

at laboratories. Evaluation of infection status on the basis of a single isolate per swab rather than multiple isolates may also have contributed to differences.

Our study showed a significant crude association between purchase of more than 5 pigs from herd SA and isolation of toxigenic *P. multocida*. Of 3 herds in this category, the 2 that purchased the greatest number of breeding pigs (9 and 13) from herd SA, were classed as infected. The probability of introducing infected pigs is a function of prevalence of infection in the source herd and the number of introductions. For example, if 10% of pigs in herd SA were infected, the probabilities of these 2 herds not introducing infection ((1-P)ⁿ) were 0.39 and 0.25, respectively.

Owners of both infected herds in the high risk group also introduced more than 50 pigs from other sources in the 2 years prior to the study but this factor was unlikely to be causal. All other herds except one, which supplied pigs to the 3 positive herds in the 2 years prior to the survey were sampled in a similar fashion and found free from clinical or cultural evidence of infection. Samples were not collected from one source, a large breeding company, but the consulting veterinarian indicated that the herd was free from infection (B Munro, personal communication 1988). Therefore, we believe that introduction of more than 5 pigs from herd SA was causally associated with the risk of isolation of toxigenic *P. multocida* and that the observed statistical association between introduction of more than 50 pigs from other sources and isolation was a spurious one.

Evaluation of factors other than introductions was limited by the small number of herds from which toxigenic isolates were recovered and the small number of herds in some exposure categories. Smith (1983) reported that management and housing factors such as poor ventilation, continuous throughput in farrowing and weaner accommodation, and high stocking densities were important risk factors for atrophic rhinitis. Such evaluations will be more appropriate should atrophic rhinitis become more prevalent in NSW.

Acknowledgements

The authors gratefully acknowledge the co-operation of piggery owners and the assistance of Pastures Protection Board and Department of Agriculture and Fisheries veterinarians with farm visits. Staff at Regional Veterinary Laboratories kindly cultured nasal swabs and identified *P. multocida* isolates. H Ridings provided valuable assistance with the statistical analysis. The study was financially supported by the Swine Compensation Fund of New South Wales.

References

- Cowan ST (1974) — In: *Cowan and Steel's Manual for the Identification of Medical Bacteria*. 2nd Ed. London: Cambridge University Press, p 78
- DiGiacomo RF and Koepsell TD (1986) — *J Am Vet Med Assoc* **189**: 22
- Eamens GJ, Kirkland PD, Turner MJ, Gardner IA, White MP and Hornitzky CL (1988) — *Aust Vet J* **65**: 120
- Fleiss JL (1981) — *Statistical methods for rates and proportions*. 2nd ed, New York: Wiley and Sons, p 14
- Pedersen KB (1983) — *Cultural and serological diagnosis of atrophic rhinitis in pigs*, Agriculture — Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen, 25-26 May, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 22
- Pedersen KB and Barfod K (1981) — *Nord Vet Med* **33**: 513
- Rutter JM (1983) — *Res Vet Sci* **34**: 287
- Rutter JM (1987) — *Pig News Inform* **8**: 385
- Rutter JM and Rojas X (1982) — *Vet Rec* **110**: 531
- Rutter JM, Taylor RJ, Crighton WG, Robertson IB and Benson JA (1984) — *Vet Rec* **115**: 615
- Sawata A, Nakai T, Tuji M and Kume K (1984) — *Jap J Vet Sci* **46**: 141
- Schoess P (1983) — *Clinical diagnosis of atrophic rhinitis*, Agriculture — Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen May 25-26, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 13.
- Smith WJ (1983) — *Infectious atrophic rhinitis — non-infectious determinants*, Agriculture — Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen May 25-26, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 151

(Accepted for publication 28 February 1989)

Investigations of an enteric infection of cockatoos caused by an enterovirus-like agent

SL WYLIE and DA PASS

School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6155

SUMMARY: An enteric infection in cockatoos associated with a 30nm diameter enterovirus-like agent seen in faeces and intestinal epithelial cells is described. The disease is characterised by intractable, profuse, mucoid diarrhoea, weight loss, dehydration and death. Lesions in the intestine consist of villous atrophy, villous fusion, enterocyte hyperplasia and, in some cases, chronic inflammation. Affected birds so far examined have concurrent psittacine beak and feather disease.

Aust Vet J **66**: 321-324

Introduction

Enteric disease associated with the presence of virus particles in faeces is becoming more widely recognised in birds. To date, infections have been reported predominantly in gallinaceous birds. These include rotaviruses in chickens (McNulty *et al* 1983; Meulemans *et al* 1985), turkeys (Saif *et al* 1985; Yason and Schat 1986) and pheasants (Gough *et al* 1985; Reynolds *et al* 1987); rotavirus-like particles in turkeys (Saif *et al* 1985) and pheasants (Reynolds *et al* 1987); astrovirus in turkeys (Saif *et al* 1985; Reynolds and Saif 1986); parvoviruses in chickens (Kisary 1985) and turkeys (Trampal *et al* 1983); calicivirus in chickens (Wyeth *et al* 1981) and guinea fowl (Gough and Spackman 1981); entero-like virus in chickens (McNulty *et al* 1984; Spackman *et al* 1984) and turkeys (Saif *et*

al 1985); reovirus in turkeys (Goodwin *et al* 1985); adenovirus in turkeys (Saif *et al* 1985); coronavirus in turkeys (Pomerey 1984) and enteric virus-like particles 40-55nm in diameter in chickens (Frazier *et al* 1986).

This paper describes the clinical signs and lesions of an enteric disease in galahs (*Cacatua roseicapilla*) and a sulphur-crested cockatoo (*C. galerita*) associated with the presence of virus particles in the faeces.

Materials and Methods

Examination of Naturally Affected Birds

The clinical and pathological description is based on 17 cases.

The disease was first seen in 6 young galahs in a flock of wild birds in the outer metropolitan area of Perth, Western Australia. The flock is composed largely of aviary escapees as many birds have the physical characteristics of galahs originating in the eastern states of Australia, ie. they have much paler feathers on the crown than birds from the southwest of Western Australia. (D Saunders CSIRO, Division of Wildlife and Ecology). Numerous birds in the flock also had psittacine beak and feather disease (Pbfd) (Pass and Perry 1984) and all birds subsequently examined by us that have had enteric viral infection have been diagnosed as having Pbfd concurrently. Six cases were caged birds held by us for experimental purposes and the remainder were "pets" recently purchased from bird dealers.

Faeces from some affected birds were cultured on sheep blood agar and MacConkey's agar and were also examined by negative contrast electron microscopy. Faeces were centrifuged to remove solid debris and a drop of the supernatant was placed on a formvar coated copper grid, stained with 3% phosphotungstic acid (pH 7.2) for 1 min and then examined in a Philips 301 electron microscope.

Necropsies were performed on birds that died or were killed humanely with intravenous barbiturate. Representative areas of intestine were destroyed, in cold 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). Skin, liver, kidney and bursa of Fabricius were fixed in 10% buffered formalin. Sections for both light and electron microscopy were processed routinely. Frozen sections of formalin-fixed intestine were stained for lipid with oil red O.

Blood was collected from four affected birds and the serum examined for the presence of antibodies to human, chicken, feline, canine and bovine caliciviruses and to human astroviruses 1, 2 and 4 by Dr D Cubitt, Public Health Laboratory Service, Central Middlesex Hospital, London.

Transmission Experiments

Attempts were made to transmit the infection by oral inoculation to 6 normal galahs, 2 galahs with Pbfd, 4 peach-faced lovebirds (*Agapornis roseicollis*) with Pbfd and 4 one-day-old SPF chickens. The inoculum used was faeces that contained virus. The faeces which had been stored at -20°C were clarified by centrifugation and the supernatant administered orally via a crop tube on 3 successive days. SPF chickens, *Agapornis* sp and galahs received 0.5, 2.0 and 3.0ml of inoculum respectively. The birds were kept for 21 days after inoculation. Faeces were examined for the presence of viruses by negative contrast electron microscopy before infection, daily for 7d, and every 2 to 3d thereafter.

Results

Clinical Signs

The major presenting clinical sign is profuse, green, mucoid diarrhoea. Depression and poor appetite of variable severity are apparent in most cases presented by owners. Our observations on the initial cases diagnosed in wild galahs suggests that depression and poor appetite are not apparent, or very mild, in the first few days after the onset of diarrhoea, but as the diarrhoea continues, these signs increase in severity. Weight loss and dehydration rapidly ensue.

The diarrhoea is persistent and all but one of the affected galahs that we have seen have died. The course of the disease varies from several days to 3 or more weeks and at the time of death the bird is emaciated.

In one case, diarrhoea was present for 4d and the bird recovered spontaneously. The history of this bird differed from the others. It was diagnosed as having zinc poisoning from ingestion of galvanised wire and was treated successfully but developed diarrhoea 4d after treatment for zinc toxicity was completed. This bird also had Pbfd.

The disease spreads rapidly to other birds. Two affected birds were placed in a room containing individually caged galahs and sulphur-crested cockatoos suffering from Pbfd. The enteric disease occurred within 5d in 5 galahs and one

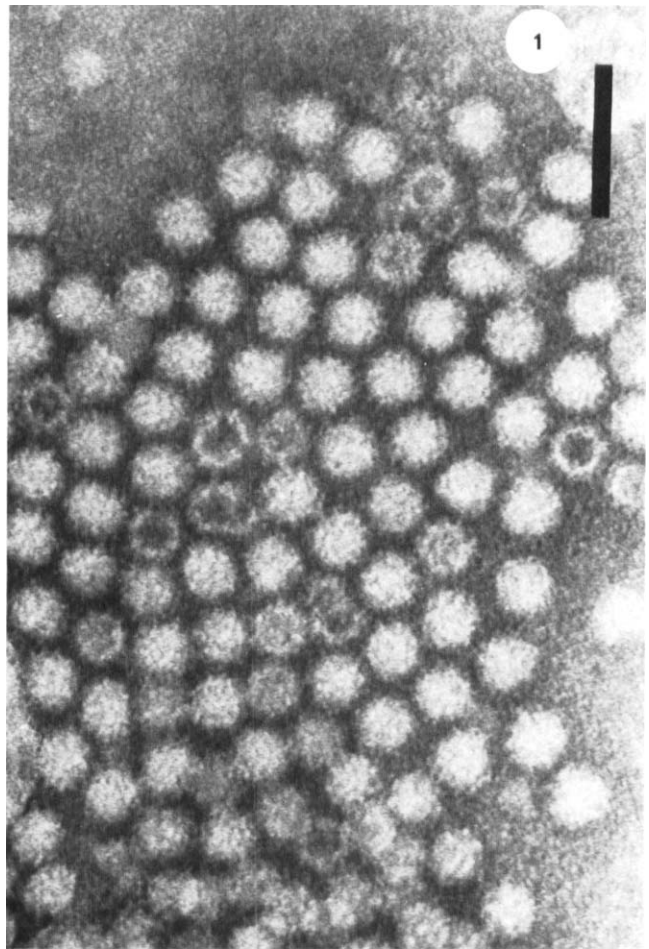


Figure 1. Virus-like particles in faeces. Bar = 0.1µm.



Figure 2. Viscera of an affected bird. Note the dilated intestine.

sulphur-crested cockatoo that previously had no evidence of diarrhoea. Diarrhoea was less severe in the sulphur-crested cockatoo than in the galahs. Five of the resident galahs died but the sulphur-crested cockatoo survived.

Negative contrast electron microscopy of faeces from affected birds revealed large numbers of round unenveloped virus particles approximately 35nm in diameter (Figure 1). The particles do not have any definite surface structure.

Pathology

Grossly, the intestine is dilated with mucoid fluid and gas and the wall is thickened (Figure 2). Microscopically, lesions are visible along the entire length of the small intestine but are most noticeable in the duodenum and upper jejunum. Lesions consist of villous atrophy, fusion of villi, elongation of crypts of Lieberkuhn and hyperplasia of epithelial cells in crypts and on shortened villi (Figure 3). Enterocytes on villi are columnar and those on the tips of villi are often vacuolated. Vacuoles contain lipid. Inflammation of variable severity may be present. In some areas, this may only be an increase in plasma cells and lymphocytes in the lamina propria but in other areas heterophils and macrophages that contain eosinophilic granular material or haemosiderin are prominent. Acute inflammation and haemorrhage into the villous tips has been seen in some cases. Overall, the mucosa is thickened despite villous atrophy.

Large numbers of bacteria are commonly present in the lumen of the gut and a heavy growth of bacteria may be cultured from faeces.

Transmission electron microscopy of the intestinal mucosa of one affected bird revealed paracrystalline arrays of round virus-like particles approximately 30nm in diameter in the cytoplasm of enterocytes (Figure 4). Many enterocytes had less numerous and shorter microvilli than normal.

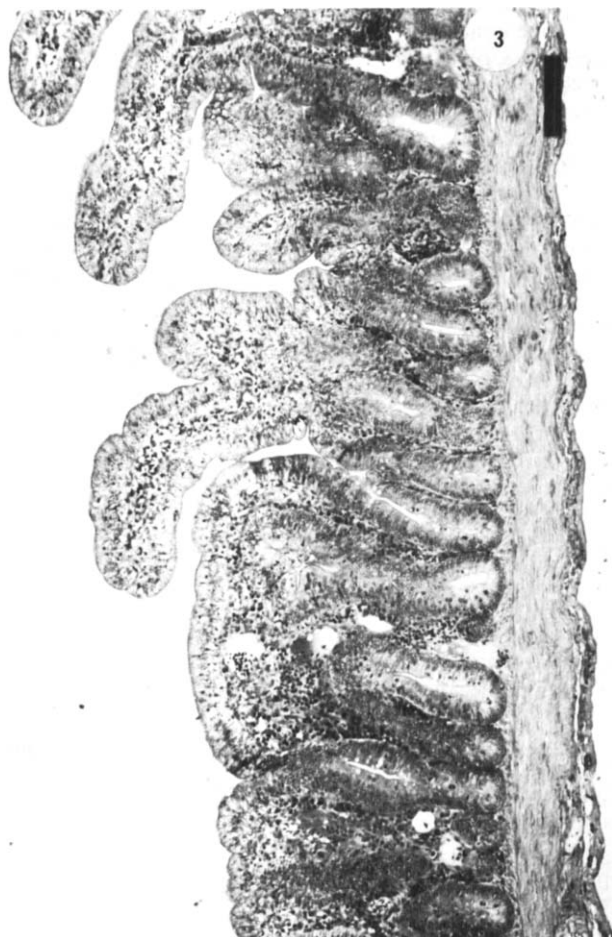


Figure 3. Villi in the small intestine are shortened, some are fused (bridging) and the crypt epithelium is hyperplastic. Bar = 100µm.

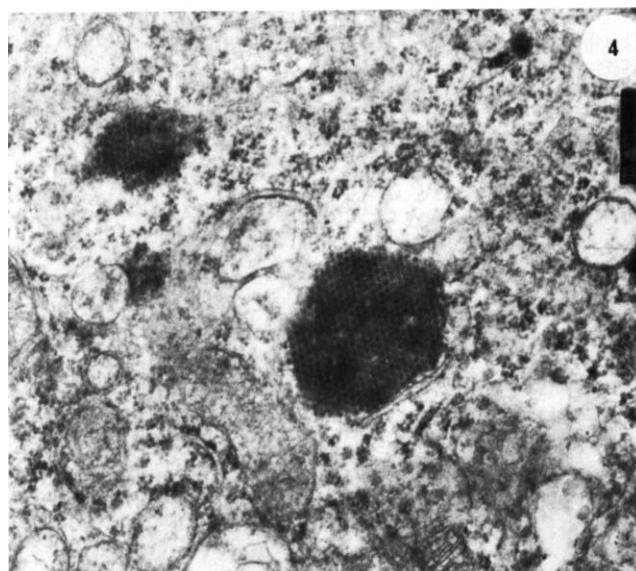


Figure 4. Electronmicrograph of an enterocyte in the small intestine. Several paracrystalline arrays of virus-like particles (arrows) are present in the cytoplasm. Bar = 0.5µm.

Experimental Transmission

Attempts to experimentally infect SPF chickens, *Agapornis* sp, normal galahs and galahs with PBFV failed. Virus was not detected in faeces of any bird.

Serology

Sera from naturally affected galahs and experimentally infected SPF chickens and *Agapornis* sp did not contain detectable antibodies against human, canine, feline, bovine or chicken Caliciviruses or Human Astroviruses 1, 2 and 4.

Discussion

The presence of virus-like particles in faeces and in the cytoplasm of enterocytes of the small intestine strongly suggest that the birds described in this report were suffering from an enteric viral infection. This is supported by the type of lesion present in the small intestine. Villous atrophy and fusion, and crypt cell hyperplasia indicate the enterocytes on the villi have been destroyed, a process typical of some viral infections, particularly coronaviruses and rotaviruses (Cheville, 1983). Although it remains unproven, we believe that the virus-like particles seen in faeces are the most probable cause of the disease.

The morphology of the virus-like particles in faeces and enterocytes closely resemble those described in an entero-like virus infection in chickens (McNulty *et al* 1985).

The association of this disease with PBFV suggests that it occurs in birds with reduced immunological competence. PBFV is due to a virus infection (Wylie and Pass 1987) and our observations on the presence of PBFV virus in the bursa of Fabricius and thymus together with the fact that affected cockatoos often develop acute bacterial infections, cryptosporidial infection of the lower gut and myeloproliferative diseases suggests that some of these birds do suffer some form of immunodepression.

Our initial experience with the disease indicated that it is very infectious as it rapidly spread to captive birds with PBFV after the first cases were introduced to the bird room. We were unable, however, to experimentally infect birds under similar conditions at a later date. The reasons for this are not known. It is possible that the birds used were not susceptible to infection because of past exposure to the virus or that the inoculum used was non-infectious even though virus particles were plentiful. Storage of faeces at -20°C, however, may have reduced infectivity of the virus.

References

- Chevill NF (1983) — *Cell pathology*, 2nd ed, Iowa State Uni Press, Ames, Iowa
Frazier JA, Farmer H and Martland MF (1986) — *Vet Rec* 118: 303
Goodwin MA, Nersessian BJ and Fletcher OJ (1985) — *J Am Med Assoc* 187: 309
Gough RE and Spackman D (1981) — *Vet Rec* 109: 497
Gough RE, Wood GW, Collins MS, Spackman D, Kemp J and Gibson LAC (1985) — *Vet Rec* 116: 295
Kisary J (1985) — *Avian Pathol* 14: 1
McNulty MS, Allan GM and McCracken RM (1983) — *Avian Pathol* 12: 45
McNulty MS, Allan GM, Connor TJ, McFerran JB and McCracken RM (1984) — *Avian Pathol* 13: 429

- Meulemans G, Peeters JE and Halen P (1985) — *Brit Vet J* 141: 69
Pomeroy BS (1984) — *Diseases of Poultry*, 8th ed, edited by MS Hofstad, Iowa State Uni Press, Ames
Reynolds DL and Saif YM (1986) — *Avian Dis* 30: 728
Reynolds DL, Theil KW and Saif YM (1987) *Av Dis* 31: 376
Saif LJ, Saif YM and Theil KW (1985) *Av Dis* 29: 798
Spackman D, Gough RE, Collins MS and Lanning D (1984) — *Vet Rec* 114: 216
Trampal DW, Kinden DA, Solorzano RF and Stogsdill PL (1983) - *Av Dis* 27: 49
Wyeth PJ, Chettle NF and Labram J (1981) - *Vet Rec* 109: 477
Yason CV and Schat KA (1986) — *Av Pathol* 15: 421

(Accepted for publication 28 March 1989)

Haematological and biochemical reference values for the koala (*Phascolarctos cinereus*)

PM CANFIELD*, ME O'NEILL† and EF SMITH‡

Department of Veterinary Pathology* and School of Crop Sciences‡, University of Sydney, NSW 2006

SUMMARY: Haematological and biochemical reference values were established from 45 clinically healthy koalas. Statistical analysis revealed no significant differences for sex and season of sampling. Immature koalas had significantly higher alkaline phosphatase and inorganic phosphate values, and significantly lower total protein concentrations due to low globulins values. Enzyme reference values tended to be wide and could limit their usefulness in detecting disease. In the reference values for leukocytes, neutrophils and lymphocytes, the inclusion of low values which were not actually seen may interfere with the detection of reduced levels due to disease.

Aust Vet J 66: 324-326

Introduction

Haematological and biochemical analyses provide a useful adjunct to the diagnosis of disease in domestic animals. It has been assumed that a similar situation applies to wildlife, especially for those animals kept in zoos or sanctuaries. However, before the usefulness of these analyses for the diagnosis of disease can be assessed, reference (normal) values have to be established. Ideally this should be done for each population with a different habitat, but in practice values are extrapolated from one group to another. In the koala, apart from unpublished data available from zoos, limited reference values are available. Two studies have been published for various wild groups from Victoria (Obendorf 1983) and New South Wales (NSW) (Dickens 1975). In this article reference values are established for a population of free living koalas centred around Port Macquarie on the north coast of NSW. This population is under threat because of urbanisation and a high level of natural occurring disease (Canfield 1987). Consequently the Koala Preservation Society of NSW and local veterinarians are utilising haematological and biochemical analyses for diagnosis and prognosis.

Materials and Methods

Animals

Forty-five apparently healthy koalas were used. These animals were captured at various times during the year for the purposes of relocation. They comprised 16 adult females, 13 adult males, 15 juvenile males and one juvenile female. For the purposes of this study adults were defined as greater than 2 to 3 years of age (based on tagging records and dentition). Juveniles were predominantly 1 to 2 years of age.

Haematological and Biochemical Testing

Koalas were physically restrained in a hessian bag and bled either at the time of capture or prior to release. The period of capture was usually less than 12 h. Venous blood was

collected from the forearm and placed in a plain serum tube and a tube containing sequestrene (EDTA). Serum was separated and a peripheral blood film prepared within one hour of collection. The samples were then transported at 4°C to a laboratory, where they were processed within 12 h of collection. Biochemical testing was done using a Hitachi 737*, a discrete, selective sample oriented analyser. Boehringer Mannheim reagents* were used for all tests except for calcium† and bicarbonate‡. Sodium, potassium and chloride analyses involved ion specific electrodes, albumin analysis utilised bromocresol green while total protein analysis involved the Biuret method. Globulins were derived from the difference between total protein and albumin. Enzyme analyses were all performed at 37°C. Anionic gap was determined by the following formula: (sodium + potassium) — (chloride + bicarbonate). Osmolality was estimated from $2 \times (\text{sodium} + \text{potassium})$. Haematological analysis (haematocrit, haemoglobin, erythrocytes, leukocytes and platelets) was performed on a Coulter S 880‡. A peripheral blood film was stained with Diff Quik§ and manually assessed for morphology and leukocyte types.

Statistical Analysis

Values were analysed using Minitab¶ and Genstat# statistical packages on an IBM personal computer. Parametric statistics were applied after assessment of normality by probability plots (N scores — Ryan *et al* 1985). In some instances square root transformations were required while in 2 (monocytes and eosinophils) no transformations could produce a normal distribution due to their limited range. Significant differences between ages, sexes and seasons were determined using anal-

* Boehringer, Mannheim Aust Pty Ltd, North Ryde, NSW

† Trace Scientific Pty Ltd, Clayton, Victoria

‡ Coulter Electronics Pty Ltd, Brookvale, NSW

§ Lab Aids, Narabeen, NSW

¶ Minitab Inc, Philadelphia, USA

Rothamsted Experimental Station, UK