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# Passage of velogenic viscerotropic Newcastle disease virus through pet birds



by

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# INTRODUCTION

Velogenic viscerotropic Newcastle disease virus (VVNDV) has caused severe economic losses in poultry in many parts of the world. Infected pet birds have been incriminated as sources of several outbreaks (Lancaster 1981). One such outbreak occurred in 1971 in Southern California (Utterback 1973). The eradication of VVND cost 56 million dollars in federal funds and almost twelve million chickens were destroyed (USDA 1978). Almost every year there are outbreaks of VVND among pet birds in the United States. Therefore, the potential for VVND is always a possibility for domestic poultry.

Parrots can shed VVNDV for over a year (Erickson 1976). Some of the isolates obtained later in the course of the infection were less pathogenic for chickens. Erickson proposed that pet birds select out less pathogenic subpopulations of VVNDV. To further study this, a highly pathogenic plaque purified clone and a nonplaque purified isolate from which it was derived were both inoculated into a species of pet birds, Platycercus eximis (golden mantled rosella).

Due to the possible introduction of VVNDV into poultry by imported birds, all birds imported into the United States are quarantined for thirty days. Tissues are collected from dead birds and submitted to the Diagnostic Virology Laboratory at the National Veterinary Services Laboratories in Ames, Iowa, for virus isolation. Tissues of the golden mantled rosella were examined for trophism and quantitation of VVNDV.

Specimen collection for virus isolation from live birds in quarantine is done by cloacal swabs. The wooden cotton tipped applicator is commonly used. However, exotic birds in quarantine vary greatly in size. The applicators are an inappropriate size for the smaller species of birds. A smaller size swab composed of calcium alginate fibers has been used for the smaller birds. Virus isolation from this swab was compared to the cotton swabs.

Grinding of tissues for virus isolation is a time consuming process. In outbreaks of VVND where large numbers of samples are processed, a fast method for handling specimens is important. Swabbing tissues instead of grinding saves time, equipment and materials. Virus isolation from tissues and swabs of theses tissues was compared.

# REVIEW OF LITERATURE

Newcastle disease virus causes four forms of disease in chickens. These four forms include Doyle's, Beach's, Beaudette's and Hitchner's. These forms are caused by four strains of Newcastle disease virus.

The most severe form is Doyle's form and this was first reported in 1926 (Doyle 1927). The virus causing this form is also called velogenic viscerotropic Newcastle disease virus. Incubation varies from 2 to 5 days. Clinical signs include marked depression, increased respiration and weakness. Diarrhea is common and may lead to dehydration. Paralysis and edema of the head may occur. Mortality is over 90%. Postmortem lesions include hemorrhage and necrosis of the gastrointestinal tract. Less commonly seen are hemorrhages in the larynx and trachea and edema of the head and wattles.

Another highly pathogenic form of Newcastle disease is Beach's form. It was first described in 1944 (Beach) and was called "avian pneumoencephalitis". It spreads rapidly and mortality is high. Neurological and respiratory signs predominate. These signs include torticollis, wing paralysis and respiratory distress. The virus is also known as neurotropic velogenic NDV.

A less pathogenic form of NDV is Beaudette's form (Beaudette and Black 1945). Clinical signs are predominantly respiratory but neurological signs may occur. Egg production is reduced. The virus is called mesogenic Newcastle disease virus.

Hitchner's form, the fourth form of Newcastle disease, was described in 1948 (Hitchner and Johnson 1948). It causes mild respiratory signs in young chickens and inapparent infections in older chickens. The causative agent is referred to as lentogenic NDV.

VVND exists as a serious threat to poultry worldwide. There have been two pandemics of VVND in the world in the 20th Century. The first occurred in Indonesia and England in 1926 (Doyle 1927; Kraneveld 1926). By 1946 the virus was present in Japan, Africa, Australia, the Middle East and many countries of Europe. The velogenic form appeared in 1935 in California (Beach 1942).

The second pandemic began in 1962. It is thought to have originated from a cockatoo in Indonesia (Lancaster 1981). Its spread resembled the first pandemic (Lancaster and Alexander 1975). In 1966 a velogenic virus was reported in Iran and spread across the Near East and Europe by 1970.

In 1971, there was an outbreak of VVND in a poultry ranch in Fontana, California. VVNDV was isolated from exotic pet birds located in a nearby pet bird store. These were suspected to be the source of this poultry outbreak which eventually spread extensively in southern California (Utterback 1973).

In 1980, VVND was diagnosed in yellow-naped parrots owned by a bird dealer in Southern California. Other birds exposed and the resulting VVND outbreak led to the depopulation of 2399 birds. Other outbreaks that year occurred in a Florida bird holding facility. Shipments had been sent to 45 states and 30,000 birds were depopulated. In 1981 there were 37 domestic cases of VVND diagnosed in the U. S. in four states. In 1982, VVND was diagnosed in pet birds on 13 premises. In 1983, the only VVNDV

isolations in the U. S. occurred in fighting cocks in Texas and in 24 cases of young yellow-naped Amazon parrots believed to be smuggled into the U. S. (Report of the Committee on Transmissible Diseases 1980, 1981, 1982, 1983). In 1984, VVND was diagnosed in exotic birds in eight states, including California, Utah, Missouri, Oklahoma, Alabama, Georgia and Florida. The birds were depopulated and there was no spread to domestic poultry (USDA 1984).

## Clinical Signs, Postmortem Lesions and Virus Shedding by Pet Birds

Clinical disease and post mortem lesions have been observed in various species of pet birds infected with VVNDV. These species include most of the psittacine species. The virus has also been isolated from some species such as finches and canaries which have no clinical evidence of infection.

The first controlled study of interaction of VVNDV and pet birds was reported by Erickson (1976). Six species were inoculated by nebulization; they were the yellow-headed Amazon parrot, halfmoon conures, budgerigars, hill myahs, canaries and black-headed nuns.

Clinical signs varied among the six species. Onset of clinical signs occurred 3-14 days postexposure in the conures, parrots, budgerigars and nuns. Clinical signs included diarrhea, serous nasal discharge, conjunctivitis and central nervous system signs of infection. These latter signs included ataxia, unilateral or bilateral wing droop, leg paralysis and tremors of the head and neck. Signs varied in severity

between the four species. The only clinical signs observed in the canaries and mynahs were death losses.

The mortality rates varied between the species - conures 55%, parrots 29%, canaries 25%, budgerigars 22% and 21% for both the nuns and the mynahs. Post mortem lesions observed included hyperemia, hemorrhage and necrosis of the gastrointestinal mucosa. These lesions were observed most frequently in the proventriculus at the junction of the proventriculus and ventriculus, and less commonly seen in small intestines. Other lesions seen included peritonitis, hepatomegaly, splenomegaly and focal hepatic and renal necrosis.

The length of time of virus shedding was 383 days for parrots, 112 days for conures, 126 days for budgerigars, 119 days for mynahs, 35 days for canaries and 84 days for nuns.

Numerous NDV isolates were obtained from this study. Of these isolates, 279 were chosen for further characterization using the mean death time (MDT) in embryonating chicken eggs and red plaque assay techniques (Schloer and Hanson 1968).

In four species of the pet birds, most of the isolates produced large red plaques which indicated these isolates were probably VVNDV. The COPI-VVNDV strain used to inoculate the birds exhibited 99% large red plaques. However, some isolates produced predominantly clear plaques, indicating a possible change in virus subpopulations. Therefore, additional studies were done on these isolates. Five sequential isolates from 5 birds (2 parrots, 3 budgerigars) that formed predominantly clear plaques were further studied by the hemagglutination-elution assay.

In the hemagglutination-elution test, in a heterogeneous culture, the rate of elution is determined by one of the genotypes. Even if this genotype is only 20% of the total population, the slow eluter will determine the elution rate. To change the elution pattern there would have to be a change in the genotype of the cultures (Spalatin et al. 1970). All 25 sequential isolates were slow eluting viruses as was the original COPI-VVNDV.

The heat stability of these 25 isolates was also examined (Hanson et al. 1949). Three isolates were thermostable at 56 C for 15 minutes and the remaining 22 isolates were unstable. The original COPI-VVNDV was unstable at 56 C for 15 minutes. Both these assays are insensitive, but they indicate that the genotype of the isolate did not change.

The third method used to evaluate if a change in genotype occurred was to inoculate NDV susceptible chickens with 15 clear plaquing isolates. The isolates did not produce signs and lesions of VNND in the chickens. Instead, chickens inoculated with 11 of the 15 isolates had no symptoms of disease. These inoculated with the remaining 4 isolates had mild respiratory disease, airsacculitis, and one chicken had neurological signs.

In addition, four isolates obtained later in the study were chosen that produced predominantly large red plaques. Three of these produced lesions typical of VVND and the fourth was classified as a probable VVNDV.

Isolates were obtained from the backpassage in chickens inoculated with the pet bird isolates. Nine of these were studied by the assays for

red plaques, MDT, hemagglutination-elution and hemagglutinin thermostability. Eight of these isolates had characteristics similar to the isolates originally inoculated.

To explain these findings, the hypothesis was proposed that after acute VVNDV infection has subsided in pet birds, the pet birds favored the propagation of small clear plaquing viral subpopulations over the large red plaquing populations. This was suggested to occur rather than viral mutation. The isolation of some large red plaquing viral isolates later in the study indicated these subpopulations can be maintained by the pet bird.

Further studies on the possible mutation of VVNDV in the avian species was done by Brugh and Beard (1984). A Newcastle disease virus isolate that could be characterized as both velogenic and viscerotropic was serially passaged in twelve sets of chickens. There was no change in pathogenicity.

## Importation of Exotic Birds into the United States

Because of the suspected link of imported birds to the outbreak of velogenic viscerotropic Newcastle disease virus in southern California in 1971, all exotic birds were banned from importation in 1972 (USDA 1978). Since 1973, importation is allowed through 86 USDA approved private quarantine stations and three USDA operated quarantine facilities. The birds are quarantined for a period of 30 days. The number of exotic birds quarantined each year has increased from 46,665 in 1974 to 631,372 in 1983. Numerous different genera of birds are imported. From 1980-1983,

7494 pet birds of the genus Platycercus have been imported (Gertrude Nilsson, Animal Welfare Institute, Washington, D.C., Personal Communication, 1984).

During this period of quarantine, tissues are collected from specified numbers of dead birds and cloacal swabs are collected from specified numbers of live birds. These are submitted to the National Veterinary Services Laboratories in Ames, Iowa, for virus isolation (Pearson et al. 1975; Senne et al. 1983). From individual birds, pieces of lung, trachea, spleen and terminal intestine are placed in brain-heart infusion (BHI) broth. Cloacal swabs from large live birds are collected with wooden cotton tipped applicators; smaller calcium alginate swabs are used for small birds.

There have been several studies quantitating NDV in tissues of chickens. Hofstad (1951) studied the effect of 4 strains of NDV on White Leghorn pullets. Chickens were killed at specified times after inoculation. A virulent field strain was distributed in the blood, spleen, lung, trachea and intestinal contents. The highest concentration of virus was in the lung and trachea. The concentrations were as high as $10^7$ embryo lethal-doses per milliliter of tissue suspension on the fourth day postexposure. Viral concentrations varied with the route used for inoculation. Higher tissue concentrations resulted when the intranasal route was used as compared to the subcutaneous route.

Asdell and Hanson (1960) studied the distribution of 2 strains of highly pathogenic NDV in chickens. Within 72 hours, virus was present in the muscle, liver, spleen, kidney, pancreas, small intestine, esophagus, lung, air sac, blood, brain and bursa of Fabricius. The concentration of

virus was determined for the spleen, kidney, liver, lung and brains. In this study, chickens were also killed at various times postexposure. Inoculation was by the subcutaneous routes. The viral concentrations after apparent signs of disease began were higher in the kidney, lung and spleen.

Sinha et al. (1952) inoculated six strains of Newcastle disease virus into chickens and titrated the viral concentrations in the lung, spleen, brain breast muscle and blood. The lung contained the highest viral titer of any tissue collected for all six strains.

There have been only limited studies of the concentration of NDV in pet birds. Hirai et al. (1981) titrated the concentration of NDV virus in an unspecified number of cockatiels that had died in a bird dealer's facility. Titers in the liver, spleen, kidney and lung ranged from $10^{7.0}$ to $10^{7.5}$ EID/0.2 ml.

# MATERIALS AND METHODS

## Virus

An isolate of velogenic viscerotropic Newcastle disease virus was used to inoculate birds of the species Platycercus eximis. This isolate was recovered from two yellow-headed parrots smuggled from Guadalajara, Mexico, to Lakewood, Colorado, in 1972. The Colorado psittacine isolate of VVNDV (COPI-VVNDV) was the same that Erickson (1976) used to study virus-host relationships in six species of pet birds.

In 1972, virus for the study was prepared by propagation of the isolate in eight- to eleven-day-old specific pathogen free (SPF) embryonating chicken eggs. Embryos dead after 24 hours incubation had their amnioallantoic fluids (AAF) assayed for the presence of NDV by the hemagglutination (HA) and hemagglutination-inhibition (HI) tests (Committee for Standard Method 1974). The propagated virus was then stored in 1.0 ml amounts at -70 C.

The virus was characterized using mean death time (Allen et al. 1980) and red plaque assay techniques (Beard et al. 1970). The red plaque assay was modified and performed on COPI-VVNDV. Monolayers of chicken embryo fibroblasts from ten-day-old embryos were grown in 60 mm plastic plates and kept at 37 C in a humidified atmosphere with 5% $CO_2$. Ten-fold dilutions from $10^{-1}$ to $10^{-8}$ of the virus were made in tris buffered tryptose broth (TBTB) with antibiotics.

The concentration of antibiotics was 10,000 units/ml penicillin G, 2,000 µg/ml streptomycin sulfate, 650 µg/ml kanamycin sulfate, 1,000 µg/ml

gentamicin sulfate and 20 µg/ml amphotericin B. One-tenth ml of dilutions $10^{-6}$ through $10^{-8}$ were inoculated onto the cell cultures. Virus was allowed to adsorb for 60 minutes and the media was removed. Then the agar overlay medium of Earle's balanced salt solution and 1.6% Ionagar No. 2 was added at 5 ml per plate. The second agar overlay that contained neutral red at a concentration of .01% was added at 72 hours. At 96 hours, large red plaques were chosen that were at least 10 mm from adjacent plaques. They were picked with a sterile glass pipette, suspended in tris buffered tryptose broth with antibiotics and then inoculated into 8- to 11-day-old embryonating chicken eggs. The eggs were incubated and the AAF from dead embryos was tested for the presence of NDV by the HA and HI tests. The procedure was repeated two more times (Hanson 1977; Lennette and Schmidt 1979).

The nonplaque purified COPI-VVNDV and one plaque purified clone were each inoculated into 6-to-8-week-old SPF White Leghorn chickens by the cloacal route to check for pathogenicity.

## Psittacine Birds Used for Inoculation

Ninety birds of the species Platycercus eximis were obtained from a California bird importer who imported the birds from Holland directly into the National Veterinary Services Laboratories (NVSL). The birds were raised in captivity in Holland. Platycercus eximis originate from Australia and are commonly known as golden mantled rosellas (Figure 1). The birds were fed a diet of parakeet mix, sunflower seeds and carrots. They were kept in two different rooms in facilities that were under high

biological security. Two birds were placed in each of 45 cages. The cages and the rooms were under negative air pressure. Each cage had a separate air inlet and air was exhausted through microbiological safety filters to the outside. Blood samples were drawn four weeks after arrival to test for susceptibility to Newcastle disease virus by the serum HI test (Committee on Standard Method 1974) and susceptibility to Chlamydia psittaci by the complement fixation test (Gustafson 1980).

## Calibration of Nebulizers

Glass nebulizers[1] with an attached rubber bulb, to be used for inoculation of the birds, were calibrated. This was done by adding 1 ml AAF to the nebulizers and pulsing 10 times in a vertical laminar flow microbiological safety cabinet. The remaining AAF was measured. There was a 10-minute pause and the procedure repeated 14 times. The average ml of AAF per 10 pulses was calculated.

## Avian Inoculation

The rosellas were inoculated six weeks after the arrival at NVSL. Thirty-seven birds in room 6F were inoculated with $10^6$ nebulized plaque purified COPI-VVNDV directed at the nares. Thirty-eight rosellas in room E-16 were inoculated with $10^6$ nebulized nonplaque purified COPI-VVNDV.

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[1]Ted Pella Company, Tustin, CA.

## Specimen Collection from Live Birds

Cloacal swabs were collected three days postexposure from all birds still alive. Calcium alginate swabs[1] were used for collection and were then placed in sterile brain-heart infusion broth (BHI).

## Specimen Collection from Dead Birds

Cotton tipped wooden applicators were used to swab the cloaca and trachea of 22 rosellas. The tracheal and cloacal swabs from each bird were pooled into one sterile tube of BHI broth.

A group of 30 dead birds was selected to evaluate two types of swab materials used for specimen collection. The tracheas of fifteen birds were swabbed with calcium alginate swabs and the cloacas of the same birds were swabbed with wooden cotton tipped applicators. These swabs were placed in sterile BHI. Another 15 birds were swabbed with wooden cotton tipped applicators used for the tracheal swab collection and calcium alginate swabs used for the cloacal swab.

All swab samples were centrifuged at 1500 x g for 20 minutes. Then, 1.3 ml of supernatants from all these specimens were transferred to 3 ml vials containing 1.3 ml of tryptose-antibiotic broth. Then 0.3 ml of each sample was inoculated into each of four 8- to 11-day-old embryonating chicken eggs (Pearson et al. 1975).

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[1] VMR Society, San Francisco, CA.

## Specimens for Titrations

Various tissues and tissue swabs were collected from each of 15 birds to check for virus trophism and quantification in tissues. Kidney, liver, brain, spleen, terminal intestine, lung and trachea and swabs of the cloaca, trachea, brain, lung and spleen were collected from each of 15 birds. The tissues were weighed and 1% or 10% suspensions made in tryptose-antibiotic broth. Ten-fold dilutions were made in tryptose-antibiotic broth. One-tenth ml of each dilution was inoculated into each of 4 eggs. The 50% embryo lethal-dose ($ELD_{50}$) was calculated by the Kärber method (Kärber 1931).

## Assay for Newcastle Disease Virus Activity

All embryos inoculated were candled daily. Chicken embryos that died in less than 24 hours were discarded. The amnioallantoic fluids (AAF) from eggs dying after 24 hours were harvested and tested for hemagglutination. If there was no hemagglutination activity present, the AAF was tested for bacterial contamination by streaking the AAF on blood agar. Those not contaminated were diluted 1:10 in tryptose-antibiotic broth and inoculated into each of 4 embryos.

## Tissue Collection for Histopathology

Tissues were collected from 8 birds. Pieces of liver, spleen, kidney, brain, lung, small intestine, large intestine, proventriculus and ventriculus were fixed in 10% formalin, processed by routine paraffin technique, sectioned and stained with hematoxylin and eosin.

# RESULTS

## Chicken Inoculation

The four 8-week-old chickens inoculated with the nonplaque purified virus had lesions of VVND. Typical lesions such as hemorrhage of the proventriculus, hemorrhage and erosion of the ventriculus and hemorrhage of the small intestine were seen. Lesions were scored according to the severity of lesions using the tabulation described by Pearson et al. (1975). Using this system of scoring, birds scored 1+, 1+, 4+, and 1+. At NVSL, lesions of +4 or +3 in any one of 4 chickens classifies a NDV isolate as VVNDV. If lesions of 2+ or more are observed in 2 or more chickens, the isolate is also classified as VVNDV (Senne et al. 1983).

The chickens inoculated with the plaque purified virus also had typical lesions of VVND. Lesions include hemorrhage of the proventriculus, erosion of the ventriculus, hemorrhage and necrosis of the small intestine and hemorrhage at the cloaca. Scores for these birds were 1+, 1+, 2+ and 3+.

## Mean Death Time

The MDTs of the plaque purified and nonplaque purified isolates were 64 hours.

## Clinical Signs and Mortality of the Rosellas

Clinical signs were the same for both groups of birds inoculated with the nonplaque purified and the plaque purified virus. By postexposure day 3, 38 birds were depressed, and had ruffled plumage and diarrhea. By day 4 the remaining live birds were depressed and had diarrhea. There were signs of central nervous system involvement in 21 birds. They had head tremors, were unable to fly and were ataxic if they tried to move. Four birds exhibited torticollis. Six birds also had unilateral wing paralysis. By six days postexposure, all the rosellas were dead (Figures 2-9).

## Postmortem and Histopathological Examination

Postmortem examination was performed on all birds. The most common gross lesion was the fluid filled, distended small and large intestines. This was seen in 65 birds. The fluid was yellow in 45 birds and was hemorrhagic in 20 birds. Hemorrhage was seen on the serosa and mucosal surfaces of the small intestine in 19 birds. Hemorrhage was present in the mucosa of the proventriculus in five birds and in the mucosa of the ventriculus in four birds. Edematous lungs were found in 17 birds. In eight birds, peritracheal and subcutaneous edema and hemorrhage were seen. Hemorrhage of the eyelid was seen in six birds and tracheal edema seen in three birds. Abdominal fluid was seen in one bird.

Histopathology was performed on the tissues of eight birds. In four birds, there was a necrotic enteritis of the small intestine. There was

congestion of the liver in six birds, and congestion of the lung in four birds (Figures 10-12).

## Virus Isolation in Swab Study

Newcastle disease virus was isolated from the 15 rosellas from both the cloaca and the trachea in the group where cotton tipped applicators were used for the cloacal swab and calcium alginate swabs used for the tracheal swab. Results are listed in Tables 1 and 2.

In the group where cotton tipped applicators were used to collect specimens from the trachea, NDV was isolated from all tracheas. However, for the cloacal swabs of these same birds with calcium alginate swabs, Newcastle disease virus was not isolated from rosella 10. Results are listed in Tables 3 and 4. In the comparison of the efficiency of NDV isolation from the cloaca and trachea of the rosellas, there is no significant difference ($p > 0.30$) between the 2 swabs (Harold Ridpath, Biometrics and Data Systems Section, NVSL, U. S. Department of Agriculture, Ames, Iowa, Personal Communication, 1984).

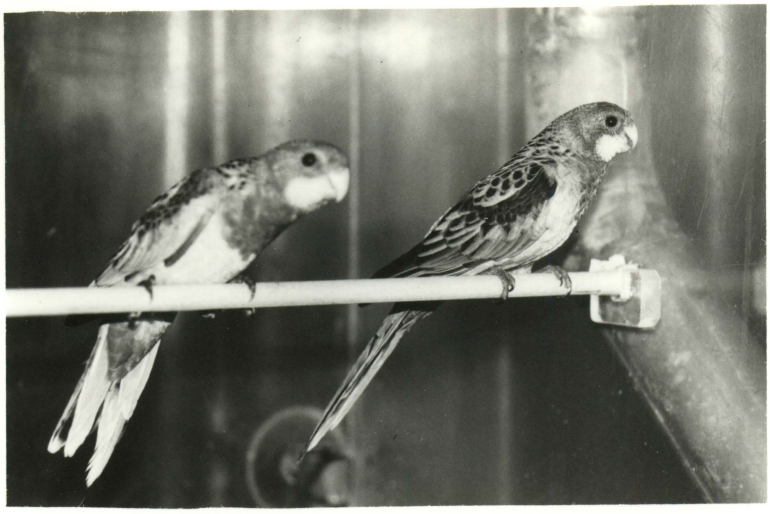
## Virus Isolation from Pooled Tracheal and Cloacal Swabs

The 22 pooled cloacal and tracheal swabs were all positive for NDV isolation.

Figure 1. Healthy rosella

Figure 2. Clinical signs observed for rosellas - ruffled plumage

Figure 3. Clinical signs observed for rosellas - depression



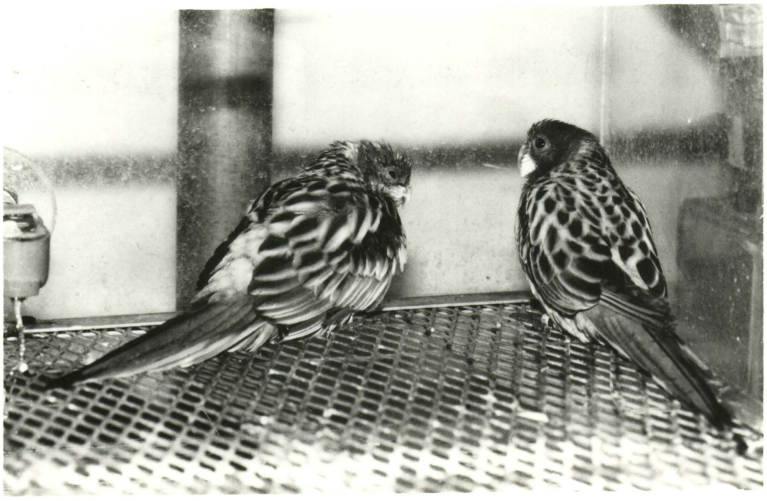
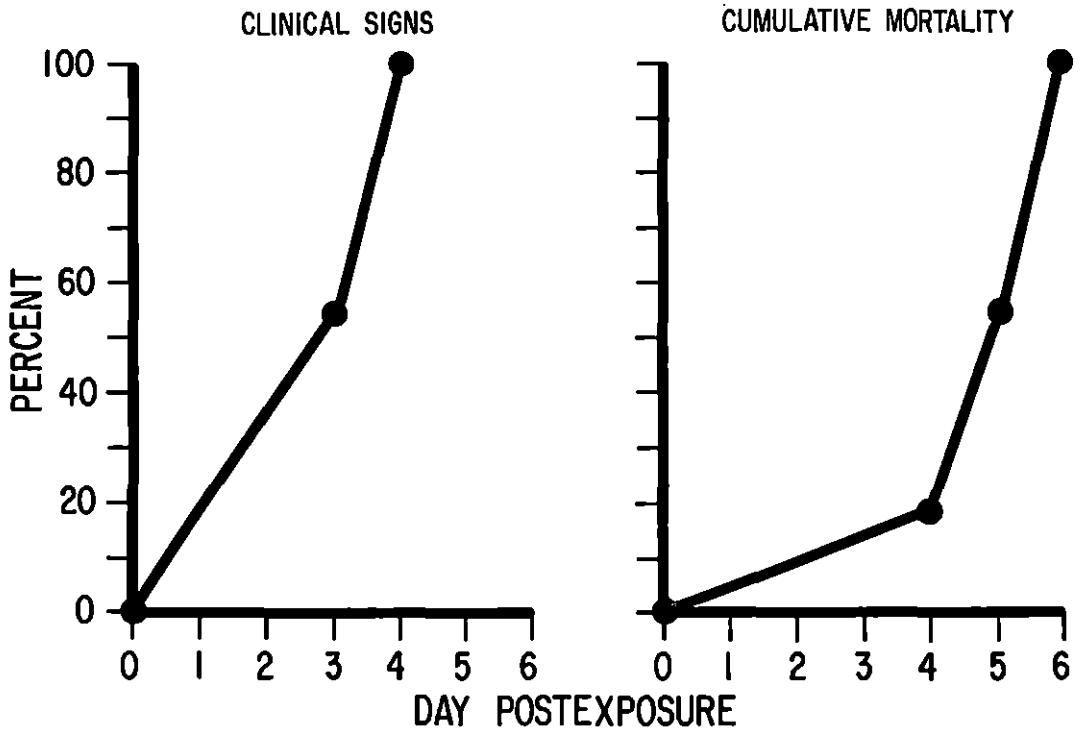


Figure 4. Clinical signs of rosellas following inoculation with plaque purified COPI-VVNDV (upper left)

Figure 5. Number of rosellas dead following inoculation with plaque purified COPI-VVNDV (lower left)

Figure 6. Cumulative mortality of rosellas following inoculation with plaque purified COPI-VVNDV (upper right)


CLINICAL SIGNS
CUMULATIVE MORTALITY
PERCENT
100
80
60
40
20
0
0 1 2 3 4 5 6
0 1 2 3 4 5 6
DAY POSTEXPOSURE

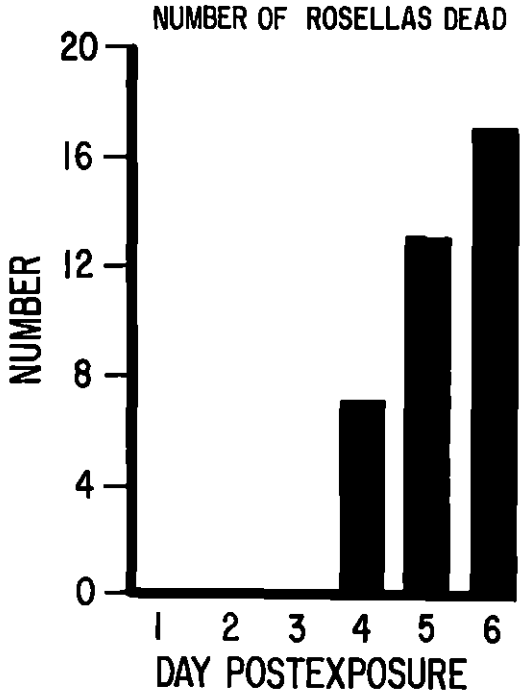

NUMBER OF ROSELLAS DEAD
NUMBER
20
16
12
8
4
0
1 2 3 4 5 6
DAY POSTEXPOSURE

Figure 7. Clinical signs of rosellas following inoculation with nonplaque purified COPI-VVNDV (upper left)

Figure 8. Number of rosellas dead following inoculation with nonplaque purified COPI-VVNDV (lower left)

Figure 9. Cumulative mortality of rosellas following inoculation with nonplaque purified COPI-VVNDV (upper right)

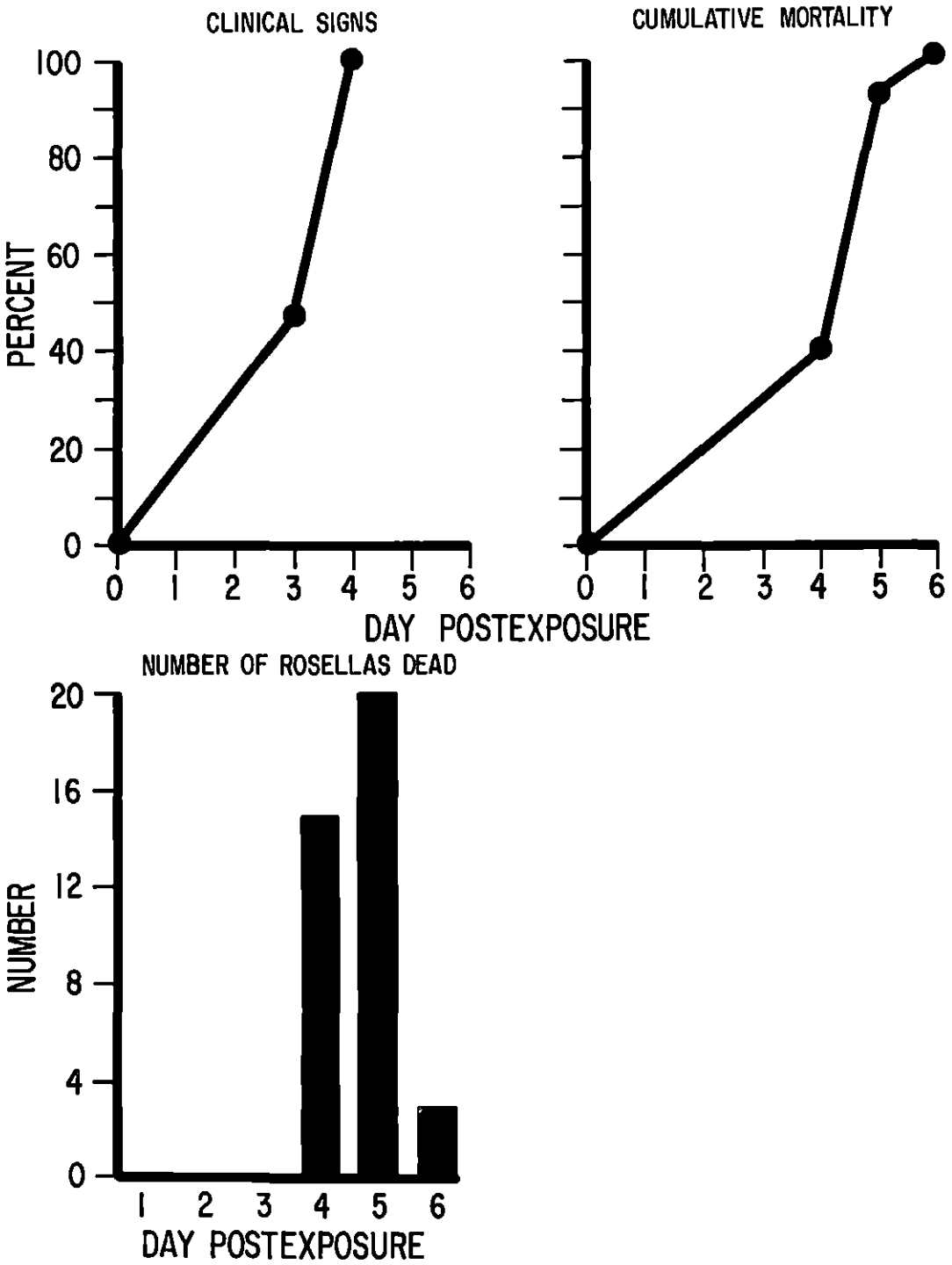
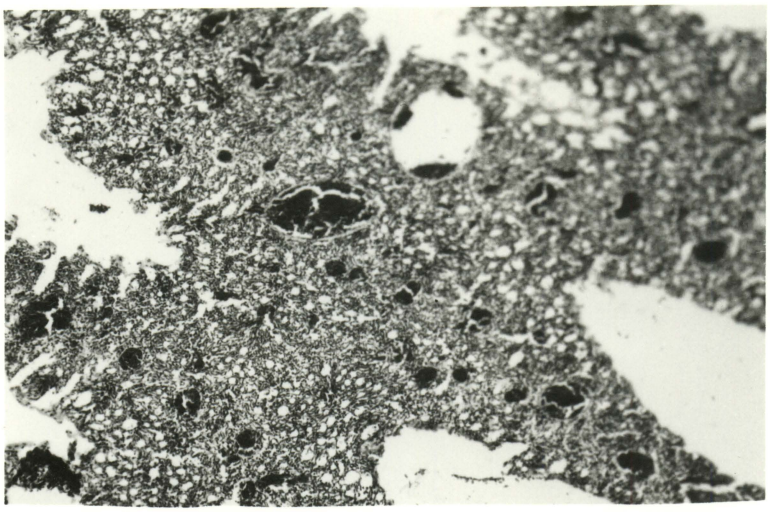
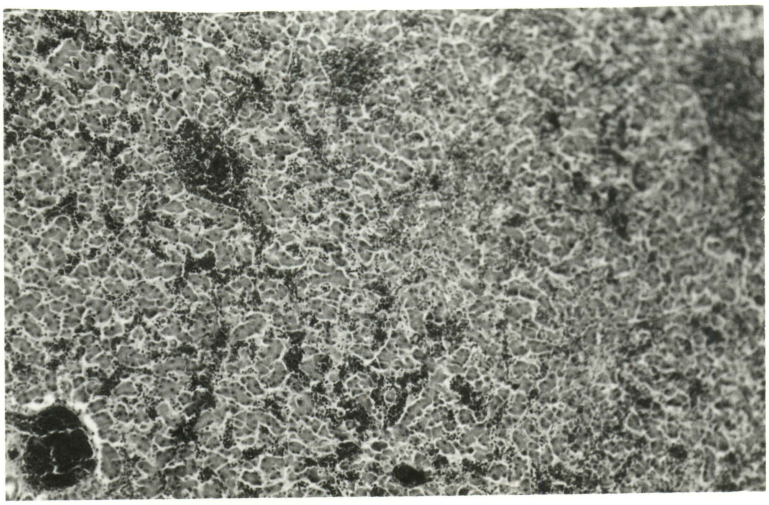

CLINICAL SIGNS
CUMULATIVE MORTALITY
PERCENT
100
80
60
40
20
0
0 1 2 3 4 5 6
DAY POSTEXPOSURE
NUMBER OF ROSELLAS DEAD
NUMBER
20
16
12
8
4
0
1 2 3 4 5 6
DAY POSTEXPOSURE

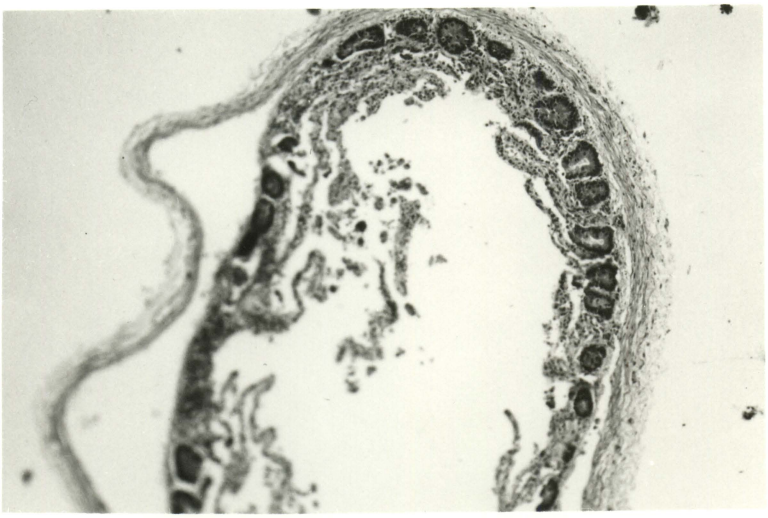
Figure 10. Histopathology of rosella tissues - congestion of lung

Figure 11. Histopathology of rosella tissues - congestion of liver

Figure 12. Histopathology of rosella tissues - necrotic enteritis







## Titrations of Tissues and Swabs

Newcastle disease virus was isolated from all 15 birds in the titration study. The results are listed in Tables 5 and 6. An analysis of variance was done to compare the NDV concentrations in the rosella tissues and swabs (Harold Ridpath, Personal Communication, 1984). The logarithms of the mean $ELD_{50}$'s were used. These are listed in Tables 7 and 8.

The average of the means of the logarithms of the 5 swab samples, 3.72, was significantly lower ($p < 0.005$) than the average of the means of the logarithms of the 7 tissue samples, 4.31.

For the remaining comparisons, the differences were considered significant ($p < 0.05$) if the means differed by at least 0.87. The comparisons were done by the Least Significant Difference Method. In the titrations of NDV in tissues, the viral concentrations in decreasing order are those in the lung, trachea, kidney, spleen, terminal intestine, liver and brain. There were 3 subgroups of tissues whose means were statistically different from each other group. These were: (1) trachea and lung, (2) liver, intestine, spleen and kidney and (3) brain. The means within the subgroup did not differ statistically.

In the titrations of NDV in the swabs, the concentrations of virus in decreasing order were lung, tracheal, cloacal, spleen and brain. In the titration of swabs, there were also 3 subgroups whose means were statistically different from each other ($p < 0.05$). These were: (1) trachea and lung, (2) spleen and cloacal and (3) brain. The means within the subgroup did not differ significantly.

Comparison of virus concentrations in a tissue and a swab of that same tissue was performed. This was done for 4 tissues. These were the lung, trachea, spleen and brain. The lung and trachea tissues had significantly higher virus concentrations than the swabs of the same tissue. The spleen and brain tissues and swabs did not have significantly different concentrations.

Table 1. NDV isolation from cloacal swabs using wooden cotton tipped applicators

| Rosella Identification | NDV Isolation[a] |
|---|---|
| 31 | + |
| 11 | + |
| 21 | + |
| 41 | + |
| 14 | + |
| 4 | + |
| 17 | + |
| 34 | + |
| 23 | + |
| 24 | + |
| 1 | + |
| 16 | + |
| 18 | + |
| 22 | + |
| 28 | + |

[a] + = virus isolation.

Table 2. NDV isolation from tracheal swabs using calcium alginate swabs

| Rosella Identification | NDV Isolation[a] |
|---|---|
| 31 | + |
| 11 | + |
| 21 | + |
| 41 | + |
| 14 | + |
| 4 | + |
| 17 | + |
| 34 | + |
| 23 | + |
| 24 | + |
| 1 | + |
| 16 | + |
| 18 | + |
| 22 | + |
| 18 | + |

[a] + = virus isolation.

Table 3. NDV virus isolation from tracheal swabs using wooden cotton tipped applicators

| Rosella Identification | NDV Isolation[a] |
|---|---|
| 45 | + |
| 49 | + |
| 46 | + |
| 85 | + |
| 52 | + |
| 57 | + |
| 78 | + |
| 56 | + |
| 83 | + |
| 87 | + |
| 10 | + |
| 25 | + |
| 42 | + |
| 26 | + |
| 38 | + |

[a] + = NDV isolation.

Table 4. NDV virus isolation from cloacal swabs using calcium alginate swabs

| Rosella Identification | NDV Isolation[a,b] |
|---|---|
| 45 | + |
| 49 | + |
| 46 | + |
| 85 | + |
| 52 | + |
| 57 | + |
| 78 | + |
| 56 | + |
| 83 | + |
| 87 | + |
| 10 | - |
| 25 | + |
| 42 | + |
| 26 | + |
| 38 | + |

[a] + = NDV isolation.

[b] - = No virus isolation.

Table 5. Concentration of NDV collected from rosella swabs

| Rosella Identification Number | $ELD_{50}$ per .1 ml swab suspension | | | | |
|---|---|---|---|---|---|
| | Cloacal Swab | Tracheal Swab | Brain Swab | Lung Swab | Spleen Swab |
| 35 | $10^{5.05}$ | $10^{5.05}$ | $10^{2.05}$ | $10^{5.05}$ | $10^{4.8}$ |
| 2 | $10^{5.8}$ | $10^{4.8}$ | $10^{3.8}$ | $10^{6.05}$ | $10^{3.3}$ |
| 37 | $10^{2.05}$ | $10^{4.8}$ | $10^{2.05}$ | $10^{5.8}$ | $10^{3.8}$ |
| 20 | $10^{4.05}$ | $10^{4.55}$ | $10^{3.05}$ | $10^{5.8}$ | $10^{4.05}$ |
| 55 | $10^{4.8}$ | $10^{6.05}$ | $10^{3.3}$ | $10^{5.8}$ | $10^{2.55}$ |
| 39 | $10^{4.8}$ | $10^{6.8}$ | $10^{2.3}$ | $10^{3.05}$ | $10^{4.8}$ |
| 30A | $10^{4.05}$ | $10^{4.55}$ | 0 | $10^{6.3}$ | $10^{2.05}$ |
| 5 | $10^{2.05}$ | $10^{5.05}$ | $10^{3.05}$ | $10^{4.8}$ | $10^{1.3}$ |
| 40 | $10^{5.3}$ | $10^{6.8}$ | $10^{2.55}$ | $10^{5.05}$ | $10^{4.55}$ |
| 27 | $10^{1.8}$ | $10^{3.05}$ | $10^{1.8}$ | $10^{5.05}$ | $10^{3.05}$ |
| 3 | $10^{1.8}$ | $10^{3.3}$ | $10^{1.8}$ | $10^{5.55}$ | $10^{2.8}$ |
| 1 | $10^{2.3}$ | $10^{2.05}$ | $10^{1.3}$ | $10^{4.05}$ | $10^{2.05}$ |
| 13 | $10^{4.8}$ | $10^{4.55}$ | $10^{2.8}$ | $10^{5.3}$ | $10^{3.3}$ |
| 30B | $10^{1.8}$ | $10^{2.8}$ | 0 | $10^{1.8}$ | $10^{4.05}$ |
| 8 | $10^{5.05}$ | $10^{4.8}$ | $10^{2.3}$ | $10^{4.55}$ | $10^{1.8}$ |
| Average Concentration | $10^{3.7}$ | $10^{4.6}$ | $10^{2.14}$ | $10^{4.93}$ | $10^{3.22}$ |
| Range | $10^{1.8}$ - $10^{5.8}$ | $10^{2.05}$ - $10^{6.8}$ | 0 - $10^{3.8}$ | $10^{1.8}$ - $10^{6.3}$ | $10^{1.3}$ - $10^{4.8}$ |

Table 6. Concentration of NDV in rosella tissues

| Rosella Identification Number | $ELD_{50}$ per .1 ml of 10% tissue suspension | | | | | | |
|---|---|---|---|---|---|---|---|
| | Liver Tissue | Spleen Tissue | Terminal Intestine Tissue | Lung Tissue | Tracheal Tissue | Kidney Tissue | Brain Tissue |
| 35 | $10^{1.75}$ | $10^{2.5}$ | $10^{4.75}$ | $10^{5.5}$ | $10^{4.75}$ | $10^{3.0}$ | $10^{2.0}$ |
| 2 | $10^{4.25}$ | $10^{2}$ | $10^{5}$ | $10^{6.0}$ | $10^{5.5}$ | $10^{2.25}$ | $10^{3.0}$ |
| 37 | $10^{4.25}$ | $10^{5}$ | $10^{4.5}$ | $10^{6.25}$ | $10^{6.25}$ | $10^{3.75}$ | $10^{3.0}$ |
| 20 | $10^{3.25}$ | $10^{3.75}$ | $10^{1.5}$ | $10^{5.5}$ | $10^{4.25}$ | $10^{3.5}$ | $10^{3.25}$ |
| 55 | $10^{3.0}$ | $10^{3.75}$ | $10^{3.5}$ | $10^{6.5}$ | $10^{5.25}$ | $10^{7.0}$ | $10^{3.75}$ |
| 39 | $10^{3.25}$ | $10^{2.5}$ | $10^{1.75}$ | $10^{6.25}$ | $10^{5.75}$ | $10^{3.0}$ | $10^{3.25}$ |
| 30A | $10^{1.75}$ | $10^{4.25}$ | $10^{3.25}$ | $10^{6.25}$ | $10^{5.5}$ | $10^{5.25}$ | $10^{1.5}$ |
| 5 | $10^{6.5}$ | $10^{6.5}$ | $10^{6.0}$ | $10^{6.5}$ | $10^{4.25}$ | $10^{6.25}$ | $10^{3.5}$ |
| 40 | $10^{3.75}$ | $10^{4.5}$ | $10^{4.5}$ | $10^{5.25}$ | $10^{4.5}$ | $10^{2.75}$ | $10^{2.25}$ |
| 27 | $10^{5.0}$ | $10^{4.25}$ | $10^{3.5}$ | $10^{5.5}$ | $10^{6.0}$ | $10^{4.25}$ | $10^{3.0}$ |
| 3 | $10^{3.75}$ | $10^{3.25}$ | $10^{4.5}$ | $10^{5.25}$ | $10^{5.5}$ | $10^{5.25}$ | $10^{3.5}$ |
| 1 | $10^{3.25}$ | $10^{2.25}$ | $10^{6.0}$ | $10^{6.5}$ | $10^{6.5}$ | $10^{6.75}$ | $10^{3.75}$ |
| 13 | $10^{3.25}$ | $10^{5.75}$ | $10^{4.25}$ | $10^{5.75}$ | $10^{7.75}$ | $10^{4.75}$ | $10^{2}$ |
| 30B | $10^{4.5}$ | $10^{4.0}$ | 0 | $10^{5.5}$ | $10^{5.25}$ | $10^{4.25}$ | $10^{2}$ |
| 8 | $10^{4.5}$ | $10^{6.0}$ | $10^{4.5}$ | $10^{5.75}$ | $10^{6.25}$ | $10^{3.25}$ | $10^{2.25}$ |
| Average Concentration | $10^{3.73}$ | $10^{4.02}$ | $10^{3.83}$ | $10^{5.88}$ | $10^{5.55}$ | $10^{4.35}$ | $10^{2.8}$ |
| Range | $10^{1.75}$ - $10^{6.5}$ | $10^{2.00}$ - $10^{6.5}$ | 0 - $10^{6.0}$ | $10^{5.25}$ - $10^{6.5}$ | $10^{4.25}$ - $10^{7.75}$ | $10^{2.25}$ - $10^{7.0}$ | $10^{2.0}$ - $10^{3.75}$ |

Table 7. Concentration of NDV in rosella tissues

| | Logarithm of the mean $ELD_{50}$ per .1 ml of 10% tissue suspension |
|---|---|
| Lung | 5.88 |
| Trachea | 5.55 |
| Kidney | 4.35 |
| Spleen | 4.02 |
| Terminal Intestine | 3.83 |
| Liver | 3.73 |
| Brain | 2.8 |

Table 8. Concentration of NDV in rosella swabs.

| | Logarithm of the mean $ELD_{50}$ per .1 ml of swab suspension |
|---|---|
| Lung | 4.93 |
| Trachea | 4.6 |
| Cloacal | 3.7 |
| Spleen | 3.22 |
| Brain | 2.14 |

# DISCUSSION

The clinical signs observed in the rosellas were consistent with those observed in studies by Erickson (1976). The signs resembled those shown in the conures, parrots, budgerigars and nuns. However, the course of the disease was shorter. In Erickson's study of six species of pet birds the highest mortality was 55%. There was 100% mortality in this study. VVNDV produced more acute clinical disease in the rosellas than any of the six species studied by Erickson even though the same virus, dosage and route of inoculation were used. In this aspect, they resemble chickens more than the other six species.

Although the mortality resembled that of chickens, the post mortem lesions did not. Hemorrhages in the chicken extend throughout the gastrointestinal tract and are prominent in the proventriculus and the ventriculus. However, the gross lesions and diarrhea in these birds indicates that the major site of predilection of NDV for this species is the gastrointestinal tract.

Since 100% of the birds died, it was not possible to study the difference in passage of a plaque purified virus as compared to a nonplaque purified virus. There were no differences in the clinical signs in either group.

In the study comparing virus isolation by two different types of swabs, there was no difference between the two swabs in their efficiency for use in specimen collection. It would appear that the calcium alginate swabs are suitable for use in small birds for Newcastle disease virus isolation. However, as virus was isolated from all but one of the

swabs, definitive quantitative information is not available to evaluate the differences.

The tissues presently collected from pet birds in quarantine stations are lung, trachea, spleen and terminal intestine. Highest concentrations of virus are found in the lung and trachea. There is no statistical difference in the concentrations of virus in the kidney, spleen, liver or terminal intestine. Since the intestinal tract has a bacterial flora, it is the tissue most likely to lead to bacterial contamination of a virus sample. Therefore, if two other tissues are to be selected besides the lung and trachea, kidney or liver would seem a more appropriate choice. However, since there are high concentrations of VVNDV in both the trachea ($10^{5.55}$) and lung ($10^{5.88}$), time might be saved by collecting only these two tissues. Additional studies of virus levels in tissues of chronically infected pet birds is needed before changing collection procedures.

For routine viral isolation of Newcastle disease virus from domestic birds (poultry or exotic), tissue swabs are collected rather than grinding tissues. The isolation technique used is to collect tracheal and cloacal swabs in one tube of tryptose-antibiotic broth and to collect a swab of the lung in another tube. This amounts to considerable saving of time rather than to grind the lung. The average virus titer of the lung tissue is significantly higher than the average titer of the lung swab. However, the average titer of the lung swab ($10^{4.93}$) is so high that this seems a very appropriate procedure to follow.

During outbreaks, there are large numbers of samples to be processed. At those times the cloacal, tracheal and lung swabs are all three placed

in one tube of antibiotic-tryptose broth. This again results in savings of time and materials and seems a very appropriate procedure to follow.

## SUMMARY

An isolate of velogenic viscerotropic Newcastle disease virus (VVNDV) was plaque purified. The plaque purified and non plaque purified viruses were inoculated into chickens to observe if any change in pathogenicity had occurred. Chickens inoculated with the plaque purified virus had lesions typical of VVND. The plaque purified clone and the nonplaque purified isolate of VVNDV were then inoculated into golden mantled rosellas (Platycercus eximis). The incubation period was three-four days. The predominant clinical signs were depression and diarrhea. Neurological signs were present in some birds. There was no difference in clinical signs in the rosellas between those inoculated with the plaque purified clone and those inoculated with the nonplaque purified virus. VVNDV produced acute clinical disease in this species; within six days postexposure there was 100% mortality. Virus was isolated from all birds using calcium alginate swabs or wooden cotton tipped applicators.

Seven tissues and five tissue swabs were collected from each of 15 birds. The VVNDV in each specimen was titrated and the concentrations compared. The lung and trachea had the highest concentrations of virus in both the tissue suspensions and swab suspensions. For the lung the average virus concentrations were $10^{5.88}$ for the tissue suspension and $10^{4.93}$ for the swab. For the trachea, the average virus concentration was $10^{5.55}$ for the tissue suspensions and $10^{4.6}$ for the swab.

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