haemagglutination Inhibition Test and Enzyme Linked Immune Sorbent Assay in Evaluation of Newcastle disease Antibodies in Japanese Quails. *J. World's Poult. Res.* 3(3): 83-88.
Journal homepage: http://jwpr.science-line.com/

Previous work by Oladele *et al.* (2008) tested sera from experimentally infected quails with NDV Kudu 113 strain by HI test using four Haemagglutinating units of La Sota antigen and 1 % chicken red blood cells and found a rise in HI antibody titer from zero to 10.56 $\log_2$ and 9.89 $\log_2$ in quails administered 0.3 ml of the virus intramuscular and per-os on day 7 post infection respectively. Paulillo *et al.* (2009) when evaluated heat treated serum from NDV vaccinated quails found that, high antibody response (6.0 - 8.6) $\log_2$ were obtained by inactivated vaccine, while moderate level of antibody (3.4 - 5.2) $\log_2$ were obtained by Ulaster 2 and Hitchner B1 live vaccines. Romao *et al.* (2011) reported poor antibody response in hatched quail at 10 - 15 days post vaccination in ovo with Hitchner B1 vaccine using homologous virus and 1 % chicken red blood cells. Alternatives to HI test, Enzyme Linked Immune Sorbent Assays (ELISA) were used to measure NDV antibodies in chicken and proved to be well correlated to HI test for evaluation of NDV antibodies in poultry serum (Brown *et al.*, 1990; Richtzenhain *et al*., 1993). In quails, monoclonal –blocking ELISA was used to evaluate immune response of quail vaccinated with inactivated Newcastle disease vaccine (Fair *et al.*, 1999; Fair and Ricklefs, 2002). Kiss *et al.* (2003) developed direct ELISA to measure ND antibodies in vaccinated quails feed deferent levels of beta - carotene. The aim of the present study is to evaluate the performance of serological assays (HI and ELISA) for evaluation of NDV antibodies in vaccinated quails and make a clear decision on the recommended diagnostic tests for serology of ND in quails.

MATERIALS AND METHODS

Newcastle disease vaccines

1. Live Newcastle Disease vaccine (La Sota strain).
2. Live Newcastle Disease vaccine (Hitchner B1 strain).

Newcastle disease Antigens

1. Newcastle Disease vaccine (La Sota strain).
2. Newcastle Disease vaccine (Hitchner B1 strain).

They are prepared by inoculating live La Sota and Hitchner B1 vaccines in separate Specific Pathogens Free (SPF) embryonated eggs, Harvest allanotic fluid from inoculated SPF eggs was used as antigens in HI test after measuring its Haemagglutinating activities.

Red blood cells

- Chicken red blood cells, (CRBCs) 1.0 %.
- Chicken red blood cells, (CRBCs) 10.0 %.
- Quails red blood cells (QRBCs) 1.0 %.

Quails

Seventy five day old Japanese quail were obtained from a commercial quail farm located in Riyadh, Saudi Arabia. They were reared on litter floor and supplied with feed and water *ad libitum*.

Serum Treatment

Heat treatment: Serum samples were treated by heat at 56˚C for 30 minutes according to (Moro *et al*., 2000).

Washing serum with (10%) chicken red blood cells: Washing sera with RBC solution was performed according to (Toffan *et al*., 2010): 50 ml phosphate-buffered saline (PBS) were dispensed into wells in the first column of a microplate (wells A1 to H1), and the second row (A2 to H2) was left empty. Then 25 ml PBS were subsequently dispensed into all other wells of the microtitre plate. A 50 ml sample of test sera was added to the first wells of the microplate (column 1) and then 50 ml of a 10% RBC suspension was added to the first wells (column 1). The plates were incubated for 30 to 40 min at room temperature (20 to 24˚C), to allow to the 10% RBC suspension to settle. Subsequently, 25 ml supernatant of the samples in the first column was transferred to the wells of the second column. An additional 25 ml supernatant from the wells of the first column was transferred to the wells of the third column. Two-fold serial dilutions of the samples in the third column were performed, and the last 25 ml were discarded. From this point onwards the sera were processed as chicken sera. The first column was excluded from the test.

Haemagglutination Inhibition (HI) test

- HI test was performed according to (Allan and Gough, 1974).
- The HI test was performed using 4 UHA of Both antigens (LaSota and HB1) against each serum sample.
- Results were recorded as log2 X values of the highest reciprocal of the dilution which showed complete hemagglutination inhibition.

Enzyme-Linked Immunosorbent Assay (ELISA)

Two commercially available ELISA kits were used to measure the amount NDV antibodies in serum of vaccinated and non vaccinated quails.

1. ELISA for measuring antibody to NDV in serum of chickens and turkeys, Part Number: 99-09263, IDEXX Laboratories, Inc. USA
2. ELISA for measuring antibody to NDV in serum of chickens and turkeys, product code: CK116 NDV, BioChek BV, Netherlands

Experimental design:

Experiment 1: It was designed to evaluate the effect and influence of using Homologous and Heterologus antigens (LaSota and Hitchner B1) on the outcome of HI results (Table 1).

- Forty five quails at 21 day of age were randomly divided into three groups 15 birds for each.

G1: Quails vaccinated with HB1 vaccine by 1x chicken dose/ quail by intraocular route.

G2: Quails vaccinated with LaSota vaccine by 1x chicken dose /quail by intraocular route.

G3: Non vaccinated quails (control group).

To cite this paper: Abdel Rhman S.S., Al Jassem A.H., Hussein G.M. and M.H. Al-Blowi. 2013. Comparison between Haemagglutination Inhibition Test and Enzyme Linked Immune Sorbent Assay in Evaluation of Newcastle disease Antibodies in Japanese Quails. *J. World's Poult. Res.* 3(3): 83-88.
Journal homepage: http://jwpr.science-line.com/

Blood samples were collected from the control group as well as the other two vaccinated groups at Zero, 14 and 28 days post vaccination**.**

Serum samples were heat treated at 56˚C /30 minutes and evaluated against homologous and heterologous antigens using 1% chicken red blood cells and 1% quail red blood cells.

Experiment 2: It was designed to evaluate the effect of serum treatment by Heat or by washing with 10% Chicken RBCs) against different red blood cells indicators systems (chicken and quail red blood cells) on the outcome of HI results and compare it with different commercial ELISA kits (Table 1).

Thirty quails at 21 day of age were randomly divided into two groups 15 birds for each.

G4: Non vaccinated quails (control group).

G5: Quails vaccinated with live La Sota vaccine (1× chicken dose /quail) by intraocular route.

- Five and Ten serum samples were collected from non vaccinated and vaccinated groups respectively at (21) days post vaccination
- Each serum sample was divided into sex parts and tested in the following manner.

1. First part was tested by HI using chicken red blood cells (CRBCs) 1.0 %.
2. Second part was tested by HI using quail red blood cells (QRBCs) 1.0 %.
3. Third part was washed by 10.0 % CRBCs and then tested by HI using CRBCs 1.0 %.
4. Fourth part was heat treated at 56 c/ 30 minutes and then tested by HI using CRBCs 1.0 %.
5. Fifth part was tested by NDV commercial ELISA kit (Idexx
6. Sixth part was tested by commercial ELISA kit (Biochek).

Table 1: Experimental design for different vaccinated quail groups

Experiment	Groups	Type of vaccine	Dose	Age at vaccination	Method of administration	Test
1	1	Hitchner	1× chicken dose/ quail	21	Eye drop	HI
	2	Lasota		21		
	3	Non vaccinated	--	--	--	
2	4	Lasota	1× chicken dose/ quail	21	Eye drop	HI + ELISA
	5	Non vaccinated	--	--	--	

RESULTS AND DISCUSSION

In the present study, comparison between Haemagglutination Inhibition (HI) test and Enzyme Linked Immune Sorbent Assay (ELISA) in evaluation of humoral immune response to Newcastle disease in experimentally vaccinated quails was investigated. To collect as much information as possible, we studied the effect and influence of using homologous and heterologus antigens (La Sota and Hitchner B1) and different red blood cells indicators systems (chicken and quail red blood cells) as well as treated and non treated serum on the outcome of the HI test results. The obtained results in Table 2 showed higher NDV antibody titer (2, 5 and 8) $\log_2$ at zero, 14 and 28 day post vaccination in La Sota vaccinated quails when the antigen used was Homologous (La Sota), Whereas lower antibody titers (2, 4 and 6) $\log_2$ were obtained when the antigen used in HI test was heterologous (Hitchner B1). Similar pattern of test result to homologous antigen was observed by (Stone, 1989) who reported that, in chickens and pigeons vaccinated with La Sota, Ulster, and Pigeon Paramyxovirus Type 1, higher titers in the HI test were obtained when the antigen used was homologous to the virus used in immunization procedure. Also, Mass *et al.* (1998) reported that, the use of homologous La Sota antigen in the HI assay after Clone 30 and La Sota vaccination of Specific Pathogen Free (SPF) chickens resulted in significantly higher titer than the use of heterologous Ulster virus with a mean difference of 1.4 $\log_2$. Also, Silva *et al.* (2010) found that, higher antibody titers in HI test were obtained with samples from Ulster vaccinated pigeon using homologous antigen. Our results revealed that, the antibody titers in serum sample from Hitchner B1 vaccinated quails were (2, 4 and 6) $\log_2$ at zero, 14 and 28 day post vaccination against both homologous (Hitchner B1) and heterologous (La Sota) antigens.

Our finding agreed with Mass *et al.* (1998), who reported that, when chicken was immunized with Ulster live vaccine, statistical difference between the use of homologous (Ulster) and heterologous (La Sota) antigen were not noticed.

Table 2: Mean antibodies titer (×$\log_2$) of Newcastle disease vaccinated quails against La Sota and Hitchner B1 antigens using 1% chicken red blood cells.

Groups	Treatment	Days post vaccination					
		0		14		28	
		La Sota	Hitchner B1	La Sota	Hitchner B1	La Sota	Hitchner B1
G1	La Sota	≤ 2	≤ 2	5	4	8	6
G2	Hitchner B1	≤ 2	≤ 2	4	4	6	6
G3	Control (Non vaccinated)	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2

To cite this paper: Abdel Rhman S.S., Al Jassem A.H., Hussein G.M. and M.H. Al-Blowi. 2013. Comparison between Haemagglutination Inhibition Test and Enzyme Linked Immune Sorbent Assay in Evaluation of Newcastle disease Antibodies in Japanese Quails. *J. World's Poult. Res.* 3(3): 83-88.
Journal homepage: http://jwpr.science-line.com/

The influence of red blood cells type on the HI test results was investigated. Interestingly, the obtained results in Table 3 compared to results obtained in Table 2, revealed that, no difference between chicken and quail red blood cells was observed when used as red blood cells indicators in HI test. So, we could recommend using quails red blood cells in HI test in case of unavailability of chicken red blood cells especially in veterinary diagnostic laboratories established in quail farms. Similarly, (Manvell *et al.,* 1998) successfully used ostrich RBCs in HI test to evaluate avian influenza antibodies in ostrich sera, but they did not recommend this type of red blood cells as the test has not been validated. The results of the second experiment in Table 4 revealed that, non treated sera from control non vaccinated quails when tested in HI against 1.0 % chicken red blood cells gave false positive results which reach up to 6 $\log_{2,}$ whereas, no false results was observed when using quail red blood cells against control non treated serum. Heat and chicken red blood cell treatment of serum samples acted equally and reduced the number of false positive reaction in the HI test. These results agreed with that obtained by (Williams *et al.,* 1997; Sakai *et al.,* 2006) who reported that ostrich sera are highly cross-reactive when tested by HI for the presence of non-specific inhibitors of haemagglutination with the consequence of a high number of false positives. In comparing between sensitivity of different test to measure ND antibodies in the same serum sample from vaccinated and non vaccinated quails, the obtained results in Table 4 showed that, all serum from non vaccinated control gave negative results except those tested by HI without treatment.

Table 3: Mean antibodies titer (× log2) of vaccinated quails against La Sota and Hitchner B1 antigens using 1% quail Red Blood cells

Groups	Treatment	Days post vaccination					
		0		14		28	
		La Sota	Hitchner B1	La Sota	Hitchner B1	La Sota	Hitchner B1
G1	La Sota	≤ 2	≤ 2	5	4	8	6
G2	Hitchner B1	≤ 2	≤ 2	4	4	6	7
G3	Control (Non vaccinated)	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2

Table 4: HI antibodies (x log2) and ELISA titers of the same serum samples from Newcastle disease vaccinated (21 days post vaccination) and non vaccinated quails

Groups	Sample No.	HI				ELISA	
		CRBCs			QRBCs	Idexx	BioCkek
		Serum treatment					
		Non treated	Heat	10 % RBCs	Non treated		
G4	1	6	2	2	2	Negative	
	2	5	1	2	2		
	3	6	2	2	2		
	4	6	2	1	2		
	5	5	1	1	2		
G5	6	9	7	8	8		
	7	9	7	8	8		
	8	8	5	6	5		
	9	8	5	6	5		
	10	7	5	5	2		
	11	7	5	5	2		
	12	7	6	7	7		
	13	7	6	7	7		
	14	8	5	5	4		
	15	8	5	5	4		

Group 4: Non vaccinated control; Group 5: Vaccinated with La Sota vaccine at 21 day old by eye drop.

Table 5: Percent of positive NDV antibodies in serum samples from vaccinated and non vaccinated quails measured by different tests*

Vaccination	HI				ELISA	
	CRBCs			QRBCs	Idexx	BioCkek
	Serum treatment					
	Non treated	Heat	10 % RBCs	Non treated		
Vaccinated	100 %	100.0 %	100.0 %	80.0 %	0.0 %	
Non vaccinated	100 %	0.0 %	0.0 %	0.0 %		

*Serum samples: Number of serum samples were (5) from non vaccinated control and (10) from vaccinated quails

Also, all samples from vaccinated quails were correlated well to each other except for samples number (10 and 11) which were negative when tested without treatment by HI using quail red blood cells, while it gave positive results when treated by heat or 10% chicken RBCs and tested by HI. Surprisingly, the obtained results in our study (Tables 4 and 5) showed that both commercial ELISA kits failed to detect any positive sera (0.0 %) for ND antibodies in both vaccinated and non vaccinated groups of quails at different time periods. This finding revealed that, using currently available commercial ELISA to measure ND antibodies in vaccinated quails will result in false negative results. So, we cannot conclude correlation between ELISA and HI in measuring ND antibodies in vaccinated quails. In contrary, Kiss *et al.* (2003) used direct ELISA to measure ND antibodies in vaccinated quails feed deferent levels of beta – carotene, Also, (Fair *et al*., 1999; Fair and Ricklefs, 2002) developed monoclonal –blocking ELISA to evaluate immune response of quail vaccinated with inactivated Newcastle disease vaccine. Whereas, Moro *et al.* (2000) reported that liquid phase ELISA and HI test with heat and koalin treated serum showed good agreement. We could conclude that, conduction of HI test using pretreated quail serum with heat (56$^{\circ C}$ /30 minutes) or 10% chicken RBC, and using the virus to which the birds have been vaccinated gave the real immune response of quail vaccinated with Newcastle disease vaccine. We recommend developing specific ELISA to measure antibodies of ND in quails to avoid false negative results obtained by currently available commercial ELISA.

REFERENCES

Allan WH and Gough RE (1974). A standard haemagglutination Inhibition test for Newcastle disease 1. A comparison between macro and micro methods. Vet. Rec., 95: 120-123.

Brown J, Resurreccion RS and Dickson TG (1990).The relationship between the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. Avian Diseases, 34**:**585–587.

Chandrasekaran S and Aziz HA (1989). Outbreak of Newcastle disease in Japanese quail. J. Vet. Malay, 1: 9-15.

Czirják G.A, Köbölkuti LB, Cadar D, Ungvári A, Niculae M and Bolfã P (2007). An outbreak of the Newcastle disease in Japanese quail (coturnix coturnix japonica). Bulletin USAMV-CN, 64: (1-2).

Fair JM and Ricklefs RE (2002) .Physiological, Growth, and Immune Responses of Japanese Quail Chicks to the Multiple Stressors of Immunological Challenge and Lead Shot. Arch. Environ. Contam. Toxicol., 42: 77–87.

Fair J, Hansen E and Ricklefs R (1999). Growth, developmental stability and immune response in juvenile Japanese quails (Coturnix coturnix japonica). Proc. R. Soc. Lond B., 266:1735–1742.

Islam HA, Ito J, Tanakuwa H, Takada A, Itrakura C and Kida H (1994). Acquisition of pathogenicity of Newcastle disease virus isolated from Japanese quail by intracerebral passage in chickens. Japan. Vet. Res., 42: 147-156.

Kiss ZL, Bárdos C, Szabó L.and Szabó LM (2003). Effect of beta-Carotene Supplementation on Plasma and Yolk IgY Levels Induced by NDV Vaccination in Japanese Quail. Int. J. Vitam. Nutr. Res., (4): 285-9.

Manvell RJ, Jorgensen PH, Nielsen OL and Alexander DJ (1998). Experimental assessment of the pathogenicity of two avian influenza A H5 viruses in ostrich chicks (Struthio Camelus) and chickens. Avian Pathology, 27:400-404.

Mass R.A, Oei HL, Kemper S, Kough G and Visser L (1998). The use of homologous virus in the haemaglutination- inhibition assay after vaccination with Newcastle disease virus strain LaSota or Clone 30 leads to an over estimation of protective serum antibody titers. Avian Pathology, 27: 625- 631.

Mayo MA (2002). Summary of taxonomic changes recently approved by ICTV. Archives of Virology, 147:1655–1656.

Merino R, Villegas H, Quintana JA and Calderon N (2009). Characterization of Newcastle disease viruses isolated from chicken, gamefowl, pigeon and quail in Mexico. Vet. Res. Commun., 33(8):1023-30.

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

Moro de Sousa RL, Montassier HJ and Panito AA (2000). Detection and quantification of antibodies to Newcastle disease virus in ostrich and Rhea sera using liquid phase blocking Enzyme Linked Immunosorbant Assay. Clin. Diagn. Lab. Immunol., 7: 940-944.

Nguyen TH, Nguyen DK, Nguyen T and Pham TL (2000). Newcastle disease virus transmission in quails. Veterinary Sciences and Techniques, 7: (1) 38-42.

OIE . Newcastle disease. In : Office International Des Epizooties – Manual of standards for diagnostic tests and vaccines. Chapter 2.3.14 http://www.oie.int/ Newcastle dis.pdf .2012.

Oladele SB, Enoch I and Ibrahim NG (2008). Changes in Histopathology, Haematocrit, Haemoglobin, Haemagglutination Inhibition Antibody Titer and Total Protein of Japanese Quails (*Coturnix coturnix japonica*) Administered Different Doses of Newcastle Disease Virus. Journal of Animal and Veterinary Advances, 7 (4): 418-424.

Paulillo AC, Moreira SE, Denadai J, Silva FL and Luciano DJ (2009). Experimental Vaccination against Newcastle Disease in Japanese quails (Coturnix coturnix japonica): Clinical and Immunological Parameters.

International Journal of Poultry Science, 8 (1): 52-54.

Romao JM, Vasconcelos de Moraes TG., Ramos Salles RP, Cardoso WM. and Buxade CC (2011). Effect of in ovo vaccination procedures on japanese quail embryos (coturnix japonica) and incubation performance. Ciência Animal Brasileira, 12 (4): 584 - 592.

Richtzenhain LJ, Paulillo AC, Pinto AA and Kronca SN (1993). Relation between the hemagglutination inhibition test and the indirect ELISA in the serologic monitoring of laying hens submitted to different systems of vaccination against Newcastle disease. Rev. Microbiol., 24:187–191.

Sakai K, Yada K, Sakabe G, Tani O, Miyaji K, Nakamura M and Takehar K (2006). Serological and virological studies of Newcastle disease and avian influenza in slaughter age ostriches (Struthio camelus) in Japan. Journal of Veterinary Medical Science, 68: 491-494.

Silva RJ, Pinto AA and Carrasco AOT (2010).The use of homologous and heterologous antigens in Hemmaglutination- Inhibition (HI) test for the detection of antibodies against Newcastle disease virus in vaccinated and experimentally infected pigeons (Columbia livia). Virus Reviews and Research, 15 (1) http://www.virusreviewsandresearch.com/ISSUE15-1.html.

Stone, HD (1989). Efficacy of oil emulsion vaccines prepared with pigeon paramyxovirus -1, Ulster and La Sota Newcastle disease viruses. *Avian Diseases*, 33: 157-162.

Toffan A, Olivier A, Mancin M, Tuttoilmondo V, Facco D, Capua I and Terregino C (2010). Evaluation of different serological tests for the detection of antibodies against highly pathogenic avian influenza in experimentally infected ostriches (Struthio camelus). Avian Pathology, 39 (1): 11-15.

Williams R, Boshoff CH, Verwoerd D, Schoeman M, Wyk AV, Gerdes TH and Ross K (1997). Detection of antibodies to Newcastle disease virus in ostrich (Struthio camelus) by an indirect ELISA. Avian Diseases, 41: 864-869.