The Prevalence of Psittacine Circovirus in Native and Exotic Parrots in New Zealand

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Abstract

Psittacine circovirus (PCV) has been identified in more than 60 psittacine species worldwide in both aviary and wild populations. The virus is a causative agent of psittacine circoviral disease (PCD), a highly infectious disease characterised by beak and feather dystrophy, high juvenile mortality or long-term immunological suppression. The virus is known to be very difficult to control or eradicate and among wild Australian parrots, the prevalence of infection is 10-20 %. No information on the incidence of PCV in parrots in New Zealand was available. The aims of this study were 1) to determine the prevalence of PCV in wild exotic parrots, 2) to determine the prevalence of PCV in wild native parrots, 3) to identify the incidence of PCV in captive native parrots, and 4) to suggest recommendations for the future conservation management of native parrots populations in New Zealand. Two species of exotic parrots; eastern rosella and sulphur-crested cockatoo, and four species of native parrots; kakapo, kaka, kea and parakeet were examined. Feathers of these parrots were collected from different regions in New Zealand and PCR assay was conducted to identify the presence of PCV.

The prevalence of PCV in wild exotic parrots in New Zealand was considerably high in both species of exotic parrots, as the prevalence of PCV at the 95% confidence intervals ranged from 19.17 - 44.02% in eastern rosellas and 22.04 - 33.07% in sulphur-crested cockatoos. No wild native parrots showed any evidence of PCV in PCR assay and given the sample sizes in this study, the prevalence of PCV was estimated as less than 4-7% if PCV is present in the populations. However, the first isolation of PCV in

native parrots occurred in two species of parakeets in captivity; red-crowned parakeets and Antipodes Island parakeets. No significant abnormalities were detectable in the red-crowned parakeets but the Antipodes Island parakeet died shortly after translocation. The presence of PCV was confirmed in contact birds in both cases.

Recommendations

I recommend the following future avenues of investigation, from the results of this study.

1. Attempts to determine the population size, distribution and ecology of exotic parrots in the wild are recommended. This will allow us to identify the risk of native parrots from contact with exotic parrots.
2. Further sampling of both wild and captive parrots to identify the prevalence and seroprevalence of PCV is required.
3. Intensive monitoring programmes should be undertaken for PCV positive parakeets to identify the impact of PCD in native parrots.
4. Experimental infection of native parrots will provide crucial information not only on the susceptibility, sensitivity, and immunity of native parrots to PCV but also on the ecology of PCV in those species.

The following recommendations for the management of native parrots can be concluded from this study. Prior to translocation or reintroduction of native parrots, the presence of exotic parrots, the prevalence and seroprevalence of PCV should be investigated. Psittacine circovirus should be included in health check and quarantine protocols in the management of native parrots in the wild and in captivity. Nature reserves and captive facilities should be aware of the presence of exotic parrots in the environment and the prevalence and seroprevalence of PCV through regular sampling efforts. The identification of cause of death of parrots that may happen around nature reserves or captive facilities is also essential to identify the presence of PCV.

Additionally, strict hygiene protocols, isolation of native parrots from exotic parrots, and disease screening for PCD in the event of importation of new parrots should be applied in captivity. Vaccination should be investigated as a preventative measure.

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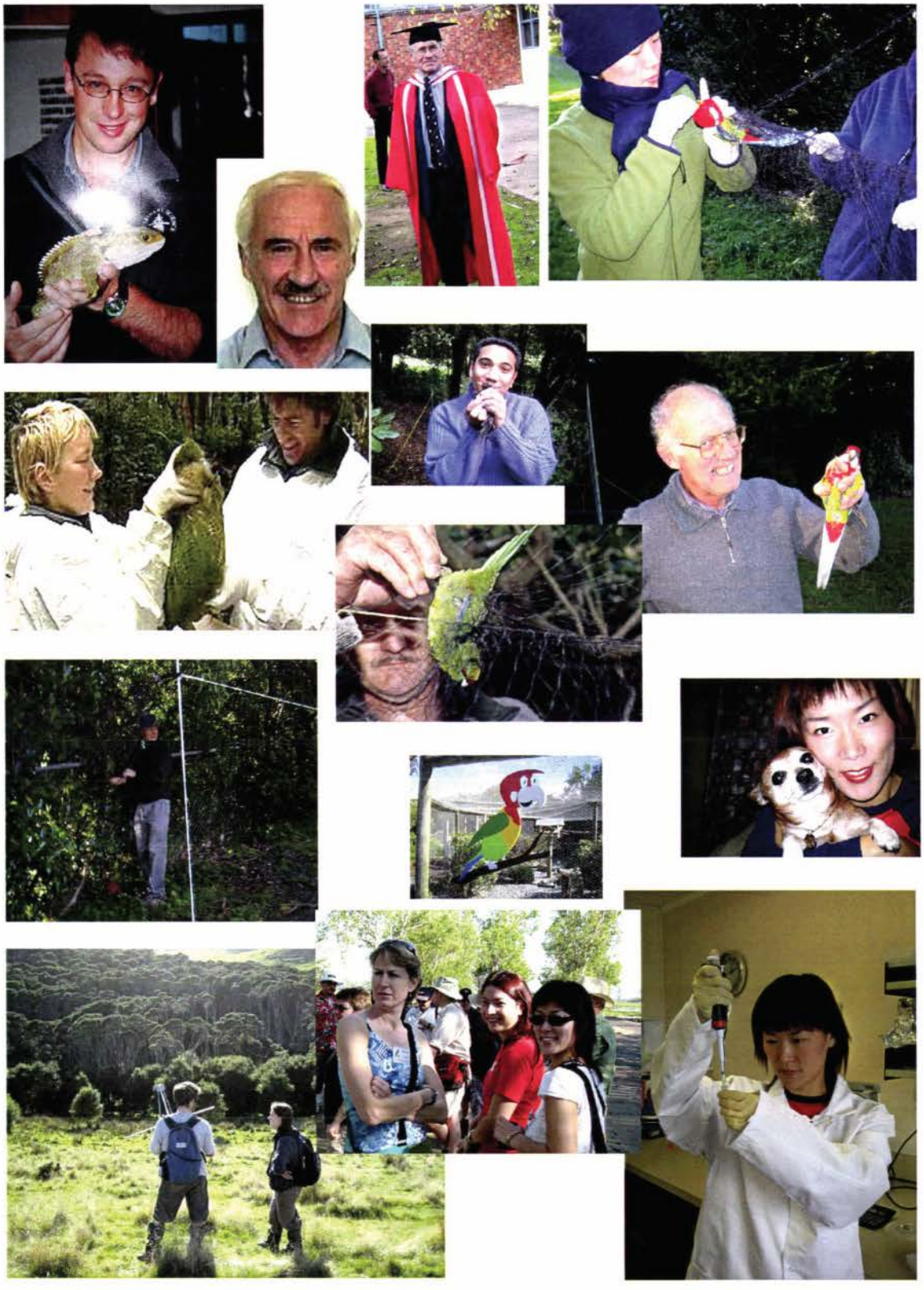

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Chapter 1: General Introduction

A sulphur-crested cockatoo suffering from psittacine circoviral disease

(Photo courtesy of S. Raidal)

Introduction

Psittacine circovirus (PCV) has been widely reported in wild and captive psittacine birds throughout the world. More than 60 free-ranging and captive species of parrots have been found to be susceptible to circoviral infection (Bassami et al 2001; Ritchie et al 2003; Todd 2004). It causes a highly infectious disease characterised by high juvenile mortality, long-term immunological suppression, or feather dystrophy and beak necrosis. It was thought that this virus only affected birds in the psittacine family. However, recent records suggest that the virus may affect a non-psittacine species, Senegal wild doves (*Streptopelia senegalensis*) (Pass et al 1994; Schultz et al 1996; Raidal and Riddoch 1997).

The first record of psittacine circoviral disease (PCD) was the description of clinical cases in sulphur-crested cockatoos (*Cacatua galerita*) in Australia in the early 1970s (Perry 1981 cited in Ritchie and Carter 1995). Among cockatoos, PCD shows as typical feather dystrophy and beak necrosis as the old name of this disease 'psittacine beak and feather disease' indicates. In Australia, it is the most common viral disease among wild psittacine birds (Pass and Perry 1984; McOrist et al 1984; Raidal et al 1993; Bassami et al 2001, Ritchie et al 2003). In addition, it is a worldwide problem in captive psittacine birds (Rahaus and Wolff 2003 and references therein).

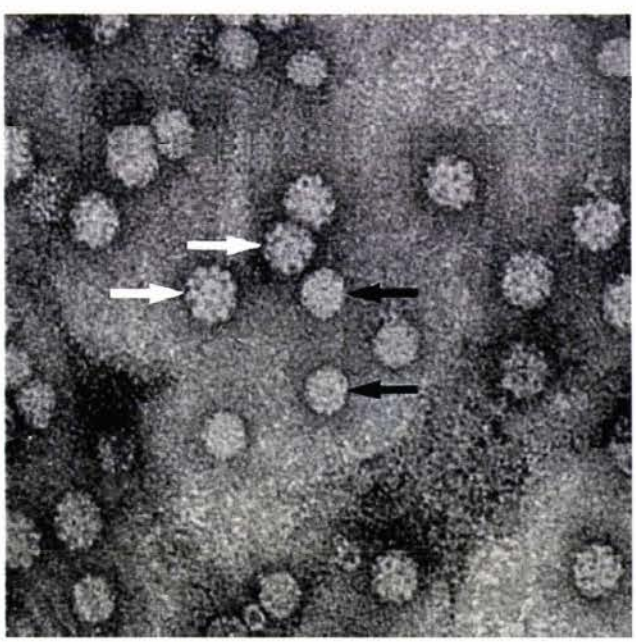
In New Zealand, PCV infection is known to exist among cage and aviary birds (Ritchie et al 2003). Recently, it has been found in wild eastern rosellas (*Platycercus eximius*) and sulphur-crested cockatoos trapped for export in the North Island of New

Zealand (Mander et al 2003). Through contact with these exotic parrots, New Zealand's endemic parrots are at risk of contact with this virus. The prevalence of PCV in wild native and exotic parrots in New Zealand is unknown.

Psittacine Circovirus (Psittacine Beak and Feather Disease)

Psittacine circovirus

Psittacine circovirus is icosahedral, non-enveloped, 14-26nm in diameter, 1.7-2.3kb in size and contains a single-stranded circular DNA molecule (Ritchie and Carter 1995; Todd 2000; Ritchie et al 2003; Ball et al 2004). It is a member of virus family *Circoviridae* which includes porcine circovirus (PCV1 and PCV2), and chicken anaemia virus (CAV) and pigeon circovirus (PiCV) as a tentative member (Todd 2004).



PCV shares the same genus *Circovirus* with porcine circovirus, while CAV is categorized as *Gyrovirus* (Todd 2000; Todd 2004). It is known as the smallest animal virus (Ritchie et al 2003) being 20% smaller than CAV when examined by electron microscopy (Todd 2000).

Figure 1-1. Micrograph of a negatively stained preparation of a mixture of CAV (white arrow) and PCV (Black arrow) (P. Ritchie 2003).

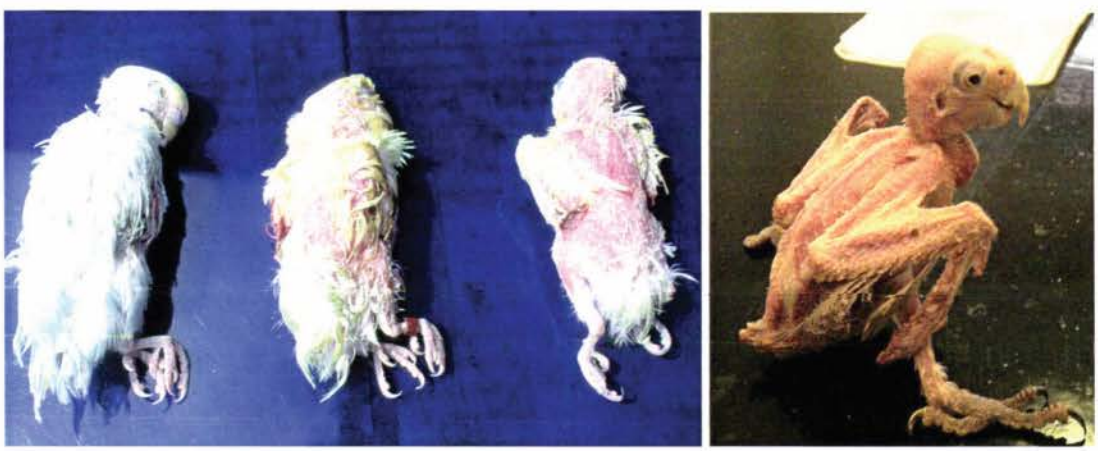
The virus is shed in the feather dust, crop secretions and faeces of infected birds and transmission occurs through direct contact (Gerlach 1994 cited in Ritchie et al 2003). Oral transmission is the most common form of transmission (Todd 2004). Vertical transmission occurs which is a cause of embryonic death (Todd 2004). The virus remains viable in the environment for up to 2-3 years (McWhirter 2000 cited in Mander et al 2003) and is resistant to many control measures (Ritchie et al 2003). Thus, inactivation or eradication of the virus is not likely to be an effective option in terms of disease control (Todd 2004). Recent research has demonstrated the possibility of vaccination as a method of disease prevention using inactivated virus (Raidal and Cross 1994; Todd 2000; Todd 2004).

Until recently, PCV has been described in wild parrot populations only in Australia and Indonesia (Mander et al 2003). The virus appears to have developed in Australia with subsequent global spread through trade of birds for aviculture (Mander et al 2003). It is assumed that this virus has been introduced into New Zealand through the importation of Australian parrots as pets or for commercial breeding (Ritchie et al 2003).

General clinical signs of psittacine circoviral infection

Psittacine circoviral disease is more common in young birds less than 3 years old (Todd 2000; Todd 2004). Clinical signs of PCD can be divided into three different forms such as per-acute, acute and chronic forms (Ritchie and Carter 1995).

Neonatal psittacine birds often show a per-acute form of PCD which causes rapid weight loss, enteritis, septicaemia, pneumonia and death (Pass and Perry 1984; Ritchie and Carter 1995; Ritchie et al 2003). Particularly, young cockatoos and African grey parrots appear to be more susceptible to the per-acute form (Schoemaker et al 2000). Also, this form of PCD is suspected to cause embryonic deaths (Perry 1981 cited in Ritchie and Carter 1995). In many cases, birds may die before feather abnormalities are noticed. Since birds that have died of the per-acute form of PCV commonly show no feather abnormalities, it is difficult to detect the disease without histology, serology or PCR testing (Ritchie and Carter 1995).



(a) (b)

Figure 1-2. PCD in neonatal psittacine birds:
Budgerigars (a: photo courtesy of Bob Schmidt)
And a galah (b: photo courtesy of Lubica Necasova).

The acute form of PCD usually occurs in young birds during the formation of the first feathers (Perry 1983 cited in Ritchie and Carter 1995). The first signs include depression for several days (Ritchie and Carter 1995 and references therein). Then feather abnormalities such as necrosis, fracture, bending, haemorrhage or premature

shedding of feathers may occur, but the gross feather lesions can be subtle and difficult to recognise (Ritchie and Carter 1995 and references therein). The clinical signs may also include anaemia (in African grey parrots), crop stasis, diarrhoea and eventual death (Pass and Perry 1984; Pass and Perry 1985; Ritchie and Carter 1995). Various kinds of parrots including cockatoos, galahs, lovebirds and African grey parrots have been found to develop acute form of PCD (Pass and Perry 1985; Ritchie and Carter 1995 and references therein).

Chronic PCD usually occurs in birds that have survived from the acute form of this disease (Ritchie and Carter 1995). It is the most common form and the clinical signs include feather loss, abnormal feathering such as retained feather sheaths, short clubbed feathers and deformed feathers (Todd 2000). As a result, baldness can occur as well as deformities of beak and claws (Todd 2000). Infected birds can live for years, but more often they are likely to die of secondary bacterial, chlamydial or fungal infection because of immunosuppression (Todd 2000).

(a) (b)

Figure 1-3. A budgerigar (a) and a sulphur crested cockatoo (b) showing typical clinical signs of PCD such as feather abnormalities and beak necrosis (photo courtesy of S. Raidal).

Older birds (three years to twenty years old) show much less susceptibility to PCV and PCD than younger ones (Ritchie et al 1989 cited in Ritchie and Carter 1995). In an experimental trial to provoke PCD in older birds, the birds have been found to produce antibodies against PCV or develop a transient viraemia instead of showing any signs of PCD (Raidal et al 1993; Ritchie and Carter 1995). Many clinically normal birds, therefore, have been diagnosed with PCD which indicates the evidence of virus carriers (Ritchie et al 1989 cited in Ritchie and Carter 1995). These infected but clinically normal birds have a great potential to spread the virus widely in the environment.

Species variability of clinical signs of PCD

In addition to the difference in the susceptibility to PCD in different age groups, the clinical signs of PCD vary between species (Doneley 2003). Cockatoos are known to be the most susceptible to PCD as they show typical chronic forms of PCD such as beak and feather abnormalities (Doneley 2003 and references therein). Sulphur-crested cockatoos showed more severe signs of disease by PCV than galahs as they died after experimental infection by PCV while galahs only presented typical feather lesions (Raidal et al 1993; Ritchie and Carter 1995). Also, sulphur-crested cockatoos commonly show feather abnormalities as well as beak necrosis (Pass and Perry 1984; Pass and Perry 1985; Ritchie and Carter 1995).

Other species can present with chronic forms that are different from that seen in cockatoos. For instance, the princess parrot (*Polytelis alexandrae*) and the king parrot (*Alisterus scapularis*) show untidy plumage with colour changes but no beak changes (Doneley 2003). Lovebirds (*Agapornis* spp.) also present feather abnormalities similar to those in sulphur-crested cockatoos but less beak necrosis (Pass and Perry 1985; Ritchie and Carter 1995). In lorikeet species (*Trichoglossus* spp.), there might be a temporary loss of feathers in wings and tail (Doneley 2003 and references therein). Many South American parrots are thought to be less susceptible to PCV (Fudge AM, personal communication in Doneley 2003). African grey parrots often show abnormal colouration of feathers (Ritchie and Carter 1995 and references therein). Rosellas commonly show subtle colour changes instead of feather or beak deformities (Gartrell BD, personal communication, figure 1-4 and 1-5).

Figure 1-4. A normal crimson rosella (Anonymous 1985).

Figure 1-5. The colour and feather changes in PCV infected crimson rosellas (Photo courtesy of B. Gartrell).

Differences in the clinical and pathological manifestations of PCD between psittacine bird species that are known to be susceptible have been thought to be due to host factors rather than antigenic or genetic variation in PCV (Bassami et al 2001; Rahaus and Wolff 2003; Ritchie et al 2003). Different isolates in PCV perform

antigenically similarly in haemagglutination (HA) and haemagglutination inhibition (HI) assays (Rahaus and Wolff 2003; Ritchie et al 2003).

Ypelaar et al (1999) demonstrated that there was no strain variation in PCV isolates from different genera of psittacine birds based on ultrastructural characteristics, protein composition and antigenic comparisons (Ritchie et al 1990 cited in Ypelaar et al 1999). Viruses attained from infected birds were found to develop PCD or stimulate immunologic response in different psittacine species, reinforcing the theory that antigenically related virus causes PCD in various Psittaciformes (Raidal et al 1993; Ritchie and Carter 1995).

However, Ritchie et al (2003) investigated the relationship between viral genotypes and their hosts. Three lineages were found which were related to a group of psittacine species. These are the cockatoo lineage, budgerigar lineage and lorikeet lineage (Ritchie et al 2003). This supports the result of Bassami et al's (2001) research that there is a close relationship between viral genotypes and their hosts. While Bassami et al (2001) demonstrated the relationship between viral genotypes and regions, e.g. PCV isolates from Australia and PCV isolates from America, there was no detectable difference between Australian and New Zealand isolates (Ritchie et al 2003).

Immunity

Parrots that recover from natural infections or vaccination of birds have antibodies to PCV that can be demonstrated by serological tests (Ritchie and Carter 1995; Rahaus and Wolff 2003). The existence of virus-carrying subpopulations of birds with no symptoms has been demonstrated (Rahaus and Wolff 2003). The birds could be in the early stage of viral development such as the viral incubation period, develop a subclinical infection or carry virus chronically (Ritchie et al 1991; Ritchie and Carter 1995; Rahaus and Wolff 2003). In the latter cases, the birds have the potential to be virus carriers and transmitters. This fact indicates the importance of appropriate monitoring and quarantine programs (Rahaus and Wolff 2003).

The presence of antibodies does not always match with clinical signs. Detecting antibodies is considered the most sensitive diagnostic test for previous exposure but not for disease. For example, in a survey of two wild flocks of sulphur-crested cockatoos in New South Wales, 73% and 94% of the birds sampled were sero-positive but clinically normal (Raidal et al 1993). 4.4% and 6% of birds in the respective flocks showed clinical signs of PCD with no detectable serum HI activity (Raidal et al 1993). Ritchie et al (1991 in Ritchie and Carter 1995) determined that birds with active PCD showed low or undetectable levels of antibodies. Clinically normal birds showed high levels of antibodies, suggesting a level of immunity (Ritchie et al 1991; Raidal et al 1993; Ritchie and Carter 1995). However, the mechanisms that allow immunity to develop are not fully understood.

Diagnostic Methods of PCV

Diagnosis of PCV

Suspicion of PCV infection can be made by clinical examination if there are detectable abnormalities of beak and feathers (Riddoch et al 1996 and references therein). In some psittacine species such as cockatoos, this can be a useful method to diagnose, but may be confused with other feather diseases. However, in some psittacine species which show less severe beak and feather lesions and the early stage of PCV development or per-acute infection of PCV, there are apparent limitations to detecting PCV through clinical examination (Riddoch et al 1996). For instance, in eastern rosella when they show no or subtle beak and feather lesions, the clinical examination would be meaningless. Thus other diagnostic tests should be carried out. For example, to rule out polyomavirus infection which causes similar feather disease to PCV in neonatal birds, diagnostic tests are required (Pass 1985 cited in Riddoch et al 1996).

There are several laboratory tests considered useful to detect viral infections such as isolation or propagation of PCV in cell culture, histopathology, dot blot hybridisation (DBH), polymerase chain reaction (PCR) assay, haemagglutination (HA) and haemagglutination inhibition (HI) test. However, to date there is no successful record of isolation and propagation of PCV in cell culture (Ball et al 2004; Todd 2004). Thus, histological diagnosis, dot blot hybridisation, polymerase chain reaction, haemagglutination and haemagglutination inhibition are the diagnostic tests that are useful methods for the diagnosis of PCV (Ritchie et al 1991; Raidal et al 1993; Raidal

and Cross 1994; Riddoch et al 1996). For the purpose of this study, only histopathology and PCR assay will be discussed.

Histological diagnosis

Histological diagnosis of PCV is based on the detection of basophilic viral inclusion bodies, commonly existing in macrophages or within epithelial layer of feather and feather follicles (Doneley 2003; Todd 2001). Lymphoid necrosis and atrophy have been found in the thymus and bursa of Fabricius of infected birds (Pass and Perry 1984; Todd 2004). The reduction of T and B lymphocytes commonly occurs which explains immunological suppression of affected birds (Todd 2004). Figure 1-6 present the changes in feather section in a white cockatoo resulted from the infection by PCV (Werther et al 1998). There is a necrosis in basal epithelial cells and nuclear inclusion bodies (black arrow) are found in the epithelial cells and also globular, basophilic, cytoplasmic inclusions (red arrow) present in macrophages (Werther et al 1998).

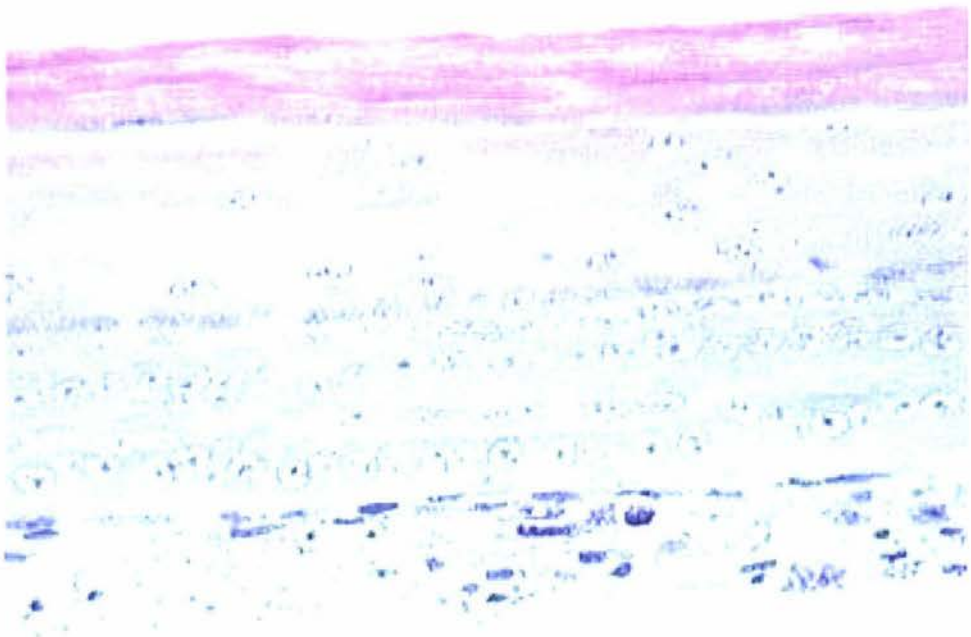


Figure 1-6. Feather section in a white cockatoo; H&E stain (Werther et al 1998).

However, the histological diagnosis requires biopsy or post mortem and it is also time consuming. The detection of viral inclusion bodies is not reliable which may result in a false negative diagnosis. The presence of inclusion bodies can be found in adenovirus and polyomavirus infection, which are very similar to those caused by PCV (Pass 1987 cited in Ritchie and Carter 1995). The histological examination is known to be less sensitive (72.4%) and specific (93.8%) than other diagnostic methods (Latimer et al 1992 cited in Ritchie and Carter 1995).

PCR assay

The PCR assay is a procedure to detect virus DNA (Ball et al 2004). By taking DNA isolated from feathers or blood as template for a PCR, PCV infection can be diagnosed (Rahaus and Wolff 2003). The PCR assay most probably detects viral DNA

present as free particles attached to the pulp and sheath of affected feathers or within intracytoplasmic inclusions of keratinocytes (Ypelaar et al 1999; Rahaus and Wolff 2003). Even though there is a possibility of false-negative or false-positive in detecting PCV using PCR assay, there are some advantages.

Feather, cloacal swabs and blood samples can be used in PCR assay (Hess et al 2004). In particular, the protocol used for the isolation of DNA from feather samples in non-invasive and rapid (Ypelaar et al 1999; Rahaus and Wolff 2003). According to Ritchie et al (2003), there is no significant difference in the results from blood samples and feather samples. Hess et al (2004) demonstrated that the feather and cloacal samples presented positive results while the blood samples prove negative ones. The point supports the hypothesis that subclinically infected birds or carrier birds may shed viruses through feather dusts or faeces to the environment (Hess et al 2004).

Handling and transportation of samples is relatively easier in PCR assays than HI or HA tests which require the secure transport of fresh blood or frozen serum samples (Riddoch et al 1996). Ball et al (2004) concluded that the sensitivity of PCR assays may be reasonably higher than other diagnostic tests as the test detects 0.10 fg amounts of virus DNA (c.f. 40 pg in DBH). The sensitivity and the specificity of DNA testing were demonstrated as 99.7% and 100% in Latimer et al (in Ritchie and Carter 1995). Although PCR assay is a different method from DNA probe testing, the sensitivity and specificity of PCR assay currently available in New Zealand appear to be as high as DNA probe testing (personal communication, Ian Anderson).

Distribution of Parrots in the wild in New Zealand

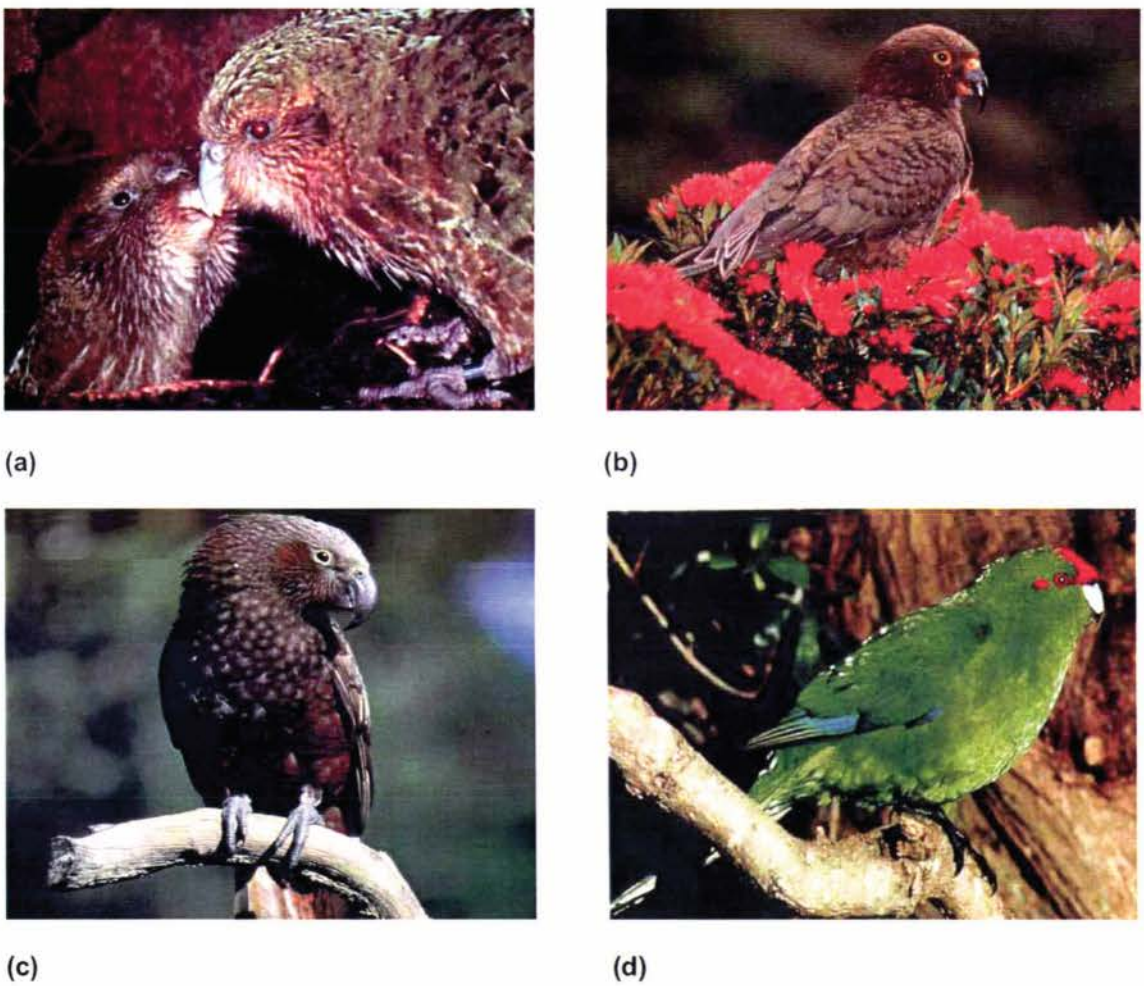
Native parrots in New Zealand

New Zealand's avifauna is unique. There are endemic native parrots in New Zealand which can be differentiated from all other parrots in appearance and characteristics. For instance, the kakapo (*Strigops habroptilus*) is a flightless parrot, endemic to New Zealand. It is a large (up to 4kg), nocturnal, herbivorous, lek-breeder which breeds only once every 2-5 years (Elliott et al 2001). Currently, only 83 kakapo exist in the world (Merton 2004). Records suggest that native parrots were once abundant prior to human settlement, however, none of native parrots is considered to be self-sustainable in the wild due to slaughtering as food source, habitat destruction, persecution as crop pests and the impact of introduced predators (Anonymous 1985).

Other native New Zealand parrots include kaka (*Nestor meridionalis* spp.), kea (*Nestor notabilis*) and parakeet/kakariki (*Cyanoramphus* spp.) (Chambers 2000). There is one species of kea and they only exist in the South Island of New Zealand. There are two sub-species of kaka known as the North Island kaka (*Nestor meridionalis septentrionalis)* and the South Island kaka (*Nestor meridionalis meridionalis).*

The New Zealand parakeets include four species: the Antipodes Island or unicolor parakeet (*Cyanoramphus unicolor*); red crowned parakeet (*Cyanoramphus novaezelandiae*); yellow crowned parakeet (*Cyanoramphus auriceps*); and orange fronted parakeet (*Cyanoramphus malherbi*) (Anonymous 1985). Additionally, there are

four sub-species of red-crowned parakeet in New Zealand: the New Zealand red-crowned parakeet (*Cyanoramphus novaezelandiae novaezelandiae*), the Kermadec parakeet (*C. n. cyanurus*), the Chatham Island red-crowned parakeet (*C. n. chathamensis*) and Reischek's parakeet (C. *n. hochstetteri*) (Anonymous 1985). Kearvell et al (2003) suggested that the orange fronted parakeet is a distinct species from other *Cyanoramphus* spp. based on mitochondrial DNA, assortative pairing, bill morphology, vocalization and comparative ecology.



(a) (b)

(c) (d)

Figure 1-7. Native parrots in the wild in New Zealand
(a: kakapo, b: kea, c: kaka and d: red-crowned parakeet;
J. Warne 2004).

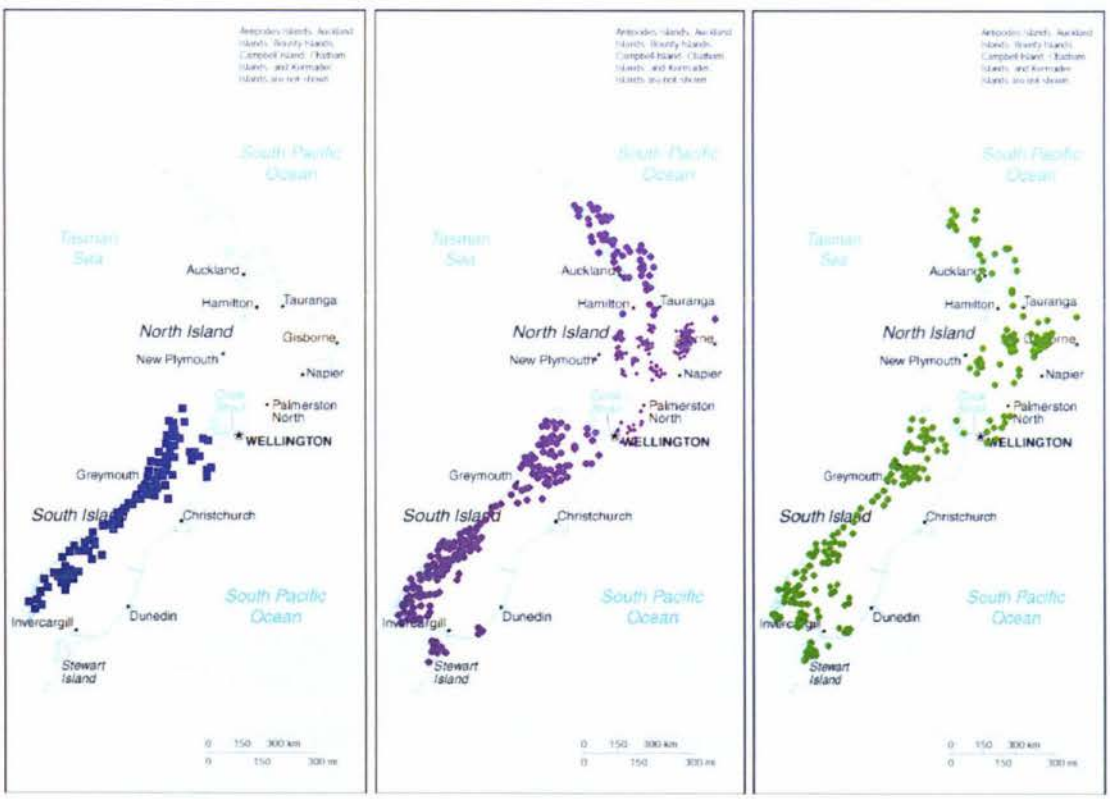
The distribution of native parrots in the wild in New Zealand

Kakapo are being intensively managed by a Department of Conservation species recovery program. The population is restricted to several offshore islands: Codfish Island/Whenua Hou; Chalky Island/Te Kakahu; and Pearl Island (Merton 2004). Largely due to the recovery programme, the population rose from 62 in 2001 to 86 in 2002 (Warne J in Parrots in New Zealand website 2004). The number of current population is 83 as a result of the loss of three kakapo due to infection with *Erysipelothrix rhusiopathiae* (Merton 2004).

Kaka is a relatively common species among native parrots in New Zealand. Good populations of kaka exist in the centre of North Island and many off shore islands including Kapiti Island and Little Barrier Island (Chambers 1989; Chambers 2000). Recently, there have been releases of kaka from captivity to the wild at Mt. Bruce wildlife sanctuary. At Karori sanctuary, the kaka population are being managed by means of supplementary feeding and releases to the wild. South Island kaka are well spread through South Island and Stewart Island (Chambers 2000). The total population has been estimated at 10,000 (Jackson et al 2000).

Wild kea populations can be found only in the South Island, especially in alpine areas (Chamber 1989). The range includes Marlborough-Lake Rotoiti, West Coast-Franz Josef Glacier, Canterbury-Arthurs Pass and Fiordland-Eglinton Valley (Chambers 2000). The estimated number of total population was less than 5,000 (Jackson et al 2000).

Among parakeet species, red crowned parakeet and yellow crowned parakeet are relatively common but there are more yellow crowned then red crowned in the wild. The distribution of red crowned parakeet and yellow crowned parakeet is overlapping in many areas. Red crowned parakeets have been occasionally found on mainland islands in New Zealand, and are abundant on off-shore islands (Chambers 2000). They are found in the forests of the central North Island, North Auckland and Northland and it is assumed that they might have arrived there from off-shore islands (Chambers 2000). Yellow crowned parakeet can also be found in the northern North Island and central North Island and are well spread through the forests of the South Island (Chambers 2000).



(a) (b) (c)

Figure 1-8. The distribution of native parrots in the wild in New Zealand (a: kea, b: kaka and c: parakeet, yellow crowned & red-crowned, Bull et al 1985; Chambers 1989; Chambers 2000).

Exotic parrots in New Zealand

The first recorded introduction of captive exotic parrots to New Zealand was the importation of budgerigar (*Melopsittacus undulates*) from Australia in 1928 (Parrots in New Zealand website, 2004). In the next decade, many other Australian, several African and some Asian parrot species were introduced to captivity in New Zealand (Parrots in New Zealand website, 2004). The numbers of parrots from South America and Indonesia was very small (Parrots in New Zealand website, 2004). Among them, the vast majority of the Australian parrots thrived and they are bred in relatively large numbers given the size of the avicultural base in New Zealand (Parrots in New Zealand website, 2004).

Exotic parrots in the wild have established as either escaped or intentionally released captive birds. It is likely the escape or release of exotic parrots had occurred prior to the importation of captive budgerigar, according to a record that the eastern rosellas had established the population in the wild around Dunedin since 1910s (Woon et al 2002). The status of exotic parrots in New Zealand varies in different regions. They are regarded as pests in terms of crop feeding and aggressive behaviour towards native species. The eradication of rainbow lorikeet which was carried out by Auckland regional council and Department of Conservation from 1999 is a good example of their insecure status in New Zealand. However, there are members of the public who believe their release would add "a bit of colour" to the wild (Polkanov and Greene 2000).

Exotic parrot species that can be found as established populations in the wild in

New Zealand include sulphur-crested cockatoos, galahs (*Cacatua roseicapilla*), eastern rosellas and crimson rosellas (*Platycerus elegans*). While eastern rosellas have established a large population in the wild in New Zealand, other species only exist in small numbers; less than 1,000 (sulphur-crested cockatoo), less than 20 (crimson rosella) and less than 100 (galah) (Jackson et al 2000). The Biosecurity Act which was introduced in 1997 by Ministry of Agriculture and Forestry has prohibited the new imports of breeding psittacine species (Ritchie et al 2003). Despite this law, it is another challenge to control the illegal pet trade to prevent the importation of new pathogens.

(a: Kolar and Spitzer 1990)

(b: Kolar and Spitzer 1990)

(c: H. Nicholson 2004)

(d)

Figure 1-9. Exotic parrots in the wild in New Zealand
(a: eastern rosella, b: crimson rosella, c: sulphur-crested cockatoo and d: galah).

The distribution of exotic parrots in New Zealand

Eastern rosellas are widely spread through New Zealand (Chambers 1989; Chambers 2000). The northern North Island population is spreading to the south and the Wellington population is expanding northwards (Chambers 1989; Chambers 2000). In the South Island, the population is small and found around Dunedin (Chambers 1989; Chambers 2000).

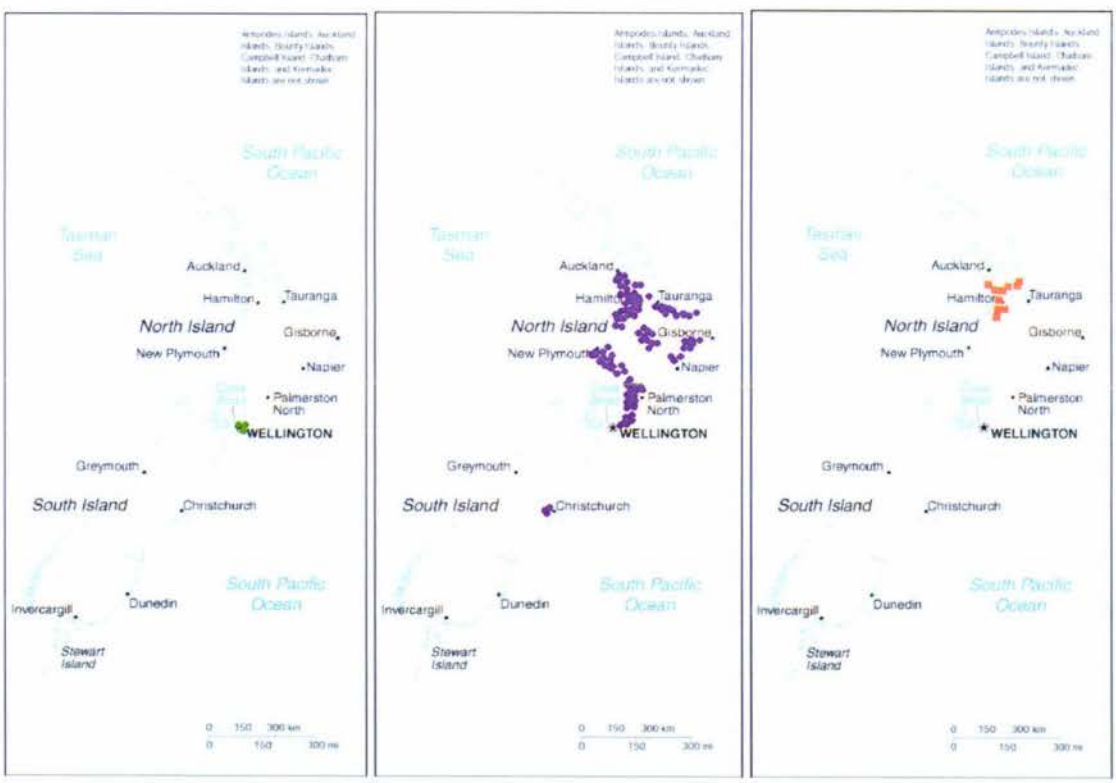
Figure 1-10. The distribution of eastern rosella in the wild in New Zealand (Bull et al 1985; Chambers 1989; Chambers 2000).

There are some reports of the presence of crimson rosella from Waiwera and South Manukau Harbour and Whangaparaoa in late 1990s (Chambers 2000). Currently, the bird occurs around the Wellington city area especially around Karori sanctuary and the botanical gardens (Chambers 1989; Chambers 2000).

The numbers of sulphur-crested cockatoos are increasing in some regions in New Zealand but only in small areas (Chambers 2000). Mainly, they inhabit the North Island of New Zealand including south Auckland-Onewhero, south Auckland-Miranda,

Waikato-Waingaro near Ngaruawahia, Waikato-Horsham Downs to Te Kowhai near Hamilton, Manawatu-Hunterville and Wellington-Waikanae (Chambers 1989; Chambers 2000). In the South Island, they can be found around Christchurch and Southland-Owaka (Chambers 1989; Chambers 2000).

Galahs are recently introduced Australian parrots and the species has established wild populations around South Auckland from Waitemata Harbour through to the lower Waikato areas and east to the Firth of Thames (Chambers 2000).



(a) (b) (c)

Figure 1-11. The distribution of other exotic parrots in the wild in New Zealand (a: crimson rosella, b: sulphur-crested cockatoo and c: galah, Bull et al 1985; Chambers 1989; Chambers 2000).

The potential for contact between native and exotic parrots in the wild in New Zealand

Widely distributed parrot species in the wild in New Zealand include native parrots such as kaka, red crowned and yellow crowned parakeet and exotic parrots such as the sulphur-crested cockatoo and eastern rosella. In fact, yellow crowned parakeet and eastern rosella are the predominant species in the wild in New Zealand.

Figure 1-8, 1-10 and 1-11 (Bull et al 1985; Chambers 1989; Chambers 2000) show the distribution of native parrots that exist on the mainland such as kaka, kea and parakeet and species of exotic parrots such as eastern rosella, crimson rosella, sulphur-crested cockatoo and galah. Kaka and parakeet populations have been found to share the same habitat as the eastern rosella population. In the current state, those native parrot species are not likely to be secure from the threat of exposure to PCV. They may become infected through the inhalation of the virus or from contact with contaminated materials.

In the South Island, kea populations exist mainly in Fiordland and throughout the western coast. Eastern rosella populations only occur around Dunedin since the first introduction of eastern rosella to the South Island. Thus, the native kea populations may be safe from the risk of exposure to PCV caused by the spread of eastern rosella populations.

The number of exotic parrots is increasing, e.g. eastern rosella (figure 12, Bull et

al 1985; Chambers 2000; The Ornithological Society of New Zealand in press) and the South Island population appears relatively stable. Now the eastern rosellas have established themselves on the North end of the South Island and it is likely that they will continue to expand south from here. This will increase the opportunity of contact with native parrots.

In particular, kaka and parakeet populations that exist on the northern North Island, around Auckland and in the Wellington region appear to have a high chance of coming in contact with sulphur-crested cockatoos and eastern rosellas. The risk of exposure to PCV is high in those populations, especially if the wild populations carry PCV at a similar incidence to that seen in Australia.

Figure 1-12. The comparison of the distribution of eastern rosella in 2000 and 2004 (left: 2000, right: 2004, Bull et al 1985; Chambers 1989; Chambers 2000; OSNZ in press).

Since the information related to the prevalence of PCD among native and exotic parrots is lacking, identifying the distribution of native and exotic parrots in New Zealand is the first step in assessing the risk to native parrots from the danger of PCV. The information on the distribution of parrots will enable conservation managers to make appropriate future management plans especially with translocation or reintroduction of endangered species.

There is a strong requirement to investigate the prevalence of PCV among both native and exotic parrot populations in New Zealand. With our current knowledge we cannot determine the risk of PCV to native New Zealand populations.

The Presence of PCV in New Zealand

The presence of PCV in native parrots

The information concerning the prevalence of PCV in native parrots of New Zealand is insufficient. There has been no official record of PCD among kakapo, kaka, kea or parakeet populations (Mander et al 2003).

However, it is unsupportable to assume that there has been no PCD in native parrots or that they are safe from the threat of PCD. The reasons why there has been no record of PCD among native parrots may include 1) the limited sampling efforts and 2) the low possibility of detecting the disease in small or isolated populations in remote

areas (Mander et al 2003). Studies to identify the susceptibility to PCV, the prevalence of PCD and immunity against PCV of native parrots should be carried out.

The presence of PCV in exotic parrots

Psittacine circovirus has been isolated in various exotic psittacine species in captivity and avieulture (Ritchie et al 2003; Stone 2004). The species include lovebird, budgerigar, lorikeet, cockatoo, cockatiel and rosella (Ritchie et al 2003). Until recently, the impact of PCV has been overlooked by scientists and the concern of PCV was more for avieulturalists and captive management of parrot species.

Psittacine circovirus has been identified in wild caught exotic parrots, mainly in sulphur-crested cockatoos (Mander et al 2003). It is thought that the transmission of PCV to wild parrots was caused by the escape of infected cage birds (Mander et al 2003). There is a record of feather disease in eastern rosellas (Julian and McKenzie 1985) but the causative agent was not positively identified. In 2003, PCV has been discovered in an eastern rosella caught in the wild and this fact confirms the presence of PCV in wild eastern rosella population (Mander et al 2003).

The increase in numbers of exotic parrots in the wild has added more concerns as to the impact of exotic pathogens being introduced. However, information relating to the prevalence of PCV in different regions or species and age groups is lacking so further study to obtain sufficient information is required.

Conservation Management of Parrots in New Zealand

To date there have been a variety of cases of intensive management of endangered species in New Zealand. Some of these cases have saved critically endangered species from extinction for example, the black robin (*Petroica Traversi*). Only five individuals were identified in the world in early 1980's and the current population counts up to 250 (Ardern and Lambert 1997 and references therein).

The main methods of intensive management of endangered bird species in New Zealand include (Elliott et al 2001):

1. Supplementary feeding.
2. Habitat restoration.
3. Nest manipulation.
4. Island translocation including predator control.
5. Captive management: artificial incubation of eggs, hand rearing of chicks.

As the techniques have improved, there are more opportunities to remedy the rapid decline of biodiversity. However, there are numerous internal and external factors that we should take into account in conservation management. The severity of wildlife diseases and the impacts have been overlooked by conservationists in New Zealand. The long history of isolation and the unique characters of New Zealand's native species have been suggested to indicate a vulnerability of New Zealand's avifauna to introduced viruses (Alley 2002).

The viral infections which have been identified or have shown any evidence of exposure in New Zealand include avian paramyxovirus (Alley 2002 and references therein), Pacheco's disease (Durham et al 1977 cited in Alley 2002), avian influenza (Stanislawek 1992 cited in Alley 2002), psittacine circoviral disease (Julian and McKenzie 1985; Alley 2002) and other circoviruses (Twentyman et al. 1999 cited in Alley 2002).

Psittacine circoviral disease is not usually a major threat to large populations of parrots but as the populations become smaller or isolated, the impacts of PCD will increase dramatically. For instance, PCD has caused high mortality amongst nestlings of Cape Parrot (*Poicephalus robustus*) of South Africa which are already endangered (Wirminghaus et al 1999; Mander et al 2003).

It is urgently required to identify the prevalence of PCV to minimise the exposure to native parrots of PCV. Thus, a surveillance program is planned to determine whether wild populations of endemic and exotic New Zealand parrots have evidence of PCV infection. This project will help conservation managers develop contingency plans to prevent or manage the disease in the future.

Project aims

The major aim of this project is to identify the risk to native parrots from circovirus infection in New Zealand. The main objectives can be summarised as:

- obtaining information on the distribution of native and exotic parrots and their interaction,
- collecting feather samples from native and exotic parrots for detecting virus DNA and
- identifying the incidence of exposure to PCV in parrot species in New Zealand using PCR assay.

The presence of PCV among exotic parrots in the wild in New Zealand will be discussed in chapter two and native parrots in chapter three. The presence of PCV among native parrots in captivity will be examined in chapter four. Through the analysis of the results, the direction of future conservation management of endangered native parrots in New Zealand will be discussed in chapter five.

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Chapter 2. The Prevalence of Psittacine Circovirus (PCV) in Wild Exotic Parrots in New Zealand

A pair of eastern rosella roosting on a tree (Anonymous 1985)

Introduction

Exotic parrots have been found in New Zealand since the early 20th century (Anonymous 1985). There is a record that the first official importation of the exotic parrots was the importation of budgerigar (*Melopsittacus undulates*) in 1928 (Parrots in New Zealand website 2004). However, the introduction of exotic parrots seems to be far earlier, as there are records of exotic parrots in the wild in 1910s (Anonymous 1985; Woon et al 2002 and references therein).

The status of exotic parrots in New Zealand varies. Their bright colour and overwhelming calls had been considered to increase the diversity in New Zealand's bush (Polkanov and Greene 2000). On the contrary, their rapid expansion, food competition with indigenous species, aggressive behaviour and damage to cultivated crops are some of the negative impacts of exotic parrots that have been seen (Polkanov and Greene 2000; Polkanov and Keeling 2002). The eradication of the rainbow lorikeet (*Trichoglossus haematodus*) undertaken from 1999 to 2002 around Auckland city is a good example of uncertain status of exotic parrots (Polkanov and Greene 2000; Polkanov and Keeling 2002).

Currently, four species of exotic parrots; eastern rosella (*Platycercus eximius*), crimson rosella (*Platycerus elegans*), sulphur-crested cockatoo *(Cacatua galerita)* and galah (*Cacatua roseicapilla*) have been identified in the wild in New Zealand (Chambers 2000). While crimson rosellas and galahs are rare and restricted to small areas, eastern rosellas and sulphur-crested cockatoos have established stable populations

in the wild (Chambers 2000). Thus, only the eastern rosella and sulphur-crested cockatoo will be discussed in this study.

There are two records of the identification of psittacine circovirus (PCV) in exotic parrots in the wild in New Zealand (Julian and McKenzie 1985; Mander et al 2003). Psittacine circovirus is known to be common among wild trapped sulphur-crested cockatoos for export or pet shops (G Knopers, personal communication). However, there has been no investigation on the incidence of PCV in these populations to date. Rapid increase in the number of several exotic parrots, the expansion of the population to larger areas as well as the isolation of PCV in wild exotic parrots have led to increased concerns on the vulnerability of New Zealand's avian species to introduced viral infections (Alley 2002; Mander et al 2003).

In consequence, this study was planned to identify the prevalence of PCV in exotic parrots in the wild in New Zealand. Two species of exotic parrots, eastern rosella and sulphur-crested cockatoo, were sampled and examined for the presence of PCV. This chapter presents the result of the investigation and discusses the implications of the result.

Exotic parrots in the wild in New Zealand

Eastern rosella *Platycercus eximius*

The eastern rosella was introduced as a cage bird to New Zealand in the early 20th century from Australia (Woon et al 2002 and references therein). The establishment of the population in the wild appears to be the result of escape or intentional release of the species (Woon et al 2002). The first establishment occurred in 1910 in Dunedin, the 1920s in Auckland and the 1960s in Hutt Valley (Anonymous 1985, Higgins 1999; Woon et al 2002).

Figure 2-1. Handling an eastern rosella.

The wild population has appeared to expand through the North Island since the 1970s, resulting in a wide distribution (Bull et al 1985; Woon et al 2002). The range covers from Northland, Auckland, Waikato, Coromandel, western Wairarapa, Hutt Valley, Wellington, Bay of Plenty, Hawkes Bay, Volcanic Palteau, Taranaki, Manawatu to Horowhenua (Higgins 1999; Woon et al 2002). In contrast, in the South Island they are restricted mainly to Dunedin (Bull et al 1985; Woon et al 2002), although there are

recent reports of rosellas on the northern end of the South Island suggesting a southwards range expansion of the North Island population (The Ornithological Society of New Zealand in press).

The eastern rosellas commonly exist in open woodlands including native forest and exotic plantations, open farmland, orchards, parks and golf courses (Anonymous 1985; Higgins 1999; Woon et al 2002). It feeds on the ground (more commonly in winter), in shrubs and trees, eating various kind of seeds as well as buds, shoots, fruits, flowers, nectars, insects and the larvae (Anonymous 1985; Higgins 1999; Woon et al 2002). Since they cause damage to crops such as citrus fruits, kiwi fruits, tomatoes and pip and stone fruits, in fruit-growing regions the species may be regarded as a pest (Anonymous 1985).

The eastern rosella usually occurs singly or as pairs (Anonymous 1985), though other reports suggest the mean flock size is 3.6 (Woon et al 2002). It is likely the flock size is slightly bigger in autumn and winter than in spring and summer which may be related to the breeding system (Woon et al 2002). The Ornithological Society of New Zealand (OSNZ) concluded that the eastern rosella may nest from spring to summer, as juveniles have been observed as late as January (Woon et al 2002).

The eastern rosella, including different species of rosellas, is known to be susceptible to PCV (Pass and Perry 1985; Ritchie and Carter 1995). Apart from this fact, the information on PCV in rosellas appears to be limited. In New Zealand, there are records of eastern rosellas with feather disease and one eastern rosella proved to be

positive to PCV in PCR assay (McKenzie and Julian 1985; Mander et al 2003).

McKenzie and Julian (1985) presented clinical cases of two eastern rosellas in Manawatu and Wairarapa regions, with no large tail feathers, no primary wing feathers and haemorrhagic feathers. The histological examination of affected feather follicles showed severe inflammation, haemorrhage, degeneration and necrosis of epidermal cells (McKenzie and Julian 1985). In 2003, an eastern rosella was found to be unable to fly (Mander et al 2003). Feather abnormalities were observed such as only one fully grown flight feather, shorter emerging replacement feathers and drop of pin feathers without bleeding during the handling (Mander et al 2003). There was, however, no abnormality in post mortem or histological examination (Mander et al 2003). In polymerase chain reaction (PCR) assay, the bird was found to be positive to PCV (Mander et al 2003) and it was the first case of confirmed PCV in eastern rosella population.

Sulphur-crested cockatoo *Cacatua galerita*

Sulphur-crested cockatoos were imported from Australia to New Zealand as cage birds. Since 1920, sulphur-crested cockatoos have established wild populations in New Zealand as a result of the escape and the release of caged birds (Anonymous 1985). Also, there are few records of wind-blown stragglers from Australia, e.g. one exhausted bird found at Kaipara heads after days of strong westerlies in May 1959 (Anonymous 1985; Higgins 1999), and this may have boosted the establishment of sulphur-crested cockatoos in the wild.

The biggest colony in New Zealand is known to exist around Wanganui (Anonymous 1985) and other areas the species inhabit include west Auckland, Bay of Plenty, Volcanic Plateau, Lake Taupo, Taranaki, southwest Hawkes Bay and Rangitikei River (Higgins 1999). The Wellington region appears to be another site that sulphur-crested cockatoos inhabit widely (Higgins 1999). In the South Island, there have been occasional records of sulphur-crested cockatoo but the size of the population seems to be small.

In New Zealand, sulphur-crested cockatoos can be found in native podocarp forests, pine plantation, coastal vegetation, farmland, orchards, golf courses and urban areas (Higgins 1999). The food items comprise seeds of the grasses, herbaceous plants, fruits, berries, nuts, grains, leaf buds, flowers, bulbous roots, insects and their larvae (Anonymous 1985; Higgins 1999). The birds are commonly found to forage on ground or in trees (Higgins 1999 and references therein). Like eastern rosellas, they are notorious as crop pests (Anonymous 1985).

Figure 2-2. A sulphur-crested cockatoo (Anonymous 1985).

Sulphur-crested cockatoos commonly exist in pairs or in small flocks during the breeding season which has been assumed to be from late Spring through Summer in New Zealand (Anonymous 1985). They appear to congregate and feed in big numbers out of the breeding season, often foraging on the ground in a big group with few watching out for intruders (Anonymous 1985).

Psittacine circovirus in sulphur-crested cockatoos is well documented. It is the most predominant viral disease in wild sulphur-crested cockatoos in Australia (Pass and Perry 1984; McOrist et al 1984; Raidal et al 1993; Bassami et al 2001; Ritchie et al 2003) and there are worldwide reports of PCD in captive sulphur-crested cockatoos. In general, infected cockatoos present typical features of beak and feather dystrophy with darkly pigmented skin (Pass and Perry 1984; McOrist et al 1984). The presence of necrosis and inflammatory changes of affected feathers in histological examination and the detection of 17-20 nm virus particles in electron microscopic examination confirmed PCD (Pass and Perry 1984; McOrist et al 1984).

Figure 2-3. A sulphur-crested cockatoo showing clinical signs of PCD (Photo courtesy of M. Alley).

McOrist et al (1984) described beak and feather deformities in 10 - 20 % of wild sulphur-crested cockatoos in Victoria. In a survey of 152 sulphur-crested cockatoos in New South Wales, seven birds showed the clinical signs of PCD (Raidal et al 1993). More dramatically, more than 70% (110) of the birds had antibodies to PCV (Raidal et al 1993). The prevalence of PCV seems to be extremely high in sulphur-crested cockatoos in captivity in Australia as one study demonstrated the presence of PCV in around 90% of the examined sulphur-crested cockatoos (Ritchie and Carter 1995 and references therein).

The introduction of PCV to New Zealand is likely to be the result of the importation of infected Australian parrots (Mander et al 2003). However, the information on prevalence or ecology of PCV is not sufficient in exotic parrots in the wild. The current information is that there are cases of PCV in wild sulphur-crested cockatoos and eastern rosellas. Recent recognition of the increase of exotic parrots in the wild and the presence of PCV among feral population evoked the concern of the potential of exotic parrots to spread the virus widely in the New Zealand environment.

Research methods

Materials and study sites

Collecting feathers from eastern rosellas and study sites

In wildlife disease investigations, it is obvious that the greater number of samples will bring more precise results. The target population in this study was the eastern rosellas in the wild in New Zealand and ideally, the study population should be all of them. However, there are limitations in assessing animals, availability of time, labour and the cost. To estimate the optimal number of samples, there was a requirement to interpret the concept of sample size in different scenarios. Table 2-1 shows the minimum number of samples required to reliably detect disease in different population sizes and at different disease prevalence.

Population size	Minimum sample size		
	Disease prevalence (%)		
	0.5	2	10
50	46	46	20
1000	448	136	27
>1,000,000	600	150	30

Table 2-1. The minimum sample size required to detect a disease at 95% confidence depends on the population size and disease prevalence (Wobeser GA 1994).

Since the isolation of PCV in wild eastern rosella was first confirmed in 2003 (Mander et al. 2003), there is no information on the prevalence of PCV in parrots in the

wild in New Zealand. We assumed that it may be similar or lower than the prevalence in parrots in the wild in Australia (10%) because Australian species comprise the exotic parrots in the wild in New Zealand.

Three study populations were chosen for mistnetting based on the population density according to the distribution map of eastern rosella by Chambers (2000). Three areas were selected such as Te Puke (Bay of Plenty, Northern North Island), Hutt Valley (Wellington, Southern North Island) and Dunedin (Otago, South Island) which were assumed to be representative of different regions in New Zealand.

Under the hypothesis of population size of 1,000, it was concluded that a minimum of 27 samples were required from each study area to detect the disease at 95% confidence interval if the disease prevalence was ~10%. Overall, 54 eastern rosellas were captured by mistnets; 18 from Te Puke, 26 from Wellington and ten from Dunedin. This includes four carcasses of eastern rosella found injured (eventually dead) or dead during the sampling period around Te Puke.

The feather tips of eastern rosellas were collected for Polymerase Chain Reaction (PCR) test. Wild eastern rosellas were captured using polyester mistnets (38 mm mesh/ 4 shelves/ 75 denier/ 2-ply, Avivet Inc., Dryden, NY, USA). To prevent contamination during the process, sterilization of mistnets using alcohol and Virkon was conducted after handling of each bird. The period for mistnetting was between April 2004 and September 2004.

Eastern rosellas were examined for feather condition, evidence of skin lesions, external injury and total body condition. Two feathers from each individual were plucked for PCR testing and were kept in separate plastic bags.

Figure 2-4. Mistnetting of eastern rosellas;
A mistnet set up and an eastern rosella captured.

Collecting feathers from sulphur-crested cockatoos and study site

While mistnetting was used to collect feather samples from eastern rosellas, feathers of sulphur-crested cockatoo were collected by professional bird trappers and exporters. Aotearoa Wildlife has provided feather samples from trapped sulphur-crested cockatoos and also allowed access to the results of PCR assays for PCV for the period of 2001-2004. The birds were trapped for export from the Turakina valley, near the watersheds of the Turakina and Rangitikei Rivers near Wanganui where the biggest population of sulphur-crested cockatoo exists in New Zealand (Anonymous 1985). The period for trapping was November, 2001 - September, 2004. Feathers were plucked and

kept in plastic bags when the birds were handled from the traps and then the feathers were submitted to Equine Blood Typing and Research Centre, Massey University for the detection of PCV.

In addition, there was one post mortem case of a wild sulphur-crested cockatoo submitted to Veterinary Teaching Hospital, Massey University and a feather was submitted for PCR assay. Overall, 255 individual sulphur-crested cockatoos were examined.

PCR assay

DNA extraction

DNA was extracted from feather tips by placing them in 50 μl of sterile deionized water in 1.5 ml microcentrifuge tubes for 30 minutes. Centrifugation at 12,000 rpm for 3 minutes was followed, 40 μl was removed and discarded. 40 μl of Biorad instagene (BioRad, Hercules, CA, USA) and 1 μl of Proteinase K (BDH, Poole, England) were added. The tubes were vortexed at high speed for 3 seconds. Then they were incubated at 37°C for 30 minutes and boiled for 8 minutes.

PCR assay

A 605-nt segment of the PCV genome was amplified. The forward primer sequence was 5′ -TTAACAACCCTACAGACGGCGA-3′ and the reverse primer sequence was 5′ -GGCGGAGCATCTCGCAATAAG-3′ as described in Ritchie et al

(2003). The PCR was conducted in a GeneAmp PCR 2700 thermocycler (Applied Biosystems Inc., Foster City, CA, USA) in 2.5 μℓ 10x PCR Gold Buffer (Applied Biosystems Inc., Foster City, CA, USA); 0.1 μℓ GeneAmpliTaq Gold (IU) (Applied Biosystems Inc., Foster City, CA, USA); 0.25 μℓ 100 mM dNTPs (Roche Diagnostics, Mannheim, Germany); 1.5 μℓ 25 mM $MgCl_2$; 1.5 μℓ forward primer; 1.5 μℓ reverse primer; BSA 1.0 mℓ and deionized water to 25 μℓ. The thermocycling conditions were 10 minutes at 95 ℃, 30 cycles each of 10 seconds at 94 ℃, 10 seconds at 56 ℃, 1 minute at 72 ℃ and a final extension of 5 minutes at 72 ℃. The amplified DNA was separated by electrophoresis in a 1.5 % NuSieve GTG agarose (Cambrex BioScience Rockland Inc., Rockland, ME, USA) gel. The gel was visualized under ultra violet light using ethidium bromide staining and run in conjunction with a Gibco BRL 50-800 base pairs (bp) ladder (Invitrogen Life Technologies, Carlsbad, CA, USA).

Statistical Analysis

The statistical analysis in this study deals with epidemiology of disease in particular, disease prevalence and its confidence interval. The calculation method followed Dooho et al (2003).

The main point of statistical analysis was to calculate the prevalence of PCV in exotic parrots in different regions. The sample size formula used to estimate a population proportion was as below. Additionally, Win Episcope (version 2.0) was run to confirm the sample size.

$[n = 4pq/L^2]$

n = required sample size,

4 = 1.96^2 (95% confidence interval),

p = expected proportion of diseased individuals,

q = 1-p,

L^2 = an estimate of the precision required.

There were two concepts of prevalence which were the test prevalence and the true prevalence. While the test prevalence means the prevalence of samples tested, the true prevalence takes into account the sensitivity and the specificity of the test. The formula to calculate the confidence interval of test prevalence was:

$$95\% \text{ confidence interval} = p \pm 1.96^* \log [p^*(1-p)/n]$$

(where p = cases / number of samples).

The true prevalence was calculated by;

$$P = P(t) + \text{specificity} - 1 \,/\, \text{sensitivity} + \text{specificity} - 1$$

(where P (t): Test prevalence).

In the event of negative results from all samples, the required sample size to detect one diseased animal (95% confidence) was estimated. Two formulas described below and the statistical program freecalc (version 2.0, free from disease) were used for this:

$$D = [1 - \log 1/n\ (1 - CI)]^*[N-((n-1)/2)]$$

D = expected number of diseased animals in study population,

N = total number of animals in study population,

n = number of animals tested.

$$n = \log (1-CI) / \log q$$

CI = Confidence Interval,

q = 1 - (D N).

From the required sample size, then, the prevalence of PCV was assumed.

Results

Eastern rosella

The result of PCR assay

PCR assay was conducted on the feather samples from 54 eastern rosellas captured by mistnets from three regions in New Zealand such as Hutt Valley, Te Puke and Dunedin. Figure 2-5 presents the results of PCR assay in eastern rosellas. The presence of a specific DNA band in the PCR gel means positive for PCV. In 17 birds, PCV was detected which was 31.5% of the samples.

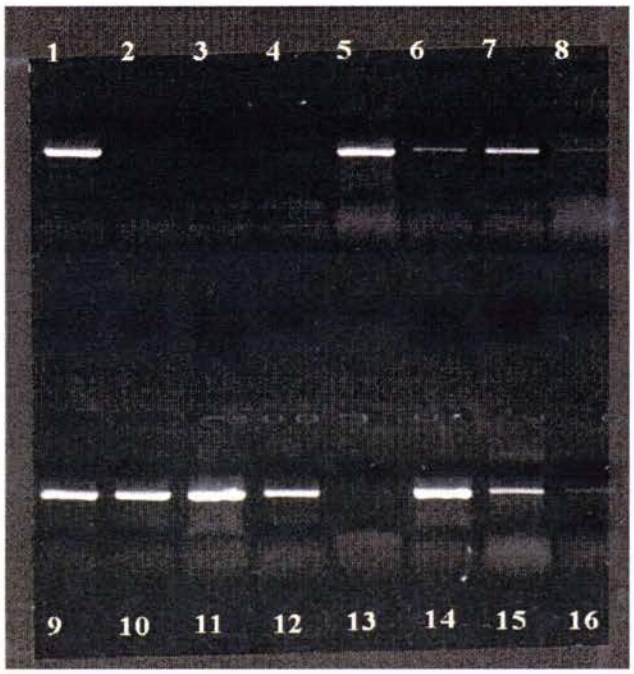


Figure 2-5. The picture of PCR gel under ultraviolet light in 15 samples (1: positive control). Sample 2, 3, 4 and 13 show negative results and others, positive.

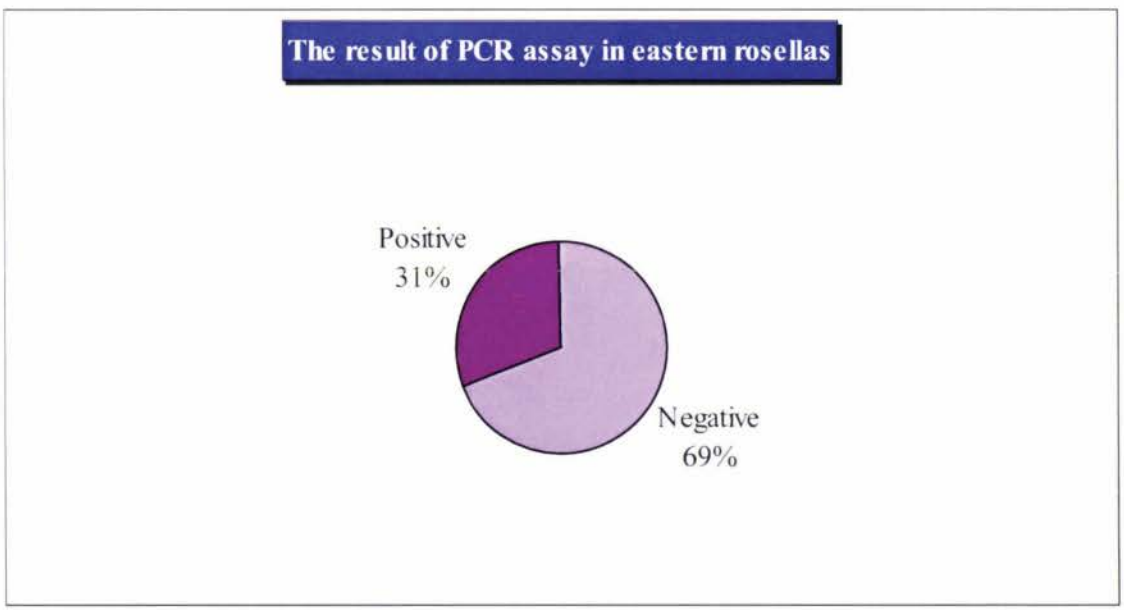


Figure 2-6. The result of PCR assay in eastern rosellas.

However, the incidence of PCR infection varied between regions. The population from Te Puke showed the highest rate of current infection by PCV. Among 18 birds sampled, PCV DNA was detected in eight samples (44.4%, Figure 2-7).

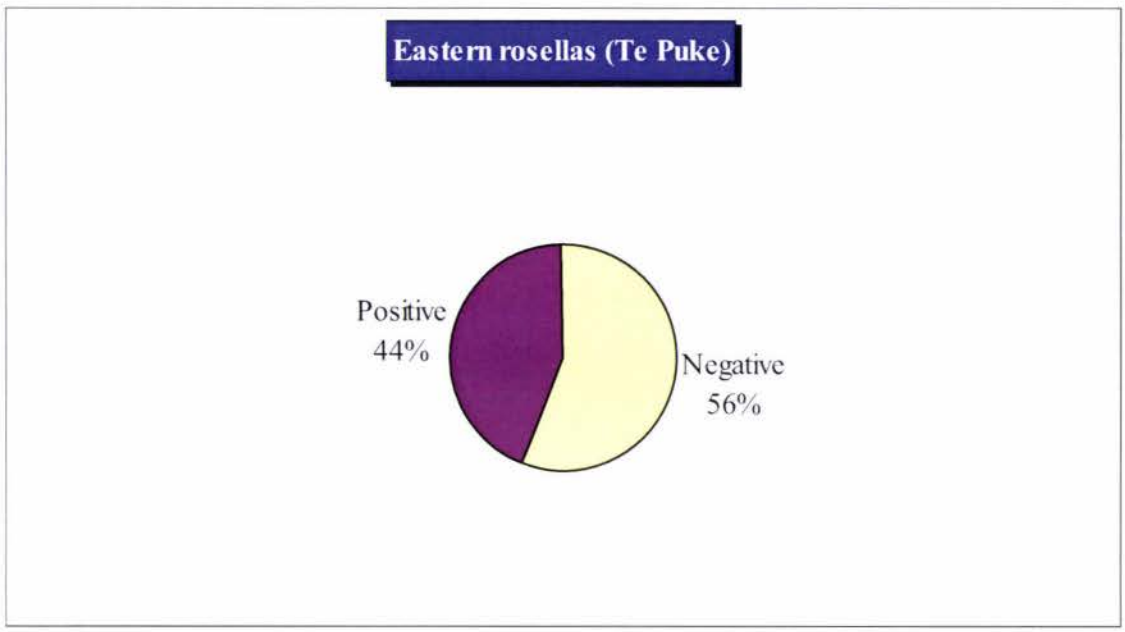


Figure 2-7. The result of PCR assay in eastern rosellas sampled from Te Puke.

The Hutt Valley population that had provoked the concern of PCV among eastern rosella population in the wild in New Zealand also showed a high incidence of PCV. Nine birds out of 26 were positive on PCR assay (34.6%, Figure 2-8).

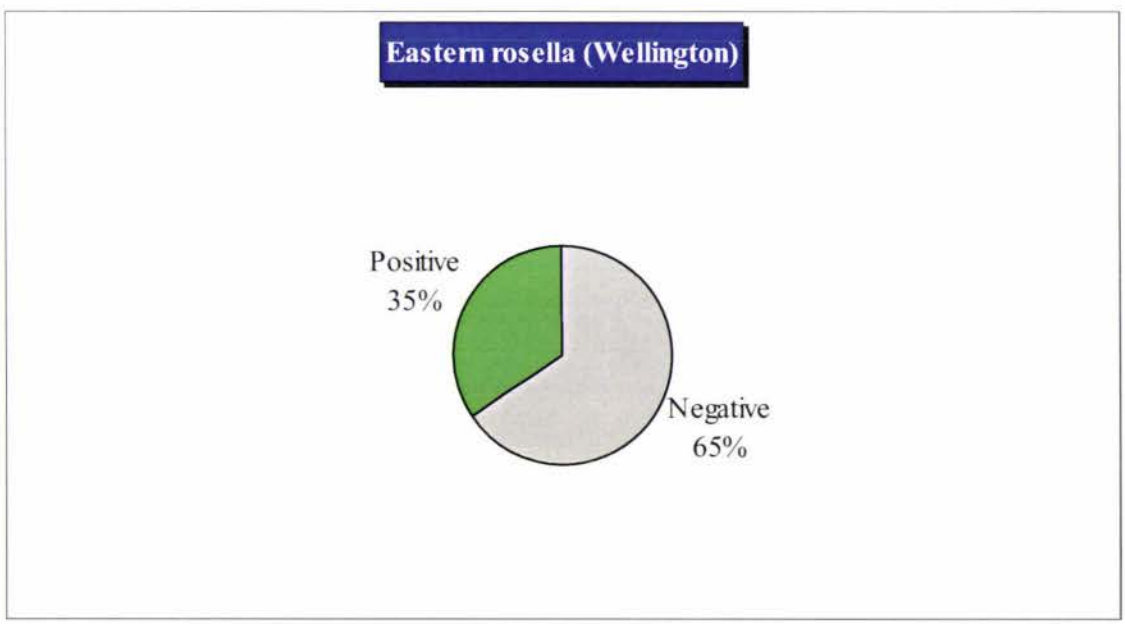
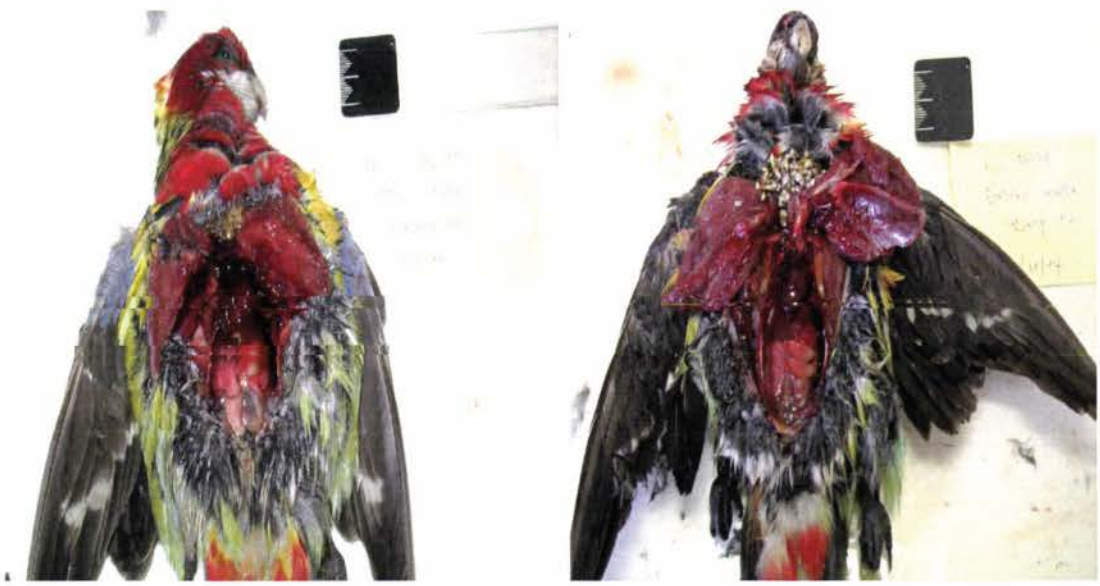


Figure 2-8. The result of PCR assay in eastern rosellas samples from Wellington.

Only ten samples were collected around Dunedin due to the relatively small population size and time limitation. No bird sampled was positive to PCV.

Post mortem and histology findings of eastern rosellas

Post mortem was conducted to examine for abnormalities caused by PCV on one eastern rosella that had previously tested negative to PCV and three eastern rosellas that had tested positive to PCV. No feather or beak abnormality was seen. No significant changes were seen at necropsy in any of the rosellas examined.



(a) (b)

Figure 2-9. The necropsy of a PCV negative eastern rosella (a) and a PCV positive eastern rosella (b); no significant changes were detected between two birds.

Figure 2-10. The feather and beak conditions of a PCV positive eastern rosella; no detectable abnormalities are present in both feather and beak lesions.

Histological examination of samples of skin including feather follicles, liver, spleen and kidney were carried out. The result was compatible with that of necropsy. No significant changes were recognized in the organs examined. No viral inclusion body was detected.

Summary statistics

The test prevalence and the true prevalence of PCV in eastern rosellas was calculated at 95% confidence interval. Table 2-2 shows the results. The sensitivity and the specificity of PCR assay were regarded as 99.7% and 100% as mentioned in chapter one.

	P value (n: sample size)	Prevalence (%)	The prevalence at 95% CI (%)	The true prevalence at 95% CI (%)
Eastern rosella (NI&SI)	0.315 (n: 54)	31.5	19.11 - 43.89	19.17 - 44.02
Eastern rosella (Te Puke)	0.444 (n:18)	44.4	21.45 – 67.35	21.51 - 67.55
Eastern rosella (Wellington)	0.346 (n:26)	34.6	16.31 – 52.86	16.36 - 53.02
Eastern rosella (NI)	0.386 (n:44)	38.6	24.22 – 52.98	24.29 - 53.14
Eastern rosella (Dunedin, SI)	0.0 (n:10)	-	-	-

Table 2-2. The estimated prevalence and the true prevalence of PCV estimated at 95% CI (NI: North Island, SI: South Island, CI: confidence interval).

A different method was used to assess the prevalence of PCV in the eastern rosellas in Dunedin because all samples showed negative result. Although negative results from all samples may imply a possibility of the population being free from PCV, the sample size was too small to confirm that conclusion. Freecalc analysis confirmed that the sample size was too small to conclude that the population was disease free. Table 2-3 presents the required sample size to detect at least one positive at 95% confidence using formulas and freecalc. Under the assumption of the population size being more than 500, the only conclusion that can be made was that the prevalence of PCV in the population may be equal to or less than 25 % (95% CI).

Population size	Prevalence							
	0.1 %	1 %	2 %	5 %	10 %	20 %	25 %	30 %
10	10	10	10	10	10	8	8	6
50	50	50	48	35	22	12	10	8
100	100	96	78	45	25	13	10	9
500	500	225	129	56	28	14	11	9
1000	950	258	138	57	29	14	11	9
10000	2588	294	148	59	29	14	11	9
infinite	2995	299	149	59	29	14	11	9

Table 2-3. Required sample size to detect at least one positive (95% CI).

Sulphur-crested cockatoo

The result of PCR assay

From 255 sulphur-crested cockatoos, PCV was isolated from 70 individuals (27.5%).

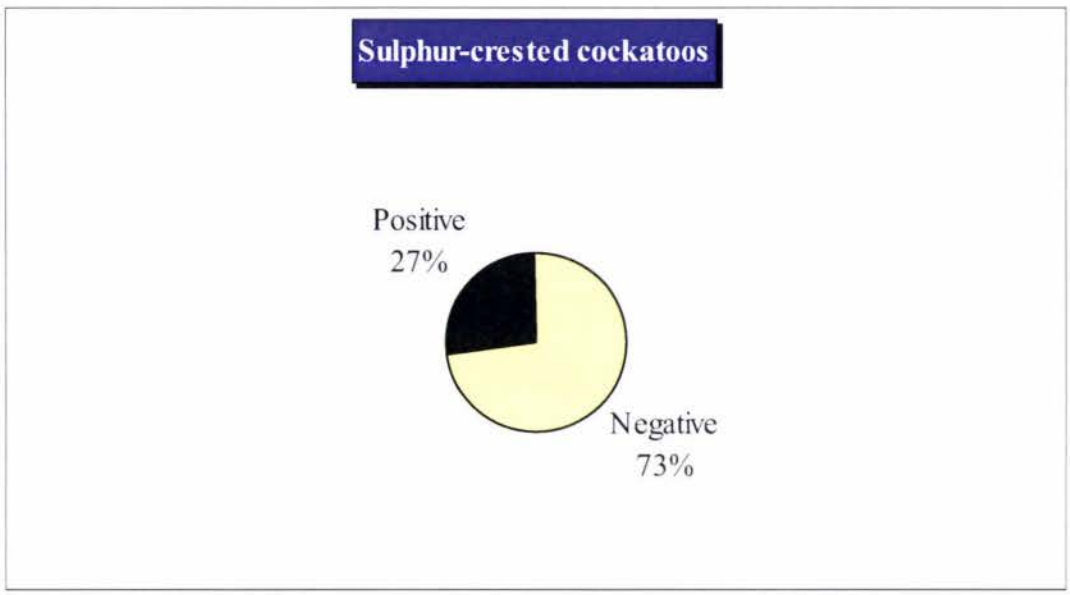


Figure 2-11. The result of PCR assay in sulphur-crested cockatoos.

Summary statistics

The test prevalence of PCV in 255 sulphur-crested cockatoos was estimated as 21.97 – 32.93 (%) at 95% confidence interval based on the test prevalence (27.45%). The true prevalence was determined as 22.04 - 33.07 (%).

Discussion

The prevalence of PCV in exotic parrots in the wild in New Zealand was estimated as 19.17 - 44.02 (%) in the eastern rosella population and 22.04 - 33.07 (%) in the sulphur-crested cockatoo population. The number of samples was too small to detect the disease or approximate the prevalence of PCV in eastern rosella population in the South Island. When samples of eastern rosellas in the North Island are only taken into

account, the prevalence of PCV is on the range 24.29 - 53.14 (%). While there have been anecdotal events of high levels of PCV infection in sulphur-crested cockatoos (personal communication, G. Knopers), the high prevalence of PCV in eastern rosellas was unexpected. The fact that there were no abnormal gross or histological changes in PCV infected eastern rosellas suggests difficulties in detecting or controlling the disease.

It can be concluded that the prevalence of PCV in exotic parrots in the wild (mainly in the North Island) is higher than 20%. This is higher than the prevalence in wild parrots in Australia. The prevalence of PCD in wild parrots in Australia has been documented at 10-20 % (McOrist et al 1984). This study has investigated the presence of PCV, while McOrist et al (1984) detected the presence of clinical signs of PCD. This fact may explain the higher prevalence of PCV in wild parrots in New Zealand than in Australia. However, the reason why the parrots in New Zealand show higher prevalence of PCV than same species in Australia is not well understood. In addition, there is no information on the seroprevalence of PCV antibodies in exotic parrots in the wild in New Zealand.

Although this study has initiated the investigation of PCV in wild parrots in New Zealand, there are strong requirements for further studies concerning PCV in exotic parrots. The first task will be to determine the population size and the distribution of exotic parrots in the wild precisely. This is fundamental to accurately assess the risk of native parrots to PCV or other dangers can be caused by the contact with exotic parrots in the wild. Further sampling and detection of PCV in the wild population is also recommended, especially the eastern rosella population in the South Island, as this study

was not sufficient to give a prevalence estimate of disease in this sub-population.

Serological examination to detect the antigen/antibody of PCV in wild exotic parrots is urgently required. The detection of PCV or the clinical signs of PCD is not always compatible with the presence of antibody (Raidal et al 1993). The wild parrots in Australia presented a high seroprevalence of 73% and 94% in New South Wales (Raidal et al 1993) which indicated that a large proportion of those parrots had exposure to PCV. Haemagglutination/Haemagglutination inhibition (HA/HI) assay is not currently available in New Zealand. However, identifying the seroprevalence is necessary to provide the information on how common or how widespread PCV exposure is among feral exotic parrots in New Zealand.

To identify the variation in virus strains between viruses isolated from sulphur-crested cockatoos and eastern rosellas is useful. Ritchie et al (2003) demonstrated the different viral lineages in three different exotic parrots. It will be useful to understand the ecology of PCV in terms of variation in virus genotypes and possibility of mutation of the virus. Without sufficient knowledge or information on the virus itself, it is not likely that the virus can be controlled.

The experimental infection of rosellas by isolated PCV will provide precise information to control the virus. There are several aims in conducting this challenge such as; 1) if a species of psittacine birds can be infected by the virus isolated from different species, e.g., the eastern rosella can be infected by PCV isolated from sulphur-crested cockatoo, 2) if 1 is possible, how susceptible or sensitive to the infection of the

species, for example, incubation period of PCV in different species and the presence of clinical signs consistent with PCD can be observed, and 3) if any immune response is developed. In particular, examining the development of immunity in parrots is crucial for future management if vaccination of PCD can be considered as a control method (Raidal and Cross 1994).

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Chapter 3. The Detection of Psittacine Circovirus (PCV) in Wild Native Parrots in New Zealand

A kakapo (Moon and Lockley 1982).

Introduction

Native parrots in New Zealand are temperate parrots who show distinctive features from other parrots. The kakapo (*Strigops habroptilus*) is one of the most unusual parrots in the world with its unique appearance, flightlessness, nocturnal behaviour and special lek breeding system (Higgins 1999). Prior to human settlement, native parrots thrived throughout New Zealand but now the species are restricted to remnant native forests or offshore islands (Moon and Lockley 1982). The reason for the dramatic decline of native parrots may vary in different species, however, there are common factors have contributed to this such as habitat destruction, slaughtering for meats, persecution as crop pests and predation by introduced predators (Anonymous 1985).

To establish self-sustainable populations in the wild and to secure the future of native parrots, rigorous management has been conducted for some species under the species recovery plans by the Department of Conservation (DOC). This has been achieved by means of predator control, habitat restoration, nest manipulation, stimulation of breeding by supplementary feeding, incubation of eggs, hand rearing of chicks or reintroduction programs and intensive monitoring (Elliott et al 2001; Clout et al 2002). Research concerning the ecology of endangered native species also plays an important role in preventing the extinction or rapid decline of species. In recent times, there has been more recognition of the impacts that exotic pathogens may have on native species (Alley 2002). Psittacine circovirus (PCV) is one of the exotic pathogens which is regarded as a major threat to endangered native parrots (Alley 2002).

Anecdotal reports suggest that PCV is present in many countries and different species of parrots (Ritchie and Carter 1995). There has been little emphasis on disease in the management of endangered native parrots. The importation or international trading of animals has resulted in the introduction of exotic pathogens to New Zealand (Ritchie et al 2003). Several species of exotic parrots such as sulphur-crested cockatoos (*Cacatua galerita*) and eastern rosellas (*Platycercus eximius*) have established self-sustainable populations in the wild in New Zealand and PCV has been isolated in those populations (Julian and McKenzie 1985; Mander et al 2003; chapter 2).

There are various routes of exposure to PCV. Oral, intracloacal, subcutaneous, intramuscular, intraocular and intranasal infections have all been discovered to be possible (Ritchie and Carter 1995 and references therein). Infected birds have been found to excrete the virus through feather dust, faeces and crop secretion (Ritchie et al 1991). Inhalation or ingestion of virus particles through direct contact with infected individuals is a common form of transmission, moreover, contaminated dust has a high potential of transmission of the virus (Ritchie et al 1991).

There are some aspects of PCV which will make control of the virus extremely difficult once it occurs. Firstly, infected birds, whether the birds are showing typical clinical signs of psittacine circoviral disease (PCD) or not, have been discovered to shed a large amount of virus particles (Ritchie et al 1991). The second point is that the route of transmission is diverse (Ritchie et al 1991; Ritchie and Carter 1995). Additionally, the virus is recognized to be very stable in the environment and resistant to control measures (Ritchie and Carter 1995).

The detection of PCV from exotic parrots in combination with the increase and spread of exotic parrots in the wild have suggested the risk of native parrots to exposure to PCV. To date, there has been no record of PCV in native parrots in the wild in New Zealand. Nonetheless, it is dangerous to conclude that native parrots are safe from the threat by PCV or that the species have no history of exposure to PCV. It is more likely to be the result of difficulty in detecting diseases in small, isolated and remote populations (Mander et al 2003).

The overall aim of this study is to determine the risk to wild native parrots from PCV, thus, there was a strong requirement to identify the prevalence of PCV among the populations. Four species of native parrots in New Zealand: kakapo (*Strigops habroptilus*), kea (*Nestor notabilis*), kaka (*Nestor meridionalis* spp.) and two species of parakeet (*Cyanoramphus* spp.), were screened for the presence of PCV. This chapter presents general ecology of native parrots and the results of sampling and testing to detect PCV in those populations.

Native parrots in New Zealand

Kakapo *Strigops habroptilus*

The kakapo are most distinctive and unusual birds. They are flightless, nocturnal and the largest of the parrots (63 cm, 3.5 kg) and have a lek breeding system (Moon and Lockley 1982; Anonymous 1985). They have short, broad wings that the birds use for

balance and support, while climbing and jumping (Anonymous 1985; Higgins 1999). The wings are used in the courtship display in breeding season (Higgins 1999). The kakapo have massive legs and a short tail (Anonymous 1985).

Figure 3-1. A kakapo (Anonymous 1985).

Evidence shows that these unique parrots were once abundant throughout New Zealand (Moon and Lockley 1982). The population has declined since pre-European settlement (Anonymous 1985). In combination with habitat destruction, the introduced predators have contributed to this decline. Their unique breeding system characterized as lek breeding, ground-nesting, sole guardian of nestlings (female only), long hours foraging of females at night time all result in the exposure of the young to predation. The kakapo's extremely slow reproductive rate also played a big role in decrease of the population (Anonymous 1985).

The last observation of the birds on the two mainland islands in New Zealand is known to be in 1927 (North Island) and 1987 (South Island) (Higgins 1999). Now only 83 individuals of this endemic parrot exist and they are restricted to several offshore islands with no predators (Elliott et al 2001). Table 3-1 shows the location, age and sex of the birds (Merton 2004). Kakapo is classified as "CR C2a(i)" by IUCN redlist (version 3.1, 2001, table 3-2).

	Female		Male		
	Sub-adult	Adult	Sub-adult	Adult	Total
Fiordland	Believed extinct since ~1987				
Stewart Island	*Population relocated 1980-97*				
Whenua Hou/ Codfish Island	7	20	5	17	**49**
Te Kakahu/ Chalky Island	10	1	8	4	**23**
Pearl Island	-	-	-	11	**11**
TOTALS	**17**	**21**	**13**	**32**	**83**

Table 3-1. The current kakapo population(Merton 2004).

Kea *Nestor notabilis*

The kea are alpine parrots (46-50 cm) that are confined to the South Island of New Zealand. They are well protected in southern national parks (Moon and Lockley 1982) and are common in alpine habitats and near sea level (Higgins 1999 and

references therein). They show a variable range of habitats as the sightings occurred in the altitude of 21-2,400 m (Wakelin 1991), in different vegetation types as beech *Nothofagus* forest, temperate rainforest, broadleaf or podocarp forest, sub-alpine scrub, tussock grassland, fellfields, meadows and herbfields (Higgins 1999 and references therein).

Figure 3-2. A kea (Moon and Lockley 1982).

Due to a reputation as sheep-killers, 150,000 kea had been killed mainly by shooting and poisoning during the period of 1870 - 1970 (Higgins 1999). It was no earlier than 1986 that the kea had been fully protected (Higgins 1999). Additional threats to kea population are food competition with introduced animals and predation by stoats *Mustella erminea* (Higgins 1999). Even though the birds damage property and vehicles near human development areas, they remain as a great attraction to the visitors to the South Island (Anonymous 1985). The conservation status of kea is "Threatened"

(Grant 1993 cited in Higgins 1999); "category B threatened" (Molloy and Davis 1994 in Higgins 1999). The IUCN redlist classified them as "VU C2a (ii)" (version 3.1, 2001). The total population was estimated as 5,000 (Jackson et al 2000).

Kaka *Nestor meridionalis* spp.

The kaka is a close relative of kea but smaller in size (38-44 cm), endemic to New Zealand (Moon and Lockley 1982). There are two sub-species of kaka which are North Island kaka (*N.m.septentrionalis*) and South Island kaka (*N.m.meridionalis*) (Anonymous 1985). Both species now exist in low number in the wild due to slaughter for meat source by early Polynesian settlers, habitat losses caused by later European settlers and predation by introduced animals (Anonymous 1985; Greene et al 2004). Moorhouse (in Higgins 1999) had demonstrated that the kaka inhabit mainly the canopy of original, indigenous, temperate rainforests.

Figure 3-3. A kaka (Jacobs 1991).

Records show that the kaka were abundant in native forests throughout New Zealand, even on Chatham Island before European settlement (Anonymous 1985). The forest clearance for farming resulted in the rapid decrease of kaka since 1860s (Higgins 1999). As a consequence, they are scattered and confined to remaining areas of native forests, coastlines and offshore islands (Higgins 1999).

Additionally, the impact of introduced predators is considered to be the biggest threat to kaka and has been disastrous for them (Greene et al 2004). The decline of kaka in the wild has been attributed to competition for food with the introduced predators such as brushtail possum (*Trichosurus vulpecula*), wasps (*Vespula* spp.) and rats (*Rattus* spp.) as well as direct predation by stoats (*Mustela erminea*) (Moorhouse 1997; Moorhouse et al 2003). In fact, the number of kaka has been increasing in several offshore islands where successful predator control had conducted such as Kapiti Island, Little Barrier Island and Stewart Island (Moon and Lockley 1982; Higgins 1999).

The estimated total population size is known as less than 10,000 (Jackson et al 2000). The conservation status of kaka has been proclaimed as "Threatened" (Bell 1986 cited in Higgins 1999) and "VU C1" (IUCN redlist version 3.1, 2001).

Parakeet/Kakariki *Cyanoramphus* spp.

Parakeet or kakariki were numerous in tall native forests in early European settlement (Anonymous 1985). There are four species of parakeet in New Zealand such as antipodes or unicolor Parakeet (*Cyanoramphus unicolor*), red crowned parakeet

(*Cyanoramphus novaezelandiae*), yellow crowned parakeet (*Cyanoramphus auriceps*) and orange fronted parakeet (*Cyanoramphus malherbi*) (Anonymous 1985). The red-crowned parakeet includes four sub-species; the New Zealand red-crowned parakeet (*Cyanoramphus novaezelandiae novaezelandiae*), the Kermadec parakket (*C. n. cyanurus*), the Chatham Island red-crowned parakeet (*C. n. chathamensis*) and Reischek's parakeet (C. *n. hochstetteri*) (Anonymous 1985).

The yellow-crowned parakeet is the most common, followed by the red-crowned. The yellow-crowned parakeets still reside in native forests on the mainland of New Zealand and are abundant on Stewart Island and a few offshore islands (Kearvell et al 2002). The red-crowned parakeet, however, is uncommon on the mainland (Kearvell et al 2002). There has been success in captive breeding of red-crowned parakeet and captive-bred birds have been released to the wild in Wairarapa, the Waitakere Rangers and on Tiritiri Matangi Island and Cuvier Island (Anonymous 1985).

Figure 3-4. A pair of yellow crowned parakeets (Kolar and Spitzer 1990).

The orange-fronted parakeet, the smallest parakeet (males 22 cm, Moon and Lockley 1982), is rare and exists only in the northern Canterbury region (Boon et al 2001). This species is known to have never been common in the wild and the information on this bird is lacking (Anonymous 1985). There had been a controversy whether orange-fronted parakeet is a fully distinct species or a colour morph of yellow-crowned parakeet (Kearvell et al 2002; Kearvell et al 2003 and references therein). At last, Boon et al (2001) and Kearvell et al (2003) have demonstrated that the orange-fronted parakeet is a distinct species from other *Cyanorampus* spp. The orange-fronted parakeet can cross breed with yellow-crowned parakeet (Anonymous 1985). Captive breeding is ongoing to identify the ecology of this species and to prevent the extinction of this rare parakeet (Anonymous 1985).

Figure 3-5. An orange-fronted parakeet (J. Warne in parrots in New Zealand website).

Persecution as crop pests (Moon and Lockley 1982, Greene 1998 and references therein) as well as deforestation, introduced predators; rats, cats and stoats, and disease probably resulted in the rapid decline of the parakeets in New Zealand (Greene 1998 and references therein). Food and habitat competition between different parakeets in modified habitats and the influence of introduced food competitors including wasps, birds, mice, rats and other herbivores and omnivores have also been recognised as threatening the survival of the parakeets in the wild (Kearvell et al 2002).

Figure 3-6. A pair of red-crowned parakeet (Moon and Lockley 1982).

The conservation status of the *Cyanoramphus* species differs from each other. For example, the yellow-crowned parakeet has been classified as "NT" (IUCN redlist version 3.1, 2001) and the oranged fronted parakeet as "En B1ab(i,ii,iv,v)+2ab(i,ii,iv,v), C2a, D" by IUCN (version 3.1, 2001). See table 3-2 for details of conservation status of New Zealand parrots.

Species	Category	Description
Kakapo	CR C2a (i) (Critically endangered C2a (i)	**CR**: A taxon facing an extremely high risk of extinction in the wild in the immediate future, **C**: population size estimated to number fewer than 250 mature individuals, **2:** a continuing decline, observed, projected, or inferred, in numbers of mature individuals, **a**: population structure in the form of, **(i)**: no subpopulation estimated to contain more than 50 mature individuals.
Kea	VU C2a (ii) (Vulnerable C2a (ii))	**VU**: a taxon is not critically endangered or endangered but is facing a very high risk of extinction in the wild in the near future, **C:** population size estimated to number fewer than 10,000 mature individuals and, **2:** a continuing decline, observed, projected, or inferred, in numbers of mature individuals, **(a):** population structure in the form of, **(ii):** all mature individuals are in one subpopulation.
Kaka	VU C1 (Vulnerable C1)	**VU**: A taxon is not critically endangered or endangered but is facing a very high risk of extinction in the wild in the near future, **C**: population size estimated to number fewer than 10,000 mature individuals and, **1:** an estimated continuing decline of at least 10% within 10 years or three generations, whichever is longer, (up to a maximum of 100 years in the future).
Yellow-crowned parakeet	NT (Near threatened)	A taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future.
Orange-crowned	EN B1ab(i,ii,iv,v)+2ab(i,ii,iv,v);	**EN:** a taxon is endangered when it is not critically endangered but is facing a very high risk of extinction in the wild in the immediate future,

parakeet	C2a(i); D (Endangered B1ab(i,ii,iv,v)+2ab(i,ii,iv,v); C2a(i); D)	**B**: geographic range in the form of, **1**: extent of occurrence estimated to be less than 5000 km², and estimates indicating, **a**: severely fragmented or known to exist at no more than five locations, **b**: continuing decline, observed, inferred or projected, in any of the following: **(i)**: extent of occurrence, **(ii)**: area of occupancy, **(iv)**: number of locations or subpopulations, **(v)**: number of mature individuals, **2**: area of occupancy estimated to be less than 500 km², and estimates indicating, **a**: severely fragmented or known to exist at no more than five locations, **b**: continuing decline, observed, inferred or projected, of the following: **(i)**: extent of occurrence, **(ii)**: area of occupancy, **(iv)**: number of locations or subpopulations, **(v)**: number of mature individuals, **C**: population size estimated to number fewer than 2500 mature individuals and, **2**: a continuing decline, observed, projected, or inferred, in numbers of mature individuals and, **a**: population structure in the form of one of the following: **(i)**: no subpopulation estimated to contain more than 250 mature individuals, **D**: population size estimated to number fewer than 250 mature individuals.

Table 3-2. The classification of native parrots by IUCN redlist (IUCN redlist version 3.1 2001).

Research methods

Materials and study sites

Collecting feathers from native parrots and study sites

Feather samples were collected in co-operation with Massey University and the Department of Conservation (DOC), New Zealand. When there were cases of post mortem of native parrots, a few feathers were collected and kept frozen in plastic containers. Also, DOC had provided the majority of the samples for health screening prior to translocation of native parrots and regular health checking of endangered parrot species.

Figure 3-7. Mistnetting of yellow-crowned parakeet.

Figure 3-8. Post mortem of a kakapo.

Additionally, there has been mistnetting of red-crowned parakeet on TiriTiri Matangi Island mainly for banding and identifying the breeding habit of the species. It was found that the species were showing signs of feather losses and related dermatitis (figure 3-9) so feathers were collected from the population for screening for PCV.

Figure 3-9. A male red-crowned parakeet (left)
and a female red-crowned parakeet (right)
showing feather losses on TiriTiri Matangi Island (photo courtesy of L. Ortiz Catedral).

The number of samples of native parrots and the origins are described in table 3-3 and figure 3-10.

Species	Origin	Number of samples
Kakapo	Codfish Island	43
Kea	Haast	1
Kaka	Eglinton Valley (8), Masterton (8), Ohakune (2), Pureora (59), St. Arnaud (1), Whangarei (1)	79
Red-crowned parakeet	TiriTiri Matangi Island (10) Waikanae (4), Wellington (5), Te Anau (1)	20
Yellow-crowned parakeet	Marlborough Sound	25

Table 3-3. Samples of native parrots and their origin.

Figure 3-10. The sampling areas for native parrots.

PCR assay

PCR assay was conducted to detect virus DNA from feather samples of native parrots following the same methods described in chapter 2.

Statistics

The statistical analysis dealing with the detection of the presence of disease and the method was as described in chapter 2.

Results

The result of PCR assay

Kakapo

The total number of 43 kakapo on Codfish Island Whenua Hou were examined for regular health checking in 2003 by DOC. Feather samples were taken for PCR assay and it was carried out at Equine Blood Typing Centre, Institute of Veterinary, Animal and Biomedical Science, Massey University. In PCR assay, all individuals were found to be negative.

Kea

The sample of kea from DOC (Haast) tested negative in PCR assay.

Kaka

79 samples from wild kaka were examined for PCV detection. No evidence of PCV was detected in any sample in PCR assay.

Parakeet/Kakariki

20 samples of red-crowned parakeets and 25 samples of yellow-crowned parakeets were examined. All the samples presented negative results in PCR assay.

Summary statistics

Only the results of samples from kakapo, kaka and parakeet were analysed because there was only one sample from kea. The required sample size to detect the presence of disease was calculated in different species. As there is no sufficient information on the numbers of kaka and parakeet, the population size was assumed to be 10,000 (kaka) and 1000,000 (parakeet, sum of all species).

Population size	Required sample size in different prevalence			
	1 %	2 %	5 %	10 %
83	79	65	43	25

Table 3-4. Required sample size to detect a diseased animal in kakapo population (95% confidence).

Population size	Required sample size in different prevalence					
	2 %	3 %	4 %	5 %	7%	10 %
500	129	90	69	56	40	28
1000	138	94	71	57	41	29
10000	148	99	74	59	42	29
infinite	149	99	74	59	42	29

Table 3-5. Required sample size to detect a diseased animal in different population size and prevalence (95% confidence).

Table 3-4 demonstrates the required sample size to detect the presence of disease in population number of 83, the number of kakapo. As there was no positive result in 43 samples of kakapo, it can be concluded that the prevalence of PCV in kakapo population is less than 5 % (95% confidence). The prevalence of PCV in kaka and parakeet populations can be estimated based on table 3-5. As the population size becomes larger, there is limited difference in the required sample size between different population sizes. 79 samples of kaka presented no positive result in PCR assay and it can be suggested that the prevalence of PCV in kaka population would be no higher than 4 %. Once again, same principle can be applied in the postulation of the prevalence of PCV in parakeet population. The prevalence of PCV in wild parakeets appeared to be less than 7% at 95% confidence level.

Discussion

All wild native parrots examined were negative to infection by PCV. This is an encouraging result considering the high prevalence of PCV in exotic parrots in the wild in New Zealand (chapter 2). In addition, the prevalence of PCV in native parrots

could be postulated to be lower than 4 ~ 7 (%) if PCV is present among them. However, the result only suggests that no native parrots examined were currently infected by PCV. In other words, the seroprevalence of PCV which is evidence of previous exposure to the virus was not assessed in this study.

It is uncertain whether native parrots have had a history of exposure to PCV or not. Accordingly, serological tests (HA/HI test) to detect antibodies to PCV are urgently required. The presence of antibodies would indicate that birds have been exposed to PCV and this differs from current infection by PCV or the presence of clinical symptoms of PCD (Raidal et al 1993). If native parrots show antibodies to PCV, it may be concluded that the birds at some stage were exposed to PCV, then have eliminated the viruses and developed immunity to PCV. On the contrary, there may be no sign of antibodies which means native parrots have never been exposed to PCV. In the latter case, this would raise serious concerns because no one can predict the impacts of PCV or PCD in native parrots.

As a consequence of results, further sampling of native parrots is necessary. Blood samples can be used both for PCR assay and HA/HI tests. A greater sample size would allow more precision in the wildlife disease investigations. Sampling from many native parrots in different regions and different age or sex groups is necessary to investigate PCV in wild native parrots accurately. In particular, further investigation on endemic parrots residing at areas known as habitats for both native and exotic parrots is highly recommended, as the native birds have more possibility of contact with exotic parrots and exposure to PCV.

Experimental infection of native parrots by PCV isolates may provide essential information to direct future management on how the virus behaves in native species. These experiments in combination with available testing methods would enable us to examine the susceptibility and sensitivity of native parrots to PCV, the impacts of PCD in native parrots as well as the immunity of endemic parrots to PCV. If native parrots show high resistance to the experimental infection and develop immunity with no trouble instead of being seriously affected, the risk to the native species from PCV can be overwhelmingly decreased. However, it is possible that native species are susceptible to PCV. The result would also examine the possibility of control of PCD by vaccination.

The result of this study has implications for the future management of native parrots in the wild. The high prevalence of PCV in feral exotic parrots strengthens the hypothesis that native parrots should not be in close contact with exotic parrots. Psittacine circoviral disease should be regarded as 'a disease of concern' in the event of translocation or reintroduction of native parrots. When translocating native parrots, identifying the presence of exotic psittacine species and assessing the prevalence of PCV in the destination population as well as the source population would enable disease risks to be anticipated. Further investigation is required before assessments can be made of the risk of PCV to native parrots, and management strategies developed. Vaccination may be a useful management tool but more research is required to assess whether it is necessary.

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Chapter 4. The Isolation of Psittacine circovirus (PCV) in Captive Native Parrots in New Zealand

A red-crowned parakeet (Anonymous 1985)

Introduction

Captive management is regarded as the last solution in the management of many endangered species. There are many factors to take into account in captive management of wild animals such as nutrition, genetic drift, understanding of anatomy and physiology, environment of the enclosure, behavioural disorders due to the stress, control of infectious diseases and other health problems (Fowler 1996). Controlling infectious diseases is one of the major problems in captive management as disease may have disastrous impacts on animals in captivity, in particular, when disease can be amplified by stress (Kirkwood 1996).

Since the status of many native parrots in New Zealand is unstable, intensive management of endangered native species has been encouraged. The kakapo (*Strigops habroptilus*) currently exists only in the wild, yet, they are being intensively managed by the Department of Conservation with the aim of establishing at least one viable, self-sustaining, unmanaged population of kakapo and two or more other populations which may require ongoing management (Creswell 1996). Captive management of native parrots may increase the numbers of a species and accelerate the re-establishment of the species in conjunction with reintroduction programs.

Little is known of infectious disease in native parrots including psittacine circoviral disease (PCD). Native parrots in captivity have been considered to be safe from the threat of PCV based on no previous history of PCV or PCD among them. However, PCV has been identified in many species of parrots worldwide (Todd 2000).

Exotic parrots in captivity in New Zealand have been documented with pathological infections with PCV (Ritchie et al 2003). In fact, Schultz et al (1996) suggested that PCV was one of the main viral infections to exotic parrots in captivity in New Zealand.

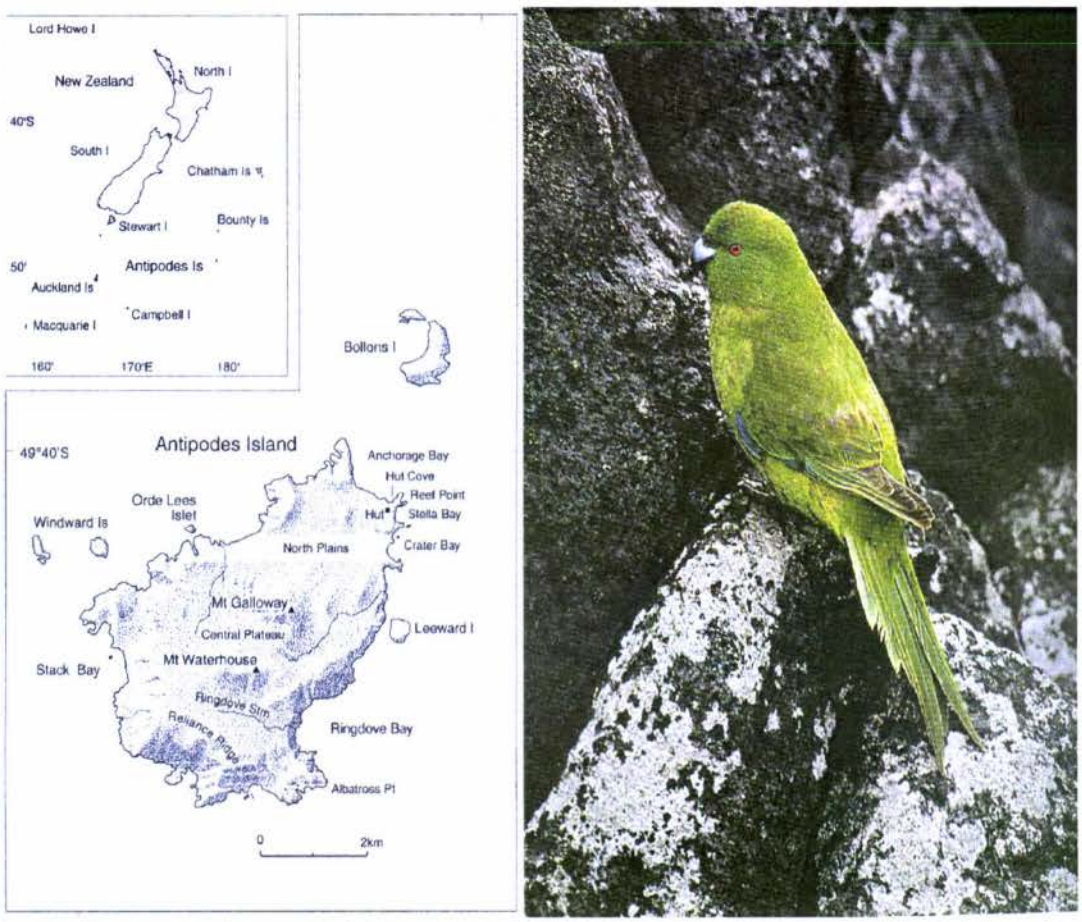
As a consequence, captive facilities in New Zealand have been investigating the presence of PCV in parrots including native and exotic species. Even though PCV has been detected in many exotic parrots (Ritchie et al 2003; I. Anderson, personal communication), there had been no isolation of PCV in native parrots. For example, Auckland Zoo Wildlife Health Research Centre tested 72 red-crowned parakeets, three kea and four kaka for PCV by PCR assay. All of these birds showed negative results in PCR assay. Also, three red-crowned parakeets, three Antipodes Island parakeets, one kaka (Wellington Zoo) and three kaka from Mt. Bruce Wildlife Sanctuary showed no evidence of the infection by PCV.

This chapter presents the first isolation of PCV in a pair of red-crowned parakeets and also a case of an acute death of an Antipodes Island Parakeet suspected to be due to PCV.

Antipodes Island Parakeet

The Antipodes Island parakeet or unicolor parakeet (*Cyanoramphus unicolor*) is a rare species of parakeet in New Zealand. They are endemic to Antipodes Island situated at latitude of 178°45'E and longitude 49°41'S, consisted with one medium size island (6.7 by 3.8 km) and few smaller islands (West et al. 1995). In 1985, the

number of the population in the wild was estimated as 2,000 to 3,000 birds (Taylor 1985 cited in West et al. 1995).



(a) (b)

Figure 4-1. Antipodes Island (a: Greene 1999) and an Antipodes Island parakeet (b: Anonymous 1985).

The Antipodes Island parakeet is the largest of the New Zealand parakeets (males 31 cm, Anonymous 1985) and commonly inhabits and nests in low scrub, dense fern and tussock grasslands (Moon and Lockley 1982; Jacobs 1991).

The Department of Conservation classified the Antipodes Island parakeets as "Category C" threatened species (West et al 1995 and references therein). The IUCN has announced the species as "Vu D2/Vulnerable D2 (IUCN redlist, version 3.1,

2001)". The category Vu D2 means the population is very small or restricted and population is characterized by an acute restriction in its area of occupancy (typically less than 100 km^2) or in the number of locations (typically less than five). Such a taxon would thus be prone to the effects of human activities (or stochastic events whose impact is increased by human activities) within a very short period of time in an unforeseeable future, and is thus capable of becoming Critically Endangered or even Extinct in a very short period.

The Isolation of PCV in Native Parrots

Case 1: The isolation of PCV in a pair of Red-crowned parakeets

Case 1 presents the isolation of PCV in a pair of red-crowned parakeets. It is the first isolation of PCV in native parrots both in the wild and captivity.

Case history

A pair of red-crowned parakeet at a captive facility in the South Island was screened for disease with the aim of the birds becoming foster parents of orange-fronted parakeet. The disease screening included PCV testing and the samples of blood from brachial vein in the wing and feathers were submitted to Equine Blood Typing and Research Centre, Massey University. In PCR assay, the male of the pair tested positive in feather sample but negative in the blood sample. The female was

negative for PCV in both blood and feather samples.

Gross findings

These results initiated the screening for PCV in all the native and some of the exotic parrots at the park. Three staff of the Department of Conservation and two park staff conducted the sampling of two kea, three South Island kaka, 16 yellow-crowned parakeets, four Antipodes Island parakeets, 23 red-crowned parakeets, four sulphur-crested cockatoos, two galahs, and five rainbow lorikeets. The pair of red-crowned parakeets that was tested previously was retested.

In addition to the sampling, physical examination of the body condition was carried out, in particular searching for any feather abnormalities. The male red-crowned parakeet presented feather lesions (figure 4-2 and 4-3). However, this fraying and wear on the feathers suggests only that they have not been replaced recently and do not indicate any virally induced damage.

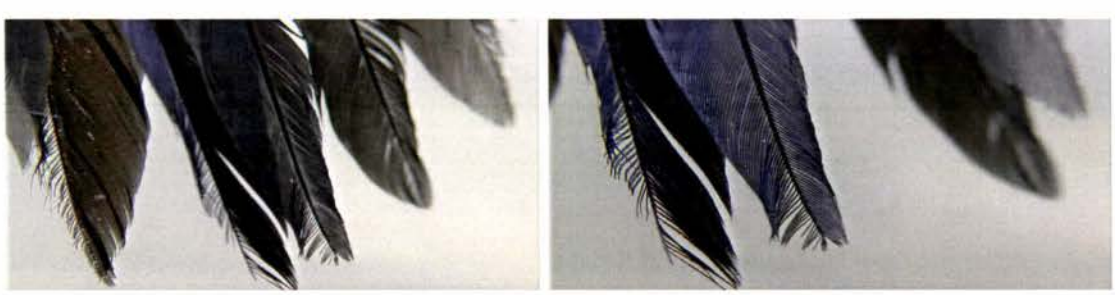


Figure 4-2. Feather abnormalities observed in a male red-crowned parakeet that tested positive to PCV.

Figure 4-3. A male red-crowned parakeet tested positive to PCV. This fraying and wear on feathers do not indicate any virally induced damage.

Several other birds also showed signs of feather losses but none of these signs were typical of PCD (figure 4-4).

Figure 4-4. A yellow-crowned parakeet showing abnormal features of feathers, however, this bird was negative on PCR assay.

PCR assay

In spite of the signs of feather abnormalities in various parrots, in the second PCR assay which was conducted in September, all the birds including a pair of red-crowned parakeet were found to be negative to PCV. A third PCR assay of the original positive bird and its mate was conducted in October. Although the male showed a consistent negative result in the assay, the female had been found to be positive to PCV.

Due to the lack of information of the life cycle of the virus in native parrots and also the difficulty to eradicate the virus in the environment, the pair of the red-crowned parakeets was quarantined and will not be foster parents for the orange-fronted parakeets.

Case 2: The isolation of PCV in Antipodes Island parakeets

Case history

In January 2004, one Antipodes Island parakeet was found dead at a captive facility in the North Island. This occurred shortly after the bird had been translocated from a captive facility in the South Island and been introduced to a group of three Antipodes Island parakeets. There was no sign of illness prior to the acute death. The bird was submitted for post mortem at Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University. Feathers were collected and kept frozen for the screening of PCV.

Gross Findings

The bird was in good body condition (score 6/9) with adequate abdominal fat reserves and body weight was 130 grams. Both eyes were sunken and clear fluid exited around both nares. The liver had a nutmeg appearance with multifocal coalescing bright red lesions measuring between 3-8 mm in diameter on the ventral aspect of the right lobe. There was a 8 mm diameter focus of consolidation on the ventrocaudal aspect of the left lung. The kidneys were deep grey colour.

Histopathology

The liver contained numerous focal areas of mixed inflammatory cells, including lymphocytes, heterophils and macrophages. There were several large areas of acute cellular necrosis near the periphery of the inflammatory cell infiltration. The spleen contained one large central area of recent inflammatory change. The lung interstitium and air capillaries were thickened with evidence of fluid accumulation. Squames and aspirated debris were present in some airways. The kidneys also showed several foci of inflammation comprised of lymphocytes, heterophils and macrophages. No viral inclusion bodies were identified.

Provisional diagnosis

Hepatitis, splenitis, nephritis and aspiration pneumonia had been identified from necropsy. The histopathology was suggestive that the bird had a septicaemia which caused severe liver damage.

PCR assay

In PCR assay, the bird presented a positive result (figure 4-5). As a result, it was decided to examine three Antipodes Island parakeets that were in contact with the bird for a short time. Feathers of the birds; one male and two females, were collected. One female Antipodes Island parakeet was PCV positive.

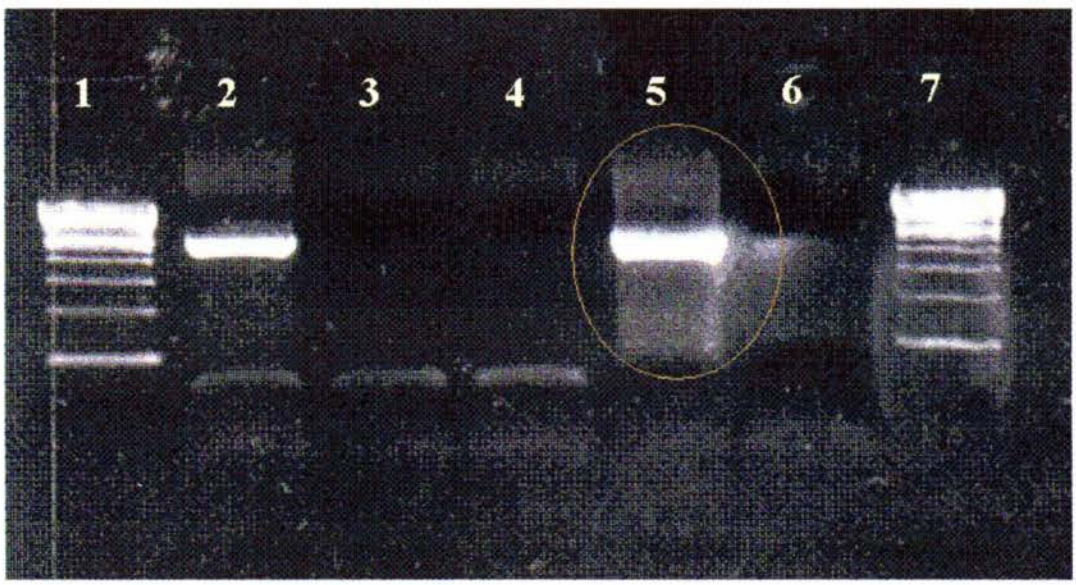


Figure 4-5. Detected virus DNA band in PCR gel (1: positive control, 5: Antipodes Island parakeet).

Discussion

These two cases of the isolation of PCV in captive endemic parrots leave several unanswered questions. The infected birds displayed totally different clinical features and it is currently impossible to foresee the impact of PCV in different native parrot species. Hence, the two cases will be discussed separately.

The isolation of PCV in a pair of red-crowned parakeet

In repeated PCR assay of feathers from a pair of red-crowned parakeets, the male presented results as initially positive, negative and negative while the female was initially negative, negative on second examination and positive one month later. The male presented no clinical signs of PCD except slight feather abnormalities which are not typical of acute PCD. A possible scenario is that the male red-crowned parakeet was recently exposed to PCV prior to the first PCR assay and was transiently positive. Then the bird, eventually, may have eliminated the virus. The female could become infected by preening or contact with virus particles in the aviary. It is more likely that the male red-crowned parakeet was subclinically infected by PCV. Hess et al (2004) demonstrated that PCV DNA was not detected in blood samples of birds who exhibited no clinical signs, while PCV was detected in samples of feathers or cloacal swabs.

Research has verified that virus-infected birds shed large amount of virus to the environment (Ritchie and Carter 1995). Sub-clinically infected birds and carriers are also known to shed the virus concentrations occasionally, indicating the difficulty to eradicate the virus (Raidal and Cross 1994; Hess et al 2004). No effective treatment method to remove the virus from infected birds is known to be possible and the eradication of the virus from the environment appears to be extremely difficult (Ritchie and Carter 1995). Consequently, the pair of red-crowned parakeet was isolated and monitoring is being carried out for further research.

It is essential to reexamine the pair of red-crowned parakeets over an extended period of time to detect virus DNA. There are several possible outcomes to repeated testing for PCV in these birds. If the results are consistently negative for both birds, it is likely that the initial positive results represented a transient infection which has since been cleared. If positive results continue to occur intermittently in these pairs, it is then likely that these birds may be PCV carriers that are shedding virus intermittently. The result will provide more information as to ecology of the virus. The presence of virus particles in the environment should be taken into account in future research.

In addition to the detection of PCV in these birds, the presence of antibodies to PCV needs to be examined. In mature birds that are exposed to PCV, immunity has been commonly observed, instead of disease (Ritchie and Carter 1995). The presence of antibodies, hence, can offer crucial information related to the susceptibility and immunity of the pair of red-crowned parakeets to PCV. For instance, if both birds are proved to be negative to PCV in PCR assay and the virus antibodies are detectable in the serum of the birds, it can be concluded that the birds had been exposed to PCV and transiently infected, followed by the development of an immune response and the elimination of the virus.

It is planned to examine offspring of the pair of red-crowned parakeets when breeding occurs. Vertical immunity is known to be possible as well as vertical transmission (Ritchie and Carter 1995). There is little doubt that the virus or the antibodies to the virus present in the parents may have an impact on the progeny. Only

further research will answer these questions concerning the ecology of the virus, the transmission routes and the susceptibility or immunity of parakeets to PCV.

The isolation of PCV in Antipodes Island parakeets

The death of the Antipodes Island parakeet leave complicated questions regarding the impact of PCV. The questions include; 1) When and how could the bird become infected by PCV?, 2) has PCV caused the acute death of the bird? and 3) if PCV had attributed to the death of the bird, why is there no evidence of viral inclusion bodies?.

As one of three Antipodes Island parakeets who were in contact with the bird previously died showed positive result in PCR assay, it became obvious that PCV is present in the enclosure. However, there is a big question of whether the virus was present in the environment prior to the introduction of the affected Antipodes Island parakeet or not. There are two possibilities. The first possibility is that psittacine circovirus was present within the destination population or the environment and the translocated bird had never been exposed to PCV before the translocation. The exposure to PCV and stress may have caused the acute death of the Antipodes Island parakeet. In fact, the impact of exotic pathogens in translocation of animals is well known, causing disastrous result to naïve source population in combination with stress.

Alternatively, the bird may have been exposed to PCV before the translocation. The subclinical or transient infection by PCV could have been amplified by stress

following the translocation by suppressing the immunity and increasing the sensitivity to PCV. In this scenario, the Antipodes Island parakeet suffered a recrudescence of viral infection and eventually died. This may have resulted in the exposure to PCV of the three resident Antipodes Island parakeets (or at least an Antipodes Island parakeet that tested positive recently) who were in contact with the bird. Serological tests to detect antibodies to PCV will identify the history of exposure to PCV of three Antipodes Island parakeets. However, it is impossible to assume which one of the possibilities was the exact case. It is likely that PCV had played a role in the acute death of the bird but it is not clear.

Post mortem examination and histology proved the presence of severe necrosis and inflammatory changes in liver, spleen, kidney and lung, indicating acute septicaemia. No inclusion bodies were detected. This result is not compatible with the one in PCR assay which presented strong positive result. Few factors that can be suspected for this matter include; 1) samples for histopathology were not fresh, 2) the bird could have been subclinically infected, 3) there were no samples of feather or skin and 4) the sensitivity of histological examination is lower than PCR assay.

Isolation of the virus from affected tissues would have added support to the hypothesis of viraemia, unfortunately no fresh tissues were available. Given that sub-clinically infected birds may shed virus through feather dust and faeces (Hess et al 2004), it can be assumed that the bird could have been infected sub-clinically. Psittacine circovirus is suggested to be epitheliotropic, the detection of intranuclear inclusions is more feasible in samples of epithelial layer of the feathers and feather follicles (Pass and Perry 1985; Todd 2004). Since PCD was not considered in the

differential diagnosis at the moment of post mortem, histopathology was not carried out on skin or feather samples. Additionally, there is a doubt regarding the sensitivity of histological diagnosis (Latimer et al 1992 cited in Ritchie and Carter 1995).

With the current information, confirming the exact reason for the acute death of an Antipodes Island parakeet appears to be impossible. I suggest that there should be ongoing surveillance of the environment and three Antipodes Island parakeets. Strict hygiene procedures should be carried out and other native parrots should be kept away from the environment. Intensive monitoring for any abnormalities caused by PCD, reexamination for PCV and the detection of antibodies to PCV in the parrots will provide crucial information on the ecology of the virus, the impact of PCD on parakeets and immunity of the birds to PCV.

Management of PCV in captivity

The strategies recommended to control PCV are mainly focused on prevention (Gerlach 1994 cited in Todd 2000) rather than eradication due to the strong resistance and resilience of PCV to various control measures (Ritchie and Carter 1995). An anecdotal report deals with the occurrence of PCD at a low prevalence in spite of strict hygiene at a captive facility, again indicating the complexity of control of the virus (Raidal and Cross 1994). Screening for PCV is required prior to animal movement to prevent the introduction of the virus into captive facilities.

The main principle to prevent the exposure of native parrots in captivity to PCV

is to keep native parrots in separate enclosures from exotic parrots. Considering the theory that PCV is viable for up to 3 years in the environment (McWhirter 2000 cited in Mander et al 2003), at least 3 years of evacuation of exotic parrots is required if enclosure is considered to be used for native parrots. Strict hygiene procedures should be implemented in handling of parrots, for example, use of disinfectants between animals and disinfection of equipments. As exotic parrots in the wild have the potential for greater environmental contamination, the enclosures of native parrots should be protected from the contact with feral exotic parrots by modifying the enclosure if necessary, such as using solid roofing, disposal of waste food and double wiring.

Virus and antibody detection should be carried out on the parrot populations in captivity including native and exotic ones. Additional trials to detect the virus in enclosures or related supplies are also useful to understand the incidence of PCV at captivity. In the event of discovery of PCV positive birds, virus-infected birds should be isolated. The area should be cleaned and disinfected repeatedly. Birds at the same flock need to be reexamined after a month, considering the minimum incubation period of PCV as 21-25 days (Ritchie and Carter 1995 and references therein). The keepers involved with infected birds should not have contact with native parrots or other parrot enclosures (Ritchie and Carter 1995).

The possibility of vaccination as a control method has been demonstrated by research (Raidal and Cross 1994; Ritchie and Carter 1995). Several facts support the feasibility of vaccination;

1. in mature birds, the exposure to PCV has led to development of immune

response (Raidal et al 1993),

2. birds developed immune response following by vaccination using inactivated virus purified from diseased tissue (Raidal and Cross 1994),
3. chicks from vaccinated hens exhibited temporary protection to infection with PCV (Ritchie et al 1992 cited in Ritchie and Carter 1995) and
4. virus isolates from different genera of psittacine birds could infect a wide range of bird in different genera (Ritchie et al 1990).

The application of vaccination will be much easier and simpler in captivity than in the wild. Recommended measures to secure native parrots from the threat of PCV include;

1. disease screening for PCD in the event of importation of new parrots into a captive facility,
2. isolation of native parrots from exotic ones,
3. testing for PCV and the antibodies in parrot populations,
4. appropriate hygiene protocols,
5. vaccination, if it appears to be available and effective.

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Chapter 5. General Discussion

Brett Gartrell conducting post mortem on a kakapo.

There is a worldwide decline in populations of parrot species. The major reasons for the decrease can be summarised as habitat destruction, insufficient food or water availability, non-human predation, commercial aviculture or pet trade, persecution as crop pests, natural occurrences and diseases (Wirminghaus et al 1999). There has been more concern about the effects of infectious diseases recently (Alley 2002; Mander et al 2003). A wide range of disease has been reported in New Zealand native species (Alley 2002), suggesting the importance of considering disease issues in wildlife management in New Zealand. Psittacine circovirus has not been considered as a threat to native parrots and the available information has not been sufficient to apply to the management of these species. The findings of this study provide the first baseline information and indicate an urgent requirement for further research concerning the effect of PCV on endangered native parrots.

There are worldwide records of psittacine circovirus (PCV) in various species of parrots (Todd 2004). Psittacine circovirus is the causative agent of psittacine circoviral disease (PCD) which has been typically described as causing beak and feather abnormalities in psittacine birds. Psittacine circovirus is known to be endemic to Australia (Pass and Perry 1985; Raidal et al 1993; Ritchie and Carter 1995) and is the most common virus among feral Australian parrots (Pass and Perry 1984; McOrist et al 1984; Raidal et al 1993; Bassami et al 2001; Ritchie et al 2003).

Psittacine circovirus may have been introduced to New Zealand through the importation of exotic parrots (Mander et al 2003). Two species of exotic parrots; sulphur-crested cockatoo and eastern rosella (*Platycercus eximius*) have established self-sustainable populations in the wild in New Zealand (Chambers 2000). If these

exotic parrots have been exposed to or infected by any of the exotic diseases, they have great potential to spread exotic pathogens to naïve endemic species. Although there were intentional releases of exotic parrots to the wild, there have been attempts to eradicate these species as a result of regarding them as crop pests (Polkanov and Greene 2000; Polkanov and Keeling 2002). Despite these attempts to control them, exotic parrots have expanded in some areas (Heather and Robertson 1996). Eastern rosellas are predominant as they have established a large population in the wild and expanded into most of the North Island as well as Otago peninsula and the tip of the South Island (Bull et al 1985; Chambers 2000; OSNZ in press).

The spread of exotic parrots suggests an increasing risk of native parrots being exposed to PCV. Yet, the interaction between native and exotic parrots within their common distribution has not been examined nor studied. That leaves a question of 'how likely is it that native parrots will be exposed to PCV from exotic parrots?'. As a consequence, several observations of coexistence between native and exotic parrots in the wild were made. Some examples are described below.

- Ada Platt reserve (Maraetai) (personal communication M. Fordham)

  Over summer one can often see eastern rosellas, and kaka are common at the Ada platt reserve at Maraetai. There were several nesting boxes in the bush for the kaka to use. However, the kaka don't appear to have nested in this reserve but all of the nest boxes have been used by eastern rosella for nesting. The kaka come to the area when the Rewarewa and Pohutukawa trees are in flower. The eastern rosella appear to be there all year around.

- Tiritiri Matangi Island (personal communication, M. Fordham)

On Tiritiri Matangi Island, there used to be eastern rosellas whose numbers slowly increased to approximately 12 or more. They used to nest on the island in the pine and gum trees near the rangers' house. Also, a nest was found in Wattle Valley. Sometimes, small mixed flocks of both species, mostly eastern rosellas with a few red-crowned parakeets had been observed feeding on the ground. There was a concern that the eastern rosellas would start to push out the red-crowned parakeets but this never happened. As the number of red-crowned parakeets has continued to increase on the island, the number of eastern rosellas dropped.

Figure 5-1. The map of areas described.

On Tiritiri Matangi Island, there are periodic visits from kaka from November through summer. However, they don't appear to have any association with the red-crowned parakeets or eastern rosellas.

- Nga Manu

Waikanae, where Nga Manu Nature Reserve is located, is known as habitats for various kinds of parrots including sulphur-crested cockatoo, eastern rosella, red-crowned parakeet and kaka. The presence of kaka appears to be periodic, occurring mainly in summer but other parrots inhabit the area all year round. While red-crowned parakeet and eastern rosella are commonly sighted, kaka and sulphur-crested cockatoo are rare. Often the red-crowned parakeet and eastern rosellas have been observed to feed on the ground in the same flock.

- Karori Wildlife Sanctuary

Karori Wildlife Sanctuary is a 252 ha of fenced reserve for native fauna and flora in New Zealand, located in Wellington city. The area has been being intensively managed under the aim of restoring the vegetation and fauna structure to the original condition prior to human interference. In cooperation with predator control programs, e.g. possums, rats, and mice, the sanctuary has been achieving its goal to provide a safe new habitat to various kinds of native species.

The only exotic vegetation in the sanctuary is a patch of pine forest. Kaka have often been observed in the pine trees, presumably feeding on the cones. There are food and water stations in the sanctuary for some species and some of these are designed for kaka. Wellington region is known as a

habitat for eastern rosellas and sulphur-crested cockatoos. The number of sulphur-crested cockatoos is low. On the contrary, the eastern rosellas can be found in many places, including Karori Wildlife Sanctuary. The rosella population is often seen feeding or roosting around the pine plantation. Also, the food and water stations for kaka are attractive to the rosellas, especially in a cold winter when the food supply is scarce.

These observations demonstrate that native parrots are sharing feeding and roosting habitat with exotic parrots and that there is a very real risk of exposure to PCV.

Even though the concern of infectious diseases has been mostly ignored in the management of these species, it is becoming increasingly obvious that the occurrence of exotic disease has the potential to cause serious perturbations in small endangered populations (Mander et al 2003). Psittacine circovirus is not a major concern in large, thriving populations; however, it can cause serious damage to already endangered species. This study provides the first baseline information on the presence of PCV in New Zealand but there is no information on the history of the exposure of native New Zealand parrots to PCV.

The major findings of this study can be summarised as three points;

1. The prevalence of PCV in wild exotic parrots in New Zealand is high, being in the range 19.17 - 44.02% in eastern rosellas using 95% confidence intervals and 22.04 - 33.07% in sulphur-crested cockatoos.

2. No evidence of PCV was detected in wild native parrots. Given the level of sampling, prevalence is likely to be less than 4-7% if PCV is present at all within these species.
3. Some native parrot species are susceptible to infection by PCV.

1. High prevalence of PCV in feral exotic parrots in New Zealand.

The prevalence of PCV in feral exotic parrots in New Zealand appears to be higher than in parrots in the wild in Australia which was demonstrated as 10-20% (McOrist et al 1984). Caution is advised when comparing these two studies however, as McOrist et al's (1984) study was based on clinical signs of disease which is much less sensitive than the PCR assay used in this study. The higher population density of exotic parrots in New Zealand than Australia may be a possible reason for the high prevalence of PCV. For instance, the mean flock size of eastern rosellas in New Zealand has been demonstrated as 3.6, with 17.0% sighting of more than 5 birds (Woon et al 2002). The mean flock size of eastern rosellas in Australia has been reported as 1.9 (in Queensland and New South Wales) and 2.9 (in Belair NP and Adelaide) (Higgins 1999).

Considering that this prevalence values simply reflect the proportion of individuals currently infected by PCV, it is very likely that PCV is widespread within feral exotic parrots. Additional information concerning the seroprevalence of PCV is lacking. The presence of antibodies indicates the history of previous exposure to PCV of the individuals, thus, the seroprevalence can be a more precise indicator of how active and widespread PCV is within a population. Results of prior research show that

mature and healthy individuals commonly develop immune response when they are exposed to PCV instead of being clinically infected (Ritchie and Carter 1995), that seroprevalence is much higher than the prevalence of current infection, and that the level of antibodies often show features inconsistent with clinical signs (Ritchie et al 1991; Raidal et al 1993).

One significant point in the incidence of PCV in exotic parrots is that infected birds may demonstrate no clinical abnormalities. This can occur not only in clinical examination but also in histological examination. Indeed, no feather or beak deformity was detected during the sampling of PCV positive parrots and this will remain a challenge in attempts to control PCV in feral exotic parrots. In this matter, it will be extremely difficult to eradicate PCV from the environment. Culling the whole population of wild exotic parrots is likely to be the only successful method to ensure eradication of PCV and the current population sizes make this proposition logistically impossible.

2. Lack of evidence of PCV in feral native parrots in New Zealand.

A hundred and sixty-eight wild native parrots tested in this study presented no evidence of current infection by PCV. There are a few possibilities to explain this result. 1) The prevalence of PCV in native parrots is low, thus, no PCV positive individual was identified in the sampling; 2) Native parrots may be free from PCV with no history of exposure to PCV; or 3) native parrots in the wild may be free from PCV despite being exposed to PCV but with low susceptibility and the development of an appropriate immune response. No baseline information in relation to the

incidence of PCV in wild native parrots is available to date and it is impossible on existing information to determine which hypothesis is correct.

If a low sample size is a reason for not detecting a diseased parrot, more sampling will allow the identification of the presence of PCV in native parrots. Being free from PCV would be the best scenario for native parrots; however, identifying if these species have a history of exposure to PCV is crucial. This can be achieved by serological examinations which are designed to detect virus antibodies. The presence of antibodies to PCV indicates previous exposure to PCV and the occurrence of a successful immune response to PCV in native parrots. If PCV antibodies are commonly present among native parrots that have tested negative to PCV, the threat of PCV to native parrots is diminished. On the other hand, if native parrots show no evidence of infection by PCV and no antibodies to PCV, the impact of PCV on native parrots is unpredictable. Exotic pathogens can cause devastating results when naive individuals are exposed to them (Wirminghaus et al 1999) and the risk to native parrots from PCV could be considerable.

3. The isolation of PCV in captive native parrots.

This study demonstrates that PCV can infect native parrots. To date, this has only occurred in parakeet species; nonetheless, it is possible that other species of native parrots will be susceptible to PCV as well. The next step will be to identify the impact of PCV in different species of native parrots and in different age groups. The infection by PCV may develop into PCD, may have little impact resulting in subclinical infection or the birds may mount an appropriate immune response to

defend themselves against PCV infection. Psittacine circovirus positive birds that remain asymptomatic have been reported (Ritchie and Carter 1995) and this was observed in this study during the sampling of eastern rosellas. While an Antipodes Island parakeet died presumably under the impact of PCV, no other PCV positive parakeets have shown serious abnormalities. Further monitoring of these parakeets will provide useful information on this matter.

There are several possible scenarios for the impact of PCV in native parrots in New Zealand.

1. Native parrots may be infected by PCV but develop appropriate immunity to it;
2. PCV may cause fatal disease in some species of native parrots or young birds; or
3. PCV may bring serious losses to a wide range of species if native parrots are extremely susceptible and have never been exposed to PCV.

However, it is impossible to predict the impact of PCV and assess the risk of native parrots from PCV from current information. This study has confirmed that exotic parrots in the wild do have a high prevalence of PCV. These parrots are increasing or expanding their range in some areas and native parrots are increasingly coming into contact with exotic parrots.

Future research avenues

There are requirements for future studies to secure native parrots from the threat of PCV. Identifying the population size, distribution and ecology of exotic parrots in the wild is essential. This will allow assessment of the possibilities of native parrots coming into contact with exotic parrots and PCV. This can be used in conservation management of native parrots, in case wild exotic parrots need to be controlled. In specific areas where exotic and native species coexist, the behaviour or interaction between species should be observed as this will increase our understanding of the possible effects of exotic parrots on native species.

Continued monitoring on the native parrots that have tested positive to PCV is essential. There is no available information on the impact of PCV and PCD in native parrots. Extended periods of monitoring on these species is the first step to understand the impact of PCV. Severe impacts of PCD in neonatal and young birds are reported (Pass and Perry 1985; Ritchie and Carter 1995). The offspring of the native birds positive to PCV should be intensively monitored and examined to detect presence of PCV or the impact of PCD and antibodies to PCV when breeding occurs.

Further investigation for the presence of PCV and antibodies to PCV in wild Antipodes Island parakeets is required. Only captive Antipodes Island parakeets were examined in this study. The result will allow us to understand the prevalence and seroprevalence of PCV in native parrots on an isolated island.

Further sampling of exotic and native parrots both in the wild and captivity is

recommended. It is possible that the prevalence of PCV may be different with different sample sizes and over different periods. Regular sampling and examination of parrots will be useful. Besides, there is an urgent requirement to identify the seroprevalence of PCV in native and exotic parrots by detecting antibodies to PCV. This will provide precise information on the epidemiology of PCV in New Zealand. The importance of identifying seroprevalence becomes greater in native parrots as it is crucial to discover the history of exposure and immunity to PCV.

Experimental infection of native parrots with PCV is recommended. This is necessary to identify the impact of PCD in native parrots and to evaluate the susceptibility or sensitivity to PCV in different species. The clinical signs should be documented in different species, age or sex groups. Experimental infection will also provide the opportunities to observe the immunity of native parrots to PCV, and thus, the possibilities of vaccination as a preventative tool can be examined.

The precautionary principle of keeping native parrots away from exotic parrots or from contaminated environments should be applied in the management of native species, although the impacts of PCV and PCD in native parrots are not yet sufficiently understood. Especially in the event of translocation or reintroduction of native parrots in the wild, the presence of exotic parrots should be investigated first. This should be followed by the examination of native parrots from the source population and exotic parrots from destination population for the presence of PCV and antibodies to PCV. In other words, PCV should be taken into account in health quarantine programmes prior to the movement of native parrots.

Regular sampling efforts for parrot species are necessary to identify the prevalence of PCV. The results will help to define appropriate management plans. Post mortem examination of native parrots is necessary, as this will enable the managers to recognise the presence of any infectious diseases and additional hazards.

In captivity, my recommendation to prevent or control PCV is to separate native parrots from exotic parrots, as PCV has been identified in different exotic parrots in New Zealand (Ritchie et al 2003). Examination of native and exotic parrots by PCR assay and HA/HI assay will be the next step. If any of the parrots examined exhibit evidence of current infection, then the birds should be isolated and strict hygiene protocols should be applied in the environment. Monitoring on PCV positive birds and contact birds for any abnormalities will provide useful information on the impact of PCD. The keepers involved with these birds should ideally not have contact with native parrots, as they have a potential to spread the virus to new environment (Ritchie and Carter 1995). In the event of importation of new parrot species, the screening for PCV should be included in the health check. This will reduce the risk of PCV to destination populations as well as the parrots being introduced to the new environment. Most of all, the feasibility of vaccination of native parrots should be investigated for prevention of infection by PCV. If the vaccination appears to be effective and safe, this method will remarkably decrease the risk of native parrots from PCV.

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