NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., June 2018 Vol 39 (2): 168 - 180.
https://dx.doi.org/10.4314/nvj.v39i2.9 ORIGINAL ARTICLE

Comparison of Blood Biochemistry Responses of Cockerels and Turkeys Experimentally Infected with a Velogenic Newcastle Disease Virus

Okoroafor, O. N.[1]; Okorie-Kanu, C. O.[2]*; Animoke, P. C.[1]; Okoye, J. A. O.[3]; Nwanta, J. A.[4] and Anene, B. M.[1]

[1.]Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. [2.] Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. [3.] Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria. [4.] Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. *Corresponding author: Email: drcokoriekanu@yahoo.co.uk; Tel No: +2348038993506.

SUMMARY

This study compared the serum biochemical responses of cockerels and turkeys infected with a velogenic Newcastle disease virus (NDV). Two hundred and fourty birds of one hundred and twenty each were used for the study. The birds were obtained at day-old and were randomly divided into eight groups of four groups for each bird species. Two groups from both bird types were vaccinated against NDV with *La Sota* vaccine at three weeks of age. The vaccinated and unvaccinated cockerels and turkeys were subsequently inoculated with the velogenic NDV after six weeks while the control groups were not vaccinated and not inoculated. Blood samples were randomly collected from five birds in each group for serum biochemical analyses at days 0, 3, 6, 10, 15 and 21 post inoculation (pi). Parameters determined included serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities, total serum proteins, albumin, globulin, blood glucose level, total cholesterol, blood urea nitrogen and creatinine. Data generated were analyzed using one-way analysis of variance (ANOVA). Results showed decreased ($p<0.05$) total serum protein, albumin, globulin, plasma glucose and total cholesterol levels in unvaccinated infected cockerels, unvaccinated and vaccinated infected turkeys and subsequent increased ($p<0.05$) serum globulin. Hypoproteinemia, hypoalbuminemia, hypoglobulinemia, hypoglycemia and hypocholesterolemia with subsequent hyperglobulinemia may be signs of velogenic NDV infection in turkeys. The absence of negative effects in some parameters in vaccinated infected turkeys further confirmed that vaccination not only prevents mortality due to velogenic NDV but also reduces pathologic effects in infected birds.

Key words: Blood biochemistry, Velogenic NDV, Cockerels, Turkeys

INTRODUCTION

Newcastle disease (ND) is a wide spread and highly contagious infectious disease of poultry. It is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) in

the family *Paramyxoviridae* and the genus *Avulavirus* (Lamb *et al.*, 2005). Virulence of NDV is greatly influenced by the host species affected and strain of the virus involved. Velogenic strains for chickens have basic amino acids at residues 113, 115 and 116 with phenylalanine at residue 117 (Alexander and Senne, 2008; Liu *et al*., 2008). Virulent NDVs are categorized into two pathotypes based on their effects on chickens into viscerotropic velogenic NDVs which cause highly virulent disease characterized by haemorrhagic lesions in the intestinal tract and neurotropic velogenic NDVs that cause high mortality with respiratory and nervous signs (Alexander and Senne, 2008). It is a reportable disease if it meets any of the criteria for virulence (OIE, 2012). Although NDV appears to be the most researched infectious disease of birds, its negative impact continues to cause a major drain on the poultry industry. The negative economic impact is due to the decline in productivity in susceptible birds and trade barriers caused by the virulent form of the disease. Also, the immense negative effects on dietary protein supply especially in the developing countries has become a major threat to public health and well-being of millions who depend on it for their livelihood (Aboe *et al*., 2006; Saidu *et al*., 2006; Olabode *et al*., 2008; Chaka *et al*., 2012; Solomon *et al*., 2012).

Although, turkeys have been reported to be more resistant to the NDV compared to chickens (Piacenti *et al*., 2006; Wakamatsu *et al*., 2006; Aldous *et al*., 2010), both bird types exhibited similar haematological responses to the virus (Okoroafor *et al*., 2018a).

Blood biochemistry evaluation is vital in detecting organ injuries in birds that show little or no clinical signs of disease even when seriously ill (Hochleithner, 1994; Harr, 2009). Our earlier studies showed increased level of markers of starvation in both susceptible and non-susceptible hosts (Okorie-Kanu *et al*., 2016 a, b). The continued replication of viruses and sometimes with lesions even in less susceptible and immunized birds has made alternative diagnostic methods inexpedient. The study was therefore designed to evaluate some blood biochemical changes associated with the velogenic NDV infection in the species. Information on the responses on the vital organs affected may help to ascertain the basis for the variation in susceptibility in these species.

MATERIALS AND METHODS

One hundred and twenty day-old cockerels and poults respectively were obtained from Ajanla Farms, CHI Limited, Ibadan, Oyo State, Nigeria. The birds were raised on deep litter and provided with water and feed *ad libitum*. The birds were randomly divided into four groups of 30 birds each and two groups of cockerels and turkeys respectively were vaccinated orally against ND at three weeks of age with ND *La Sota* vaccine (National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria). The groups were as follows: vaccinated and infected cockerels with NDV (VIC), unvaccinated and infected cockerels with NDV (UIC), vaccinated and uninfected cockerels (VUC), unvaccinated and uninfected cockerels (UUC), vaccinated and infected turkeys with NDV (VIT), unvaccinated and infected turkeys with NDV (UIT), vaccinated and uninfected turkeys (VUT) and unvaccinated and uninfected turkeys (UUT). The vaccinated and unvaccinated groups were kept far apart in different locations in research animal houses of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. The birds were challenged with a velogenic NDV, Kudu 113 (Echeonwu *et al*., 1993) obtained from NVRI, Vom, Plateau State, Nigeria after 6 weeks of age. The inoculum was reconstituted to ELD_{50} of $10^{6.46}$ per ml and each bird in VIC, UIC, VIT and UIT groups was inoculated intramuscularly (im) with 0.1ml of the inoculum while the uninfected groups received 0.1ml of phosphate buffered

saline im. The uninfected groups were kept in separate locations far from the infected groups and guidelines for the care and humane handling of animals were duly adhered to all through the study (FASS, 2010).

The birds were observed for clinical signs and lesions from day 0 to 21 pi and results recorded.

Blood biochemistry determinations were carried out on days 0, 3, 6, 10, 15 and 21 pi. Blood samples (3ml) were collected randomly from five birds in each group through the jugular vein using a 5-ml hypodermic syringe. Blood was not taken from the same birds within a week to avoid inducing anaemia. Samples were kept for 30 minutes at room temperature to clot. They were centrifuged for 10 minutes at 3000g and serum harvested for blood biochemistry analyses. The serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) was determined by the phenolphthalein monophosphate method (Klein *et al*., 1960; Babson *et al*., 1966). The total serum protein was determined by the direct Biuret method (Lubran, 1978) while the serum albumin was determined by the bromocresol green method (Doumas *et al*., 1971; Doumas and Peters, 1997). The serum globulin was determined by subtracting the serum albumin from the determined total serum protein (Colville, 2002). The serum cholesterol was determined by the enzymatic colorimetric method (Allain *et al*., 1974) while the blood urea nitrogen (BUN) was determined by the modified Berthlot-Searcy method (Fawcett and Scott, 1960). The serum creatinine was determined by the modified Jaffe method (Blass *et al*., 1974). All parameters were determined using serum sample and Quimica Clinica Aplicada test kits (Quimica Clinica Aplicada, Spain) and a Cole Palmer spectrophotometer (USA) while the blood glucose level was determined using Accu-chek Active® Glucometer (Roche Diagnostics GmbH, Nannheim, Germany) based on the glucose oxidase method (D'Orazio *et al*., 2005).

Data generated were analyzed using One-way Analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc, Chicago, IL). Post-hoc test was carried out using the Least Significant Difference (LSD) method. Significance was accepted at 5% probability level.

RESULT

Clinical signs

Clinical signs and lesions were enumerated in our earlier reports (Okoroafor *et al*., 2018b).

Liver enzymes

The mean values recorded for alanine aminotransferase, aspartate aminotransferase and alkaline phosphate activities in both cockerels and turkeys did not vary ($P>0.05$) throughout the study (TABLES I, II and III).

Total serum proteins

The mean total serum proteins of the infected cockerels and turkeys were not different ($P>0.05$) when compared with their uninfected counterparts on day 0. On day 3 pi, the mean total protein of UIC was lower ($p<0.05$) when compared with VIC, VUC and UUC. On days 3 and 6 pi, the mean values obtained for UIT was lower ($p<0.05$) when compared with VIT, VUT and UUT. The mean values of VIT and UIT were lower ($p<0.05$) when compared with the values obtained for VUT and UUT and UIT value was lower ($p<0.05$) than VIT on day 10 pi. The mean value of UIT was higher ($p<0.05$) than all the turkey groups on days 14 and 21 pi (TABLE IV).

TABLE I: Alanine aminotransferase activity (Means ± standard error; UI/L) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	35.40 ± 0.08	35.09 ± 0.19	35.20 ± 0.20	35.28 ± 0.22	33.42 ± 0.13	35.49 ± 0.10
UIC	35.49 ± 0.15	35.49 ± 0.45	AD	AD	AD	AD
VUC	35.53 ± 0.14	35.35 ± 0.25	35.23 ± 0.34	35.91 ± 0.26	33.44 ± 0.19	35.52 ± 0.18
UUC	35.32 ± 0.08	35.26 ± 0.21	35.44 ± 0.04	36.48 ± 1.06	35.46 ± 0.32	35.53 ± 0.13
Turkeys						
VIT	35.40 ± 0.15	35.42 ± 0.08	36.13 ± 00.80	35.17 ± 0.15	35.86 ± 0.32	35.49 ± 0.10
UIT	35.55 ± 0.07	35.66 ± 0.15	35.73 ± 00.07	35.58 ± 0.18	35.54 ± 0.22	35.40 ± 0.10
VUT	35.57 ± 0.04	35.50 ± 0.16	35.77 ± 00.16	35.32 ± 0.15	35.70 ± 0.06	35.36 ± 0.14
UUT	35.57 ± 0.08	35.74 ± 0.07	35.65 ± 00.30	35.44 ± 0.10	35.72 ± 0.22	35.43 ± 0.24

* No significant difference between the groups ($p>0.05$).

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

TABLE II: Aspartate aminotransferase activity (Means ± standard error; UI/L) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	55.65 ± 0.04	56.31 ± 0.31	49.83 ± 0.54	48.53 ± 0.20	49.54 ± 0.11	54.52 ± 0.12
UIC	55.52 ± 0.02	56.12 ± 0.07	AD	AD	AD	AD
VUC	55.60 ± 0.07	56.15 ± 0.10	50.10 ± 0.37	48.66 ± 0.18	49.62 ± 0.67	54.33 ± 0.14
UUC	55.54 ± 0.04	56.05 ± 0.12	48.64 ± 0.48	48.49 ± 0.17	49.30 ± 0.11	54.33 ± 0.14
Turkeys						
VIT	55.17 ± 0.52	55.91 ± 0.07	49.14 ± 00.56	48.79 ± 0.03	49.99 ± 0.11	54.32 ± 0.08
UIT	55.45 ± 0.08	56.22 ± 0.12	49.21 ± 00.10	48.85 ± 0.22	49.55 ± 0.31	54.50 ± 0.20
VUT	55.54 ± 0.04	56.10 ± 0.08	49.31 ± 00.58	48.94 ± 0.03	49.92 ± 0.10	54.35 ± 0.13
UUT	55.47 ± 0.08	56.12 ± 0.18	48.73 ± 00.15	48.79 ± 0.03	50.03 ± 0.18	54.62 ± 0.11

* No significant difference between the groups ($p>0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

Serum albumin

The mean serum albumin values of the infected cockerels and turkeys were also not different ($P>0.05$) when compared with their uninfected counterparts on day 0. On day 3 pi, the mean albumin values recorded for UIC and UIT were lower ($p<0.05$) when compared with other cockerel and turkey groups respectively. On days 6 and 10 pi the mean values obtained for VIT and UIT were lower ($p<0.05$) than VUT and UUT but UIT value was lower ($p<0.05$) than the value recorded for VIT on day 10 pi (TABLE V).

TABLE III: Alkaline phosphatase activity (Means ± standard error; UI/L) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	210.40 ± 2.45	211.88 ± 1.66	196.70 ± 1.51	219.61±13.55	231.58 ± 4.00	228.95 ± 1.93
UIC	209.21 ± 2.92	208.75 ± 1.64	AD	AD	AD	AD
VUC	210.79 ± 1.02	208.31 ± 2.34	201.82 ± 4.85	237.54 ± 5.17	243.25 ± 2.09	230.90 ± 3.45
UUC	211.18 ± 0.76	210.79 ± 1.02	203.62 ± 3.69	237.00 ± 4.48	240.88 ± 6.05	234.87 ± 3.89
Turkeys						
VIT	209.61 ± 2.36	209.50 ± 2.63	203.34±03.49	229.80±16.08	242.65 ± 2.84	225.00 ± 0.79
UIT	209.14 ± 2.10	209.94 ± 0.70	199.50±02.12	215.40±20.57	250.59 ± 3.06	234.48 ± 3.19
VUT	212.37 ± 1.02	209.21± 1.37	200.42±01.64	243.53 ± 1.02	243.53 ± 1.02	223.42 ± 2.92
UUT	210.00 ± 2.24	211.58 ± 1.12	200.62±01.13	227.90 ± 2.79	251.03 ± 1.96	232.50 ± 5.2

* No significant difference between the groups (p>0.05)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

TABLE IV: Total serum proteins (Means ± standard error; g/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	3.21 ± 0.06	3.10 ± 0.16[a]	3.22 ± 0.10	3.28 ± 0.05	3.13 ± 0.09	3.15 ± 0.20
UIC	3.33 ±0.29	2.95 ± 0.10[b]	AD	AD	AD	AD
VUC	3.33 ± 0.09	3.17 ± 0.29[a]	3.14 ± 0.13	3.25 ± 0.06	3.14 ± 0.20	3.21 ± 0.12
UUC	3.33 ± 0.12	3.21 ± 0.25[a]	3.19 ± 0.18	3.16 ± 0.09	3.20 ± 0.30	3.13 ± 0.12
Turkeys						
VIT	3.24 ± 0.09	3.08 ± 0.13[a]	3.25 ± 00.12[a]	2.91 ± 0.26[a]	3.33 ± 0.26[a]	3.18 ± 0.13[a]
UIT	3.36 ± 0.11	2.55 ± 0.09[b]	1.95 ± 00.21[b]	2.07 ± 0.18[b]	3.90 ± 0.29[b]	3.80 ± 0.23[b]
VUT	3.23 ± 0.05	3.02 ± 0.31[a]	3.39 ± 00.17[a]	3.13 ± 0.16[c]	3.28 ± 0.08[a]	3.08 ± 0.21[a]
UUT	3.40 ± 0.03	2.98 ± 0.11[a]	3.62 ± 00.11[a]	3.21 ± 0.12[c]	3.23 ± 0.10[a]	3.10 ± 0.15[a]

[abc] Different superscripts in a column in each bird type indicate significant difference between the groups (p<0.05

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

Serum globulin

The mean serum globulin of the cockerels did not vary (p>0.05) from day 0 to the end of the study. For the turkeys, the mean serum globulin values did not vary (P>0.05) on day 0 but on days 3 and 6 pi the mean values for UIT were significantly lower (p<0.05) when compared with VIT, VUT and UUT. On day 10 pi, although the values for VIT and UIT were lower when compared with the values recorded for VUT and UUT, only UIT was significant (p<0.05). The values recorded for UIT were significantly higher (p<0.05) when compared with other turkey groups on days 15 and 21 pi (TABLE VI).

TABLE V: Serum albumin (Means ± standard error; g/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	1.40 ± 0.07	1.48 ± 0.09[a]	1.43 ± 0.26	1.45 ± 0.05	1.39 ± 0.11	1.42 ± 0.14
UIC	1.39 ± 0.12	1.18 ± 0.07[b]	AD	AD	AD	AD
VUC	1.40 ± 0.06	1.43 ± 0.12[a]	1.39 ± 0.07	1.49 ± 0.06	1.35 ± 0.16	1.43 ± 0.08
UUC	1.39 ± 0.03	1.39 ± 0.06[a]	1.41 ± 0.14	1.45 ± 0.07	1.32 ± 0.20	1.37 ± 0.15
Turkeys						
VIT	1.28 ± 0.07	1.39 ± 0.08[a]	0.96 ± 00.15[a]	1.28 ± 0.29[a]	1.60 ± 0.16	1.33 ± 0.04
UIT	1.34 ± 0.04	1.04±0.13[b]	0.99 ± 00.16[a]	1.00 ± 0.07[b]	1.62 ± 0.03	1.30 ± 0.15
VUT	1.26 ± 0.07	1.36±0.14[a]	1.29 ± 00.11[b]	1.43 ± 0.16[c]	1.57 ± 0.04	1.30 ± 0.19
UUT	1.32 ± 0.04	1.24±0.04[a]	1.34 ± 00.06[b]	1.56 ± 0.07[c]	1.55 ± 0.11	1.39 ± 0.07

[abc] Different superscripts in a column in each bird type indicate significant difference between the groups ($p<0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

TABLE VI: Serum globulin (Means ± standard error; g/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	1.81 ± 0.12	1.62 ± 0.07	1.79 ± 0.21	1.83 ± 0.08	1.74 ± 0.08	1.73 ± 0.12
UIC	1.94 ± 0.19	1.77 ± 0.04	AD	AD	AD	AD
VUC	2.00 ± 0.09	1.74 ± 0.15	1.75 ± 0.12	1.77 ± 0.10	1.79 ± 0.10	1.78 ± 0.07
UUC	1.94 ± 0.12	1.82 ± 0.19	1.78 ± 0.05	1.71 ± 0.04	1.89 ± 0.09	1.76 ± 0.11
Turkeys						
VIT	1.88 ± 0.12	1.69 ± 0.07[a]	2.26 ± 00.09[a]	1.56 ± 0.56[ab]	1.73 ± 0.22[a]	1.85 ± 0.13[a]
UIT	2.02 ± 0.12	1.51± 0.15[b]	0.95 ± 00.07[b]	1.07 ± 0.11[a]	2.29 ± 0.32[b]	2.50 ± 0.38[b]
VUT	1.97 ± 0.13	1.66 ± 0.17[a]	2.20 ± 00.28[a]	1.70 ± 0.05[b]	1.74 ± 0.11[a]	1.78 ± 0.19[a]
UUT	2.08 ± 0.06	1.65 ± 0.08[a]	2.16 ± 00.19[a]	1.75 ± 0.01[b]	1.68 ± 0.06[a]	1.71 ± 0.07[a]

[ab] Different superscripts in a column in each bird type indicate significant difference between the groups ($p<0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

Blood glucose

The mean blood glucose level of the cockerels did not vary ($p>0.05$) from day 0 to the end of the study. For the turkeys, the mean blood glucose level did not vary ($p>0.05$) from day 0 to day 21 pi except on day 6 pi when although the values recorded for VIT and UIT were lower when compared with the values recorded for the VUT and UUT, only the UIT was significant ($p<0.05$) (TABLE VII).

Serum total cholesterol

The mean serum total cholesterol of the cockerels did not vary ($p>0.05$) from day 0 to the end of the study. For the turkeys, the values recorded for all the groups did not vary on day 0 but on days 3 to 10, the values recorded for UIT were significantly lower

TABLE VII: Blood glucose (Means ± standard error; mg/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	258.75 ± 4.03	254.50 ± 4.17	236.25 ± 4.66	240.00 ± 5.40	234.25± 1.80	231.75± 2.02
UIC	255.00 ± 3.81	262.50 ± 3.59	AD	AD	AD	AD
VUC	256.75 ± 4.73	242.50 ± 5.95	231.60±11.75	236.00 ± 3.03	223.50± 5.68	231.00± 2.27
UUC	264.75 ± 3.45	256.75 ± 4.52	231.00 ± 2.04	2.39.25± 9.26	230.75± 2.50	236.75± 3.64
Turkeys						
VIT	261.00±13.52	276.25 ± 5.36	254.50±08.09[a]	260.00 ± 9.12	260.25± 9.25	267.50± 9.61
UIT	257.50 ± 4.97	262.00±9.64	210.50±07.26[b]	246.75±18.15	255.00 ±1.15	272.42±14.15
VUT	263.50 ± 7.17	270.75±16.04	268.00±010.87[a]	249.50±10.14	254.50± 9.12	258.00 ± 5.61
UUT	266.50 ± 3.07	269.50±3.48	271.50 ±07.49[a]	260.00 ± 2.97	253.50± 2.40	268.25±12.89

[ab] Different superscripts in a column in each bird type indicate significant difference between the groups ($p<0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

TABLE VIII: Serum total cholesterol (Means ± standard error; mg/dl) of the cockerels and turkeys

Groups	**Days**					**21**
	0	3	6	10	15	
Cockerels						
VIC	125.00 ± 4.06	102.78 ± 9.49	115.91 ± 4.35	117.08 ± 7.98	112.22±12.75	110.81 ± 9.23
UIC	120.71 ± 3.94	100.00 ± 9.05	AD	AD	AD	AD
VUC	120.24 ± 2.28	97.22 ± 12.32	125.46 ± 5.25	108.39 ± 4.88	113.33 ± 6.66	110.81 ± 11.36
UUC	122.62 ± 4.91	110.14±10.44	124.21±16.08	115.72±11.12	107.17 ± 6.98	109.09 ± 5.25
Turkeys						
VIT	123.81 ± 6.73	107.94±19.31[a]	116.67±026.67[a]	124.04±11.36[a]	114.45 ± 8.95	117.05 ± 4.69
UIT	119.30 ± 8.01	55.07 ± 2.90[b]	69.69 ± 04.93[b]	93.87 ± 4.00[b]	131.82 ± 2.62	104.53 ± 2.62
VUT	123.81 ± 1.94	104.76±19.05[a]	118.51±016.98[a]	123.08±16.84[a]	118.13 ± 3.04	109.09 ± 8.30
UUT	117.86 ± 5.95	127.53±17.63[a]	123.15±05.27[a]	122.73±11.44[a]	114.98 ± 12.19	115.15 ± 8.02

[ab] Different superscripts in a column in each bird type indicate significant difference between the groups ($p<0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

($p<0.05$) when compared with that recorded for other turkey groups. There were no variations in all the turkey groups on days 15 and 21 pi (TABLE VIII). There were no variations in the values recorded for other parameters ($p>0.05$) between the infected and uninfected groups for cockerels and turkeys respectively throughout the duration of the study (TABLES IX and X).

DISCUSSION

The results obtained showed incubation period of 2 days pi in both unvaccinated cockerels and turkeys. Incubation periods of 8 and 10 days had been reported (Piacenti *et al*., 2006) in commercial turkeys due to infection with Californian strains of velogenic NDV. The 100% and 60% mortalities in unvaccinated infected chickens and turkeys respectively confirmed

TABLE IX: Blood Urea Nitrogen (Means ± standard error; mg/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	1.37 ± 0.26	0.82 ± 0.09	0.73 ± 0.10	0.78 ± 0.09	0.73 ± 0.10	0.64 ± 0.09
UIC	0.91 ± 0	0.73 ± 0.10	AD	AD	AD	AD
VUC	1.37 ± 0.26	0.64 ± 0.09	0.63 ± 0.09	0.61 ± 0.09	0.70 ± 0.10	0.81 ± 0.09
UUC	1.37 ± 0.26	0.64 ± 0.09	0.73 ± 0.10	1.00 ± 0.26	0.73 ± 0.10	0.70 ± 0.10
Turkeys						
VIT	0.91 ± 0	0.64 ± 0.09	0.73 ± 00.10	0.78 ± 0.09	0.70 ± 0.10	0.73 ± 0.10
UIT	0.91 ± 0	0.73 ± 0.10	0.82 ± 00.09	0.78 ± 0.09	0.73 ± 0.10	0.70 ± 0.10
VUT	1.14 ± 0.23	0.73 ± 0.10	0.82 ± 00.09	0.78 ± 0.09	0.80 ± 0.09	0.73 ± 0.10
UUT	1.14 ± 0.23	0.73 ± 0.10	0.73 ± 00.10	0.78 ± 0.09	0.73 ± 0.10	1.00 ± 0.26

* No significant difference between the groups ($p>0.05$).

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC - Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

TABLE X: Creatinine (Means ± standard error; mg/dl) of the cockerels and turkeys

Groups	Days					
	0	3	6	10	15	21
Cockerels						
VIC	0.50 ± 0.10	0.42 ± 0.09	0.50 ± 0.10	0.59 ± 0.09	0.42 ± 0.09	0.42 ± 0.09
UIC	0.50 ± 0.10	0.50 ± 0.10	AD	AD	AD	AD
VUC	0.42 ± 0.09	0.50 ± 0.10	0.42 ± 0.09	0.50 ± 0.10	0.50 ± 0.10	0.59 ± 0.09
UUC	0.50 ± 0.10	0.59 ± 0.09	0.50 ± 0.10	0.59 ± 0.09	0.59 ± 0.09	0.42 ± 0.09
Turkeys						
VIT	0.59 ± 0.09	0.42 ± 0.09	0.42 ± 00.09	0.59 ± 0.09	0.59 ± 0.09	0.59 ± 0.09
UIT	0.59 ± 0.09	0.67 ± 0.14	0.50 ± 00.10	0.50 ± 0.10	0.50 ± 0.10	0.50 ± 0.10
VUT	0.59 ± 0.09	0.50 ± 0.10	0.59 ± 00.16	0.59 ± 0.09	0.42 ± 0.09	0.50 ± 0.10
UUT	0.50 ± 0.10	0.59 ± 0.09	0.59 ± 00.09	0.59 ± 0.09	0.59 ± 0.09	0.42 ± 0.09

* No significant difference between the groups ($p>0.05$)

VIC - Vaccinated and infected cockerels, UIC - Unvaccinated and infected cockerels, VUC- Vaccinated and uninfected cockerels, UUC - Unvaccinated and uninfected cockerels, VIT - Vaccinated and infected turkeys, UIT - Unvaccinated and infected turkeys, VUT - Vaccinated and uninfected turkeys, VUT - Unvaccinated and uninfected turkeys, AD - All Dead

earlier reports of turkeys less susceptibility to velogenic NDV than chickens

The mortalities in the vaccinated chickens and turkeys were 13.3 and 0.0%, respectively, in this experiment also confirmed reports that *La Sota* vaccination did not prevent clinical signs and lesions in chickens. However, it provided full protection against mortalities in turkeys.

The clinical signs in the challenged turkeys in this experiment were mainly depression, diarrhoea and nervous signs. The lesions in the major lymphoid organs in this experiment had been described in unvaccinated infected cockerels and turkeys (Piacenti *et al*., 2006; Wakamatsu *et al*., 2006; Ezema *et al*., 2009; Okoye *et al*., 2010; Okpe *et al*., 2015; Okorie-Kanu *et al*., 2016 b) No proventricular haemorrhage, intestinal and caecal tonsil ulcers were observed in turkeys.

This is in agreement with reports of Hanson and Spalatin (1973), Piacenti *et al*. (2006) and Wakamatsu *et al*. (2008) in commercial

turkeys, Igwe *et al*. (2014) in guinea fowls and Okorie- Kanu *et al*. (2016a) in ducks.

There were no significant variations between vaccinated and unvaccinated birds. This is in agreement with our earlier reports in cockerels and ducks (Okorie-Kanu *et al*., 2016 a, b) and results of the ALT activity, blood glucose level and total cholesterol in both vaccinated and unvaccinated birds and in contrast with reports of significant reduction in the total proteins and albumin values in broilers vaccinated against NDV and significant increase in the AST activity when compared with the unvaccinated controls (El-Toukhy *et al*., 1989; Talebi, 2006; Kudair and Al-Hussary, 2010).

There were no variations in the ALT, AST and ALP activities in the infected cockerels and turkeys in this study. This is in agreement with our earlier reports in cockerels and ducks (Okorie-Kanu *et al*., 2016 a, b) but in variance with reports of increased AST activity in serum and intestines of fowl infected with a mesogenic NDV strain (Rivetz and Bogin, 1974).

These results also contrast with the reports of Rivetz *et al*. (1975) who observed reduced ALP activity in chickens infected with mesogenic strains of NDV. The decrease was attributed to damage to the intestines as the predominant isoenzyme in plasma originates in the gut (Bide, 1970), and poor feeding and starvation due to anorexia and reduced intestinal activity (Bide, 1972). Increased ALP activity due to velogenic NDV was also reported but was attributed to use of high doses of NDV (Rivetz and Bogin, 1974). The difference in the responses of these birds might be due to different organ tropism of the different strains of NDV. However, the strain used in our study had consistently not affected these liver enzymes.

The reduced total protein and albumin levels recorded in the infected groups are also in agreement with our earlier reports in cockerels and ducks (Okorie-Kanu *et al*., 2016 a, b). This is attributable to starvation and enteritis as the birds were anorexic from the onset of clinical signs. Anorexia among other clinical signs had been reported in vaccinated chickens challenged with a velogenic NDV (Ezema *et al*., 2009). Intestinal ulcers and haemorrhages associated with ND were attributed to malabsorption and protein loosing enteropathy in chickens. The reduction in total protein in the unvaccinated infected when compared with the vaccinated infected could be due to the severity of enteritis which resulted in increased malabsorption and loss of protein.

Causes of hypoproteinemia include reduced synthesis due to chronic hepatocyte disorders; malabsorption and maldigestion due to enteritis, tumors and parasitism; increased loss due to renal disorder, malnutrition and starvation (Hochleithner, 1994; Harr, 2002; Campbell, 2004; Harr, 2009.

In our earlier reports, hypoalbuminemia was attributed to hypoproteinemia (Okorie-Kanu *et al*., 2016 b) and the present report has further confirmed it. As the most abundant protein in plasma (Harr, 2009), hypoalbuminemia always results in hypoproteinemia. Hypoalbuminemia and the consequent reduction in total calcium level (Lumeji, 1990) had been attributed to decreased egg lay and other egg abnormalities in layers infected with NDV (Okorie-Kanu *et al*., 2016 b). The hypoalbuminemia on days 6 and 10 pi and hypoglobulinemia on day 10 pi in both vaccinated and unvaccinated infected turkeys also confirmed earlier reports that vaccination did not prevent infection but reduced mortality, clinical signs and lesions. The initial hypoglobulinemia recorded in the study may be due to starvation and enteritis while the subsequent hyperglobulinemia is similar to our earlier reports in cockerels and ducks and in agreement with reports of Snyder (2012). Inflammation due to microorganisms results in production of antibodies which are gamma globulins and antibody production is a response to antigenic stimulation. The increase in the

blood glucose level in the unvaccinated infected cockerels in this study though not significant compared to our earlier report could be due to stress from the NDV infection as it is among the causes of hyperglycemia (Amand, 1986; Hochleithner, 1994; Campbell, 2004. However, this significant reduction in blood glucose in unvaccinated infected turkeys 6 days pi may be due to actual hypoglycemia due to reduced feed intake. Although, there is relative resistance in turkey compared to cockerels, some turkeys actually died alongside the more vulnerable cockerels as well the reduced weight of the infected turkeys and confirmed starvation due to reduced feed intake. The reduced total cholesterol level from day 3 to 10 pi also supports this claim of starvation as the cause of hypoglycemia. This is however, in contrast to our earlier reports in ducks which was attributed to reduced feed intake (Okorie-Kanu *et al*., 2016a) Pathologic decrease in total cholesterol level had been attributed to starvation (Hochleithner, 1994). The loss of weight caused by anorexia together with increased vulnerability to pathogenic organisms following resultant immunosuppression due to lymphocyte depletion and necrosis is worthy of note as this will have immense negative impact on productivity. Hypoproteinemia, hypoalbuminemia, hypoglycemia and hypocholesterolemia and subsequent hyperglobulinemia may be signs of velogenic NDV infection in turkeys.

REFERENCES

ABOE, P. A. T., BOA-AMPONSEM, K., OKANTAH, S. A., BUTLER, E. A., DORWARD, P. T. and BRYANT, M. J. (2006): Free-range village chickens on the Accra plains, Ghana: their husbandry and productivity. *Tropical Animal Health and Production*, 38(3): 235 - 248.

ALDOUS, E. W., SEEKINGS, J. M., MCNALLY, A., NILI, H., FULLER, C. M., IRVINE, R. M., ALEXANDER, D. J. and BROWN, I. H. (2010): Infection dynamics of highly pathogenic avian influenza and virulent Paramyxovirus type 1 viruses in chickens, turkeys and ducks. *Avian Pathology*, 39(4): 265 - 273.

ALEXANER, D. J. and SENNE, D. A. (2008): Newcastle Disease. In: SAIF, Y. M., FADLY, A. M., GLISSON, J. R., MCDOUGALD, L. R., NOLAN, L. K. and SWAYNE, D. E. (eds.) Diseases of Poultry. (12th edition) Blackwell Publishing, USA, pp.75 - 100.

ALEXANDER, D. J., ALDOUS, E. W. and FULLER, C. M. (2012): The long view: a selective review of 40 years of Newcastle disease research. *Avian Pathology*, 41(4): 329 - 335.

ALLAIN, C. C., POON, L. S., CHAN, C. S., RICHMOND, W. and FU, P. C. (1974): Enzymatic determination of total cholesterol. *Clinical Chemistry*, 20: 470 - 475.

AMAND, W. B. (1986): Avian clinical haematology and blood chemistry. In: FOWLER, M. E. (ed.) Zoo and Wild Animal Medicine, Philadelphia, W.B. Sounders, pp. 272 - 274.

BABSON, A. L., GREELEY, S. J., COLEMAN, C. M. and PHILIPS, G. E. (1966): Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. *Clinical Chemistry*, 12: 482 - 490.

BIDE, R. W. (1970): Plasma alkaline phosphatase in the fowl: Differentiation in the tissue isoenzymes by urea. Technicon International Congress, Chicago, 1969 Vol III P.169. New York: Medical Inc.

BIDE, R. W. (1972): Changes in the plasma alkaline phosphatase in avian erythroblastosis. *Avian Diseases*, 16: 421- 4 27.

BLASS, K. G., THIEBERT, R. J. and LAM, L. K. (1974): A study of the mechanism of the Jaffe reaction: *Journal of Clinical Biochemistry*, 12: 336 - 343.

CFSPH (2008): Newcastle disease. The Centre for Food Security and Public Health, Iowa State University, pp. 1-7.

CHAKA, H., GOUTARD, F. GIL, P., ABOLNIK, C., SERVAN DE ALMEIA, R., BISSCHOP, S. P. R. and THOMPSON, P. N. (2012): Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. *Tropical Animal Health and Production*, DOI: 10.1007/s11250-012-0278-y

COLVILLE, J. (2002): Blood Chemistry. In: Hendrix, C. M. (ed) Laboratory Procedures for Veterinary Technicians, 4th edn. Mosby Inc., St. Louis, Missouri: pp. 75 - 103.

D'ORAZIO, P., BURNETT, R. W., FOGHANDERSEN, N., JACOBS, E., KUWA, K., KULPMANN, W. R., LARSSON, L., LEWENSTAM, A., MAAS, A. H. J., MAGER, G., NASKALSKI, J. W. and OKORODUDU, A. O. (2005): Approved International Federation of Clinical Chemistry (IFCC) recommendation on reporting results for blood glucose (Abbreviated). *Clinical Chemistry*, 51: 1573 - 1576.

DOUMAS, B. T. and PETERS JR., T. (1997): Serum and urine albumin: A progress report on their measurement and clinical significance. *Clinica Chimica Acta*, 258: 3 - 20.

DOUMAS, B. T., WATSON, W. A. and BIGGS, H. G. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, 31: 87 - 96.

ECHEONWU, G. O. N., IROEGBU, C. U. and EMERUWA, A. C. (1993): Recovery of velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. *Avian Pathology*, 22: 383 - 387.

EL-TOUKHY, N., ALY, S. A. and SOLIMAN, M. K. (1989): Physiological studies on the level of some electrolytes and enzymes in normal and Newcastle vaccinated chicks. *Assiut Veterinary Medicine*, 21: 7 - 15.

EZEMA, W. S., OKOYE, J. O. A. and NWANTA, J. A. (2009): *LaSota* vaccination may not protect against the lesions of velogenic Newcastle disease in chickens. *Tropical Animal Health and Production*, 41: 477 - 484.

FASS, (2010): Guide for the care and use of Agricultural Animals in research and teaching (3rd edition). Federation of Animal Science Societies, pp. 103 - 128.

FEWCETT, J. K. and SCOTT, J. E. (1960): A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*, 13: 156 - 159.

HANSON, R. P. and SPALATIN, J. (1973). The viscerotropic pathotype of Newcastle disease virus. *Avian Diseases*, 17:354–361.

HARR, K. E. (2002): Clinical chemistry of companion avian species: A review. *Veterinary Clinical Pathology*, 31: 140 - 151.

HARR, K. E. (2009): Diagnostic value of Biochemistry. In: Harrison, G. J. and LIGHTFOOT, T. L. (eds). Clinical Avian Medicine, International Veterinary Information Service, Ithaca, NY.

HOCHLEITHNER, M. (1994): Biochemistries. In: RITCHIE, B. W., HARRISON, G. J. and HARRISON, L. R. (eds.). Avian Medicine: Principles and application,

International Veterinary Information Service, Ithaca, NY.

IGWE, A. O., EZEMA, W. S., EZE, D. C. and OKOYE, J. O. A. (2014): Experimental velogenic Newcastle disease can be very severe and viscerotropic in chickens but moderate and neurotropic in Guinea fowls. *International Journal of Poultry Science*, 13: 582–590.

KLEIN, B., READ, P. A. and BABSON, A. L. (1960): Rapid method for the quantitative determination of serum alkaline phosphatase. *Clinical Chemistry*, 6: 269 - 275.

KUDAIR, I. M. and AL-HUSSARY, N. A. J. (2010): Effect of vaccination on some biochemical parameters in broiler chickens. *Iraqi Journal of Veterinary Science*, 24: 59 - 64.

LAMB, R. A., COLLINS, P. L., KOLAKOFSKY, D., MELERO, J. D., NAGAI, Y., OLDSTONE, M. B.A., PRINGLE, C. R. and RIMA, B. K. (2005): Family Paramyxoviridae, In: Fauquet, C. M., MAYO, M. A., MANILOFF, J., DESSELBERGER, U. and BALL, L. A. (eds) Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses, Elsevier Academic Press, San Diego, pp.655 - 668.

LIU, H., WANG, Z., WANG, Y., SUN, C., ZHENG, D. and WU, Y. (2008): Characterization of Newcastle disease virus isolated from waterfowl in China. *Avian Diseases*, 52: 150 - 155.

LUBRAN, M. M. (1978): The measurement of total serum proteins by the Biuret method. *Annals of Clinical Laboratory Science*, 8: 106 - 110.

LUMEJI, J. T. (1990): Relation of plasma calcium to total protein and albumin in African grey (*Psittacus erythacus*) and amazon (*Amazona spp*) parrots. *Avian Pathology*, 19: 661 - 667.

OIE (2012): World Organization for Animal Health. Newcastle disease. Manual of diagnostic tests and vaccines for terrestrial animals, Biological Standards Commission, World Organization for Animal Health, Paris, France, PP. 1-19.

OKORIE-KANU, C. O., OKORIE-KANU, O. J. and OKOYE, J. O. A. (2016a): Blood biochemistry responses of ducks infected with a velogenic Newcastle disease virus. *Comparative Clinical Pathology*, 25(4): 681-688.

OKORIE-KANU, C. O., OKORIE-KANU, O. J. and OKOYE, J. O. A. (2016b): Blood biochemistry responses of chickens experimentally infected with a velogenic Newcastle disease virus (Kudu 113). *Nigerian Veterinary Journal*, 37(3): 160 -174.

OKPE, G. C., EZEMA, W. S. and OKOYE, J. O. A. (2015): Vitamin A supplementation reduced the mortality of velogenic Newcastle disease significantly in cockerels. *International Journal of Experimental Pathology*, 96: 326–331.

OKOROAFOR, O. N., EZE, C. P., EZEMA, W, S, NWOSU, C., OKORIE-KANU, C., ANIMOKE, P. C., ANENE, B. and OKOYE, J. O. A. (2018b): *La Sota* vaccination may not protect against virus shedding and the lesions of velogenic Newcastle disease in commercial turkeys. *Tropical Animal Health and Production*, 50(2): 345 - 351.

OKOROAFOR, O. N., OKORIE-KANU, C. O., ANIMOKE, P. C., OKOYE, J. O. A., NWANTA, J. A. and ANENE, B. M. (2018a): Comparative haematologic responses of cockerels and turkeys infected with a velogenic Newcastle disease virus. *Nigerian Veterinary Journal*, In press.

OKOYE, J.O.A., AGU, A. O., CHINEME, C. N. and ECHEONWU, G. O. N. (2000): Pathological characterization in chickens of a velogenic Newcastle disease virus isolated from guinea fowl. *Rev. Elev. Med. Vet. Pays trop*., 53: 325 - 330.

OLABODE, H. O. K., EGHAFONA, N. O. and IYOHA, H. (2008): A retrospective (2004 - 2006) study of poultry diseases diagnosed in Benin, Edo State, Nigeria. *Nigerian Veterinary Journal*, 29: 76 - 80.

PIACENTI, A. M., KING, D. J., SEAL, B. S., ZHANG, J. and BROWN, C. C. (2006): Pathogenesis of Newcastle disease in commercial and specific pathogen free turkeys experimentally infected with isolates of different virulence. *Veterinary Pathology*, 43: 168–178.

REITMAN, S. and FRANKEL, S. (1957): A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28: 56 - 62.

RIVETZ, B. and BOGIN, E. (1982): Enzyme changes in serum and tissues in fowl infected with a neurotropic-mesogenic strain of Newcastle disease virus. *Avian Pathology*, 11: 407 – 425.

RIVETZ, B., BOGIN, E., HORNSTEIN, K. and MERDINGER, M. (1975): Biochemical changes in chicken serum during infection with strains of Newcastle disease virus of differing virulence. I. Enzyme study. *Avian Pathology*, 4: 189 – 197.

SAIDU, L., ABDU, P.A., TEKDEK, L. B. and UMOH, J. U. (2006): Retrospective study of Newcastle disease cases in Zaria, Nigeria. *Nigerian Veterinary Journal*, 27: 53 - 62.

SOLOMON, P., BISSCHOP, S., JOANNIS, T. M., SHITTU, I., MESEKO, C., SULAIMAN, L., GADO, D., OLADOKUN, A.T., OLAWUYI, K. A. and ABOLNIK, C. (2012): Phylogenetic analysis of Newcastle disease viruses isolated from asymptomatic guinea fowls (*Numida meleagris*) and muscurvy ducks (*Cariana moscata*) in Nigeria. *Tropical Animal Health and Production*, 45: 53 - 57.

SNYDER, P. W. (2012): Diseases of Immunity. In: ZACHARY, J. F. and MCGAVIN M. D. (eds) Pathologic Basis of Veterinary Disease 5th edn. Mosby Inc. USA, pp. 242- 288.

TALEBI, A. (2006): Biochemical parameters in broiler chickens vaccinated against ND, IB and IBD. *International Journal of Poultry Science*, 5: 1151 - 1155.

WAKAMATSU, N., KINGS, D. J., KAPEZYNSKI, D. R., SEAL, B. S. and BROWN, C. C. (2006): Experimental pathogenesis for chickens, turkey and pigeons of exotic Newcastle disease virus from an outbreak in California during 2002 - 2003. *Veterinary Pathology*, 43: 925 - 933.