ENZOOTIC PSITTACOSIS AMONGST WILD AUSTRALIAN PARROTS[1]

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THERE are still many obscure points in regard to the epidemiology of psittacosis, particularly concerning the distribution of the virus in apparently healthy parrots. Recently, it has become evident that knowledge concerning the condition of native Australian parrots in the wild state was urgently needed. During the European and American pandemic of 1929–30, the birds incriminated were mostly South American parrots and the great majority of the cases could be traced fairly directly to one or two definite shipments of birds. Further, these cases were mostly associated with sick and dying parrots, not with healthy birds.

With the subsidence of the pandemic small outbreaks have continued to occur, particularly in Germany and the United States of America, and, in both these countries, the birds responsible have been locally bred—*Melopsittacus undulatus*, the Australian budgerigar, shell parrakeet or Wellensittich. In Germany, there were 145 cases of psittacosis with twenty-six deaths in the first seven months of 1934, and Fortner and Pfaffenberg (1934) examined many birds associated directly or indirectly with these cases. Fifty-two birds infected with the virus of psittacosis were found, and of these forty-nine were budgerigars.

According to Meyer and Eddie (1934*a*) 123 out of 150 cases occurring in Canada and the United States of America since 1929 could be attributed to contact with infected budgerigars. Meyer and Eddie made an extensive investigation of the budgerigar breeding establishments in California and found a surprisingly high prevalence of infection by the virus. More than 100 aviaries were examined and 52 per cent. found to contain infected birds. The percentage of birds in a given aviary which were infected varied from 10 to 90 per cent. Frank outbreaks of the disease with sickness and high mortality were relatively rare, most of the infected birds being apparently healthy and showing at autopsy only an enlarged spleen. The degree of infection varied with the age of the bird, enlarged spleens containing virus being much commoner in young (< eight months) than in older birds.

In order to obtain a healthy stock of budgerigars for experimental purposes, Meyer and Eddie (1934*b*) imported 200 birds freshly caught near Adelaide.

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Certain deaths occurred amongst these parrakeets in California and, although they had been carefully isolated from any source of infection, psittacosis virus was isolated in ten instances. Although no record of psittacosis in Australia either in parrots or human beings had appeared prior to 1934, epizootics amongst parrots have been noted, and Merrilees (1934) has recently given a retrospective account of an epizootic in 1930 which he considers was almost certainly due to psittacosis. Two cases of illness in human beings were associated with this epizootic and may have been psittacosis infections. The stimulus to the present investigation was provided first by Meyer and Eddie's results and secondly by a report received by the Commonwealth Department of Health that cargoes of Australian parrots had arrived in London heavily infected with psittacosis.

PRELIMINARY SURVEY OF INFECTION AMONGST AUSTRALIAN PARROTS

During the period over which this work was carried out there were no epizootics reported from aviaries, nor were any human cases of psittacosis notified. With a few exceptions the birds examined were apparently healthy parrots and cockatoos of the common Australian species. A number of batches of parrots were purchased from dealers in Adelaide and Melbourne. These had probably all been caught in the wild state and had been in captivity for relatively short periods, but under crowded conditions which would facilitate transfers of infection. No opportunity has arisen to investigate aviaries in which budgerigar breeding is carried out. The parrots obtained from dealers were at once found to include many carriers of psittacosis virus and a few birds with definite signs of active infection. As soon as it was established that the common species from these sources were frequently infected, attention was concentrated on parrots recently caught and examined immediately on arrival from the country. Through the co-operation of the Commonwealth Department of Health representative species were obtained from each of the other states as well as a larger supply of Victorian parrots caught mostly near Kerang.

The routine method of examination was to kill the birds with coal gas, the cage being completely surrounded with a cloth soaked in lysol solution. The dead birds were then drenched liberally with lysol solution, plucked and their organs examined. The state of nutrition, presence or absence of anal soiling, size and appearance of spleen, condition of the walls of the abdominal air sacs and of the liver were noted and the spleen was removed to a tube containing 1 c.c. of broth. The spleen was emulsified in the broth by the use of a sterile glass rod and a little powdered quartz. The emulsion was plated on blood agar and injected into two mice, usually intraperitoneally; occasionally intracerebral inoculations were also made. When no pathological changes were observed, all the small spleens of each species were pooled and the combined emulsion inoculated into two mice. None of these small spleen pools contained any demonstrable virus.

The mice were kept in individual glass jars and examined when dead, or, if surviving, when killed about the tenth day after inoculation. The diagnosis was made primarily by the demonstration of L.C.L. bodies in smears from the cellular peritonitic exudate around

the liver and spleen. In a few instances typical pathological changes were present, but no L.C.L. bodies were visible in the smear. Subinoculation of peritoneal washings or liver emulsions in these instances gave typical lesions in which L.C.L. bodies could be demonstrated.

The results are tabulated according to species and locality in Table I. The birds are divided into three groups: (*a*) those with enlarged spleens (more than twice the normal diameter) from which virus was obtained by mouse inoculation, (*b*) those with enlarged spleens which contained no demonstrable virus, and (*c*) those with normal spleens also containing no demonstrable virus. In the case of two batches of grass parrots with many large spleens, a proportion only of these were tested. Since all were found to contain virus those birds in the same batches with similar enlarged spleens and air-sac lesions are included in the proved psittacosis group. Group (*b*) is shown because Meyer and Eddie found that in Californian budgerigars infected young birds as they grew older tended to lose their infectivity but to retain the large spleen. They found that the largest spleens occurred in such parrots and were much less liable to be infective for mice than the moderate enlargements found in younger birds. Several of the largest spleens found in the present work were non-infective, and it is highly probable that conditions here are similar to those in California and that the enlarged non-infected spleen in the absence of any other demonstrable cause can be taken as presumptive evidence of past psittacosis.

If we can accept the birds examined as fair samples of the species they represent, we may conclude that grass parrots and cockatiels from Victoria and Queensland lorikeets are heavily infected with psittacosis in the wild state. The rosellas are much less frequently infected, and a majority of those showing enlarged spleens are non-infective for mice as judged by the present technique. Conditions amongst the cockatoos seem to be analogous to those found with the rosellas—enlarged non-infective spleens being relatively frequent. A comparison of galahs (*Kakatöe roseicapilla*) from Queensland with those from Victoria indicates how the degree of infection of a given species may differ in different localities. Six Queensland galahs all showed very large spleens, one of which induced typical psittacosis in mice. The others had presumably also been infected in the past. On the other hand, in twenty-seven galahs caught near Kerang (Victoria) only one enlarged spleen was found and this was non-infective for mice.

Characteristic of Australian strains of psittacosis virus

There are slight differences in virulence between strains of different origin, but, on the whole, all the Australian psittacosis strains we have isolated conform to a general pattern. No infection experiments with parrots were carried out owing to the impossibility of obtaining uninfected stocks and of housing experimental birds with safety.

Table I. *Evidence of psittacosis in Australian parrots*

Genus	Species	Locality	How obtained	Proved psittacosis	Enlarged spleen without demonstrable virus	Normal spleen	Total	
Trichoglossus (lorikeets)	*chlorolepidotus*	Brisbane, Queensland	W	4	1	1	6	
	moluccanus	,, ,,	W	3	1	2	6	
			Total	7 (58%)	2 (17%)	3 (25%)		12
Kakatōe (cockatoos)	*galerita*	Kerang, Victoria	W	0	4	8	12	
	roseicapilla	,, ,,	W	0	1	26	27	
	,,	Brisbane, Queensland	W	1	5	0	6	
	,,	Melbourne, Victoria	D*	2	0	0	2	
			Total	3 (6%)	10 (21%)	34 (72%)		47
Leptolophus (cockatiels)	*hollandicus*	Adelaide, South Australia	D	2 (+2)†	0	0	4	
	,,	Kerang, Victoria	W	2	0	4	6	
			Total	6 (60%)	0	4 (40%)		10
Platycercus (rosellas)	*elegans*	Melbourne, Victoria	D	2	3	1	6	
	,,	Gippsland, Victoria	W	0	2	23	25	
	,,	Brisbane, Queensland	W	0	0	6	6	
	adelaidae	Adelaide, South Australia	D	0	0	4	4	
	adscitus	Brisbane, Queensland	W	0	0	7	7	
	eximius	Adelaide, South Australia	D	2	0	3	5	
	,,	Melbourne, Victoria	D	1	5	12	18	
	,,	North Coast, New South Wales	W	0	8	20	28	
	,,	Brisbane, Queensland	W	0	1	5	6	
	icterotis	Katanning, Western Australia	W	0	0	3	3	
			Total	5 (5%)	19 (18%)	84 (78%)		108
Barnardius	*semitorquatus*	Katanning, Western Australia	W	0	1	4	5	5
Psephotus	*haematonotus*	Adelaide, South Australia	D	2	0	9	11	
(grass parrots)	,,	Melbourne, Victoria	D	4 (+5)‡	0	3	12	
	,,	Kerang, Victoria	W	5 (+9)‡	0	16	30	
	,,	,, ,,	W	3	0	12	15	
			Total	14 (+14) (41%)	0	40 (59%)		68
Melopsittacus (budgerigars)	*undulatus*	Melbourne, Victoria	D	1	1	4	6	
	,,	Kerang, Victoria	W	0	0	15	15	
			Total	1 (5%)	1 (5%)	19 (90%)		21
Neophema	*elegans*	Katanning, Western Australia	W	0	0	3	3	3
			Grand total	50 (18·2%)	33 (12%)	191 (70%)		274

* Two galahs received dead from Melbourne households.

† All four birds had similar large spleens—two pooled emulsions each of two spleens were both infective for mice.

‡ All these birds had enlarged spleens and thickenings of air-sac walls: only a proportion were tested by mouse inoculation. Since all so tested were infective the untested spleens are presumed also to have contained virus.

D = obtained from dealers. W = examined shortly after bird caught.

Identification of species: The specific names are in accordance with the *Official Check-list of the Birds of Australia*, 2nd ed. 1926, and the species have been determined mainly by reference to *What Bird Is That?*, by N. W. Cayley (Sydney, 1933).

When mice are inoculated intraperitoneally with successive tenfold dilutions of a 5 per cent. emulsion of liver from a typically infected mouse, the undiluted material usually kills in four to six days, but it is rare for any of the other dilutions to kill within ten days, although some of the mice may look ruffled and not very active at that period. When the mice are killed and examined on the tenth day, those which received the higher concentrations of virus show gross subacute peritonitis. There is often free turbid peritoneal fluid, and the liver and spleen are more or less heavily coated with sheets of white fibrino-purulent lymph. This inflammatory exudate is frequently thickest above the liver, and more often than not the pleural cavities are to some extent involved in a similar process, particularly the mediastinal pleura. When the coating of lymph is peeled off the liver the parenchyma of the organ appears to be normal: there is no macroscopic evidence of necrotic changes. The spleen is enlarged, paler than normal and rather coarsely mottled. With the lowest infecting doses of virus the lesions may be much milder and show little more than a stickiness of the peritoneum and a trace of purulent material between the liver and the diaphragm. With most strains liver emulsions are infective in a dose of 0·5 c.c. of a $1 : 10^5$ dilution of fresh 5 per cent. emulsion. Weaker strains may only be active to $1 : 10^3$.

The characteristic L.C.L. bodies are very numerous in smears of the inflammatory lymph from a typical infection but are sometimes not found in smears from mild lesions which can be shown to be typically infective by subinoculation. Castaneda's stain has been used almost exclusively, and the appearances of the L.C.L. bodies are precisely as described and figured for classical strains. In doubtful mice the most suitable material for smears seems to be one of the peritoneal folds around the liver such as the falciform ligament or the lesser omentum. If these look at all cloudy the mouse is probably infected and smears from them will show L.C.L. bodies.

In most descriptions of the lesions produced in mice, the most prominent feature reported has been the presence of necrotic areas or other forms of parenchymatous degeneration of the liver. This has been seen in only one of the primary mouse inoculations from infected spleens in the present series. Occasionally, typical liver changes have been observed in subinoculations, but no strain has developed a capacity to produce them with regularity. Strain K44 from a freshly caught grass parrot has been used for the greatest number of mouse inoculations. Of forty-seven mice showing definite lesions forty-one presented the usual peritonitic picture without liver damage, six showed definite necrotic patches sometimes very widespread in the liver.

In most of this work, psittacosis strains were not kept continuously active by mouse passage. Growth on the egg membrane was more frequently used with an occasional mouse inoculation. Recently, three strains, K44, K121 and Q168, were subjected to continuous mouse to mouse passage. All three are now giving a high proportion of classical liver necroses, particularly when young mice are used.

The relative infrequence of liver necroses and the predominance of peritonitic changes may indicate a difference in virulence between the Australian strains and the classical strains isolated from human material in 1930. In the absence of a direct comparison with such a strain it is of course possible that the difference is due to dissimilarities in the mice used rather than in the viruses, but this seems unlikely.

Apart from mice the pathogenicity of the virus has been tested by intratracheal inoculation in monkeys and by intranasal instillation in rats. A severe pneumonia was induced in each species. Five monkeys in all have been inoculated intratracheally with from 1 to 2 c.c. of fresh mouse liver emulsion of strain K44. All appeared apathetic and sick during the period from about the third to the eighth day, although none died of the infection. One monkey was killed seven days after inoculation and showed a typical psittacosis pneumonia of the type described by Rivers and Berry (1931). Patches of bronchopneumonic consolidation were present in all lobes on the right side, the process appearing to radiate from the hilum; an extensive area was similarly situated in the left lung. The consolidation in the affected areas was not complete and the corresponding pleural surface was irregularly wrinkled and nodular when the lungs were removed from the body. L.C.L. bodies were not observed in smears, but subinoculation of an emulsion of consolidated area to mice gave typical illness and the mice were killed when moribund at six and seven days. Both showed peritonitic exudate with L.C.L. bodies and both had unusually definite parenchymatous changes in the liver.

A companion monkey similarly inoculated killed on the fourteenth day showed an extensive infarct-like consolidation of the right lower lobe rather sharply marked off from the normal lung by a haemorrhagic zone. The consolidated area in this case also contained psittacosis virus but apparently in much smaller amount. At ten days one mouse was sick and post-mortem showed typical peritonitic lesions with numerous L.C.L. bodies. The other appeared healthy; post-mortem, there was slight peritonitic exudate of typical distribution, but no L.C.L. bodies were seen in smears.

Histologically, the seven-day lesion corresponded precisely with Rivers and Berry's description (1931). A few alveoli and one bronchiole were filled with fibrin, but the consolidation was predominantly due to multiplication of alveolar cells and infiltration with mononuclear cells. A fair number of polymorphonuclears were present in some parts of the section. At the edge of the consolidated area the alveolar walls were greatly thickened with similar mononuclear cells, and areas of haemorrhage infiltrating the alveolar walls were present.

The other three monkeys were given two intratracheal inoculations with a ten-day interval and were killed eight and eleven days after the second, when their blood was collected for serological work. Two showed extensive bronchopneumonic areas of the usual type, the third insignificant lesions only.

Rats are not obviously affected by intracerebral or intraperitoneal inocu-

lation of local psittacosis strains but develop a severe pneumonia after intranasal instillation of about 0·5 c.c. of a fresh mouse-liver emulsion. The rats are inoculated under ether anaesthesia. They are held vertically and the inoculum dropped on to the external nares from a Pasteur pipette. With a proper depth of anaesthesia the inoculum is rapidly drawn into the nasal cavity and most of it appears to find its way into the lungs. The rats nearly all showed symptoms on the second day and the deaths all occurred between the second and third days. Of those not killed before the third day, five died and eleven survived. Practically all showed L.C.L. bodies present in smears from pneumonic patches of the lung removed from the first to the third day after inoculation. Subinoculations to mice in a majority of instances resulted in death from peritonitis due to a mixed infection with psittacosis virus and an organism which is almost certainly *Streptobacillus moniliformis*. Strangeways (1933) has shown that this organism is a normal inhabitant of the rat nasopharynx, and it appears likely that the pneumonia induced by psittacosis virus in the rat is in part at least due to the activity of this organism.

The characteristics of the lesion produced by psittacosis virus in the developing egg have been described elsewhere (Burnet and Rountree, 1935). All the strains which have been so far examined give similar lesions.

Fatal psittacosis infections in galahs

Two galahs (*Kakatöe roseicapilla*) have been received from unrelated households in Melbourne suburbs which had clearly died of acute psittacosis. The first had been obviously sick for a few days before death with apathy and greenish diarrhoea. Post-mortem, there was evident wasting of the pectoral muscles. The skin was covered with an erythematous rash, macules 2–4 mm. in diameter, being more or less uniformly scattered over body and legs. The lesions were about 1–2 cm. apart. Internally, there was a profuse semipurulent coating over most of the air-sac walls in smears from which typical L.C.L. bodies were numerous. The spleen was enlarged, about 12 mm. in diameter, and thickly coated with inflammatory lymph. The liver was swollen and finely mottled with shades of greenish brown. Blood from the heart was sterile and, on inoculation into a mouse intraperitoneally, had reduced the animal to a moribund condition when it was killed on the fourth day. It showed a sticky peritonitis with many L.C.L. bodies in a smear from the peritoneal exudate. Liver emulsion killed the mouse inoculated with it on the fourth day with similar findings.

The second bird had been caught about a fortnight before its death. No preceding symptoms had been noted. Post-mortem, it was emaciated but showed no rash. Internally, the most notable feature was a massive fibrinous pericarditis and similar fibrino-purulent thickening of the air sac walls. The spleen was not enlarged (7 mm. in diameter) and the liver showed only slight patchy discolorations. Smears from pericardial and air-sac exudates showed

many L.C.L. bodies. Of two mice inoculated intraperitoneally with an emulsion of the pericardial exudate, one died on the fifth day with the usual peritonitic lesions, and, in addition, definite necrotic patches scattered over the liver. The other killed on the seventh day showed the peritonitic type of lesions only.

These virus strains were evidently of fairly high virulence, both for cockatoos and mice, but no human cases were definitely associated with the sick birds. In one household a child suffered from a brief but fairly severe febrile illness at about the time of the bird's death, but no evidence that it was psittacosis was obtained.

DISCUSSION

The virus strains which have been isolated from these recently caught Australian parrots are undoubtedly true psittacosis strains. They appear to be of distinctly lower virulence for mice than most of the strains described from cases of the human disease and show a greater tendency to produce peritonitic rather than liver lesions after intraperitoneal inoculation. In all other respects, they appear to conform to the characters described for classical strains of the virus.

The results of the present survey would seem to indicate that psittacosis infection is enzootic amongst the commoner wild Australian parrots. The infection extends over such a wide geographical range and involves so many species that it is hard to imagine that it is of recent origin. Importation of parrots into Australia has been prohibited since 1930, and even before that only negligible numbers were imported, so that the likelihood of the disease having been conveyed originally to wild birds by infected parrots escaping from confinement seems remote. All the indications are consistent with the hypothesis that a low-grade form of psittacosis infection has been enzootic amongst Australian parrots for centuries.

The findings observed with wild birds are very similar to those described by Meyer and Eddie for the aviary populations of budgerigars with which they worked. It has not been possible to determine the ages of the parrots which we have examined, but it is probable that the majority are young birds less than six months old. There is no evidence which conflicts with the working hypothesis that the spread of infection in nature is essentially similar to that found in infected aviaries in California. The young birds may be infected in the nest or when they join the flocks—most of the species under consideration are of gregarious habit. The usual result is a non-symptomatic infection with an enlarged infective spleen, but it is probable that a certain proportion of deaths occur. I have been informed by bird catchers that on rare occasions noticeable numbers of dead parrots may be seen in the bush, and more frequently that large numbers of parrots may die soon after being caught. No opportunity of investigating such occurrences has arisen, but it seems very likely that some of these epizootics are manifestations of psittacosis infection. Enlarged non-infective spleens have been frequently observed, and, following

Meyer and Eddie, it is reasonable to regard them as indicative of birds which have been infected with psittacosis but have succeeded in eliminating the virus.

The widespread prevalence of psittacosis in Australia indicates that the enzootic conditions in budgerigar breeding establishments in Europe and America are probably derived from the natural infections of the original Australian birds from which they are descended. Since in both Germany and America locally bred birds of this species have been definitely responsible for human infections it seems highly probable that the Australian virus is potentially liable to cause disease in man, although quite obviously the great majority of the current strains are not highly pathogenic.

Australia can be regarded as the main centre of parrot evolution. Of the six families in the order Psittaciformes five are confined to the Australasian region and there are almost exactly as many species in this region as in the other five zoogeographical regions taken together. There is a possibility that the characteristic virus infection of the order is also a development of Australian origin, but judging from Meyer and Eddie's (1934*c*) report on the presence of psittacosis in birds arriving in California from South American ports it is more likely that psittacosis is also enzootic amongst some at least of the parrots of the neotropical region. If this is substantiated by observations in South America itself, we shall have to regard psittacosis as an almost universally present low-grade infection of parrots which only on rare occasions flares up into the dramatically infective human disease which characterised the outbreaks of 1929 and 1930.

SUMMARY

1. Psittacosis infection is enzootic amongst several of the common species of Australian parrots in the wild state. A large proportion of recently caught individuals of *Psephotus haematonotus*, *Trichoglossus* (two species) and *Leptolophus hollandicus* are infected. In other genera, demonstrable infection is rare, but enlarged non-infective spleens probably indicative of past infection are common.

2. The virus strains derived from wild Australian parrots appear to resemble closely those isolated from human and parrot infections in Europe and America.

REFERENCES

BURNET, F. M. (8. xii. 1934). *Med. J. of Australia*, **2**, 743.
BURNET, F. M. and ROUNTREE, P. (1935). *J. Path. and Bact.* (in the press).
FORTNER, J. and PFAFFENBERG, R. (1934). *Z. f. Hyg.* **116**, 397.
MERRILEES, C. R. (8. ix. 1934). *Med. J. of Australia*, **2**, 320.
MEYER, K. F. and EDDIE, B. (1934*a*). *Klin. Wochschr.* **13**, 865.
—— (1934*b*). *Proc. S. Exp. Biol. and Med.* **31**, 917.
—— (1934*c*). *Science*, **79**, 546.
RIVERS, T. M. and BERRY, G. P. (1931). *J. Exp. Med.* **54**, 129.
STRANGEWAYS, W. I. (1933). *J. Path. and Bact.* **37**, 45.

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