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Chlamydiosis in birds in Great Britain

1. Serological reactions to chlamydia in birds sampled between 1974 and 1983

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SUMMARY

A total of 6593 serum samples from birds, received for diagnostic testing or surveys between 1974 and 1983, were titrated by direct complement fixation (CF) test against *Chlamydia psittaci* antigen. The percentage of positive reactions found was variable for domestic poultry, ranging from 1·5 in chickens to 22·2 in geese, and was highest in pigeons *Columba livia* (47·3) and collared doves *Streptopelia decaocto* (51·4). A moderate incidence was found for game birds (29·0), wild ducks (23·3) and imported birds of the order Psittaciformes (15·9). The highest titres were found in pigeons, collared doves and psittacines, confirming that infection is both frequent and active in these birds in Britain. Results of isolation of chlamydia from similar sources will be reported subsequently.

MATERIALS AND METHODS

This paper summarizes the results of testing serum samples from birds for chlamydia by the CF test as performed at this laboratory during the 10 years from 1974 to 1983. These samples were obtained in two ways: either they were submitted to us with a request for aid in diagnosis or for export certification, or they were collected for us at our own request for surveys of prevalence of infection. In presenting the results, no distinctions have been made on the basis of the purpose of the tests. The species of birds in which survey work was done included turkeys, ducks, pigeons and chickens.

Sera were separated by natural clotting, centrifuged to remove red blood cells if necessary and treated at 56 °C for 30 min before the test.

A standard, direct CF test was done in micro-titre plates using sheep red blood cells and guinea-pig complement. The preparation of the antigen from culture in NCTC Clone 929 L-cells was as described by Bracewell & Bevan (1982). The general methods for growing *C. psittaci* in these cells were described by Bevan, Cullen & Read (1978).

Difficulty in getting a direct CF test to work on many species of avian sera has been reported by several authors (Page, 1978). The use of ultrasonicated antigen prepared from cell cultures may have been the reason why positive results were obtained at this laboratory with a direct CF test on turkey sera from 1975 onwards although the titres found were generally lower than those found in pigeons and

psittacines. However, during surveys in ducks in 1980–81, it was found to be necessary to reduce the dose of complement from 2·0 to 1·5 u. to detect positive reactions in that species. This lower dose then became routinely used for all species. It made no difference to previously recorded titres (Bracewell & Bevan, 1982). The indirect CF test was tried in the past but was not found to reveal any reactions that could not be demonstrated more easily by the direct test.

All sera were titrated simultaneously against a negative control antigen (prepared from non-infected cell culture) to reveal any non-specific fixation or anticomplementary effect. In the past, non-specific fixation has been frequently found in field sera up to a dilution of 1/5, but it has been very rarely seen above this dilution. For this work, the dilutions were started at 1/10, and fixation at that dilution, accompanied by a negative reading in the corresponding negative antigen well, was regarded as the minimum specific titre.

RESULTS

From Table 1, it will be seen that 16·2 % of all samples tested were positive giving titres ranging between 10 and > 1280. Titres greater than 320 were found in three groups of birds only: pigeons, collared doves and psittacines. The number of pigeons tested (1549) was sufficiently high to be regarded as a fair sample of the population so the finding that 47·3 % were positive is significant. The only species that gave a higher proportion of positives was the collared dove, with 51·4 %, a very similar figure. The imported birds gave on the whole fewer positives and lower titres than the pigeons and doves. The domestic poultry gave very few positives and low titres except for the geese, (22·2 %), which were similar to the wild ducks (23·3 %) and British game birds (29·0 %). The five positive chicken sera were from a very unusual set of samples. They were all from the same group of bantams which had been kept in close proximity to psittacine and other imported birds, with access to their droppings. The remaining chickens were from the usual commercial stocks, and were all negative.

DISCUSSION

Results on samples acquired as these were, from a variety of unrelated diagnostic requests, or statistically unplanned survey work, give a general indication of rates of infection but cannot be presented as definitive studies.

Ornithosis was shown to be present in pigeons in Britain by Andrews & Mills (1943) and Hughes (1947). A high rate of infection in pigeons and doves has been reported in several other countries: the USA (Meyer, 1965); Japan (Fukushi *et al.* 1983); France (Milon *et al.* 1983); Germany (Vogel *et al.* 1983). The results presented in this paper are in close agreement with all these authors' findings, indicating that a similar high rate of infection occurs in Britain. This suggests that pigeons should be regarded as a major potential source of human infection, in view of their close association with man.

Isolations of *C. psittaci* from collared doves in Britain were reported by Gough & Bevan (1983) and de Gruchy (1983). This species, like feral pigeons, often tends to live in close proximity to man, and seems to have increased in numbers in recent

Table 1. *Frequencies of CF titres in birds sampled between 1974 and 1983*

Species or group of birds	Total	Negative (< 10)	10	20	40	80	160	320	640	> 1280	Positive No.	Positive %
Domestic												
Chicken	354	349	2	1	2	0	0	0	0	0	5	1·5
Turkey	2411	2261	44	52	27	18	8	1	0	0	150	6·2
Duck	1802	1713	54	22	10	2	1	0	0	0	89	4·9
Goose	36	28	2	2	3	1	0	0	0	0	8	22·2
British game birds	62	44	1	4	6	4	2	1	0	0	18	29·0
British wild birds												
Duck	43	33	3	2	4	1	0	0	0	0	10	23·3
Goose	46	41	2	2	1	0	0	0	0	0	5	10·9
Pigeon (Columba livia)	1549	817	146	143	153	113	78	53	26	20	732	47·3
Collared dove (Streptopelia decaocto)	37	18	2	0	1	3	5	2	0	6	19	51·4
Other	56	54	0	0	0	2	0	0	0	0	2	3·6
Imported												
Large psittacine	119	99	4	3	0	1	4	3	3	2	20	16·8
Budgerigar	17	15	0	0	1	0	1	0	0	0	2	11·8
Other small psittacine	35	28	0	1	1	0	1	2	2	0	7	20·0
Non-psittacine	26	26	0	0	0	0	0	0	0	0	0	0·0
Total for all groups	6593	5526	260	232	209	145	100	62	31	28	1067	16·2

years. Gough & Bevan (1983) reported some mortality, with postmortem findings typical of chlamydiosis, among the group of collared doves from which these isolations were made. This species gave the highest titres and positive percentage but the number of samples tested was relatively small compared with those from pigeons.

The results for domestic poultry add some weight to the theory that infection is not usually endemic but often comes from recent exposure to wild birds. The fully housed chickens were completely negative, while low but consistent rates were found in turkeys and ducks which are partly kept in the open, and the highest rates were found in the geese which are usually kept in the open.

Evidence that ducks have been the source of some cases of human ornithosis in Britain was presented by Andrews, Major & Palmer (1981), Ministry of Agriculture (1981), Palmer, Andrews & Major (1981), and Bracewell & Bevan (1982). From the evidence presented here that turkeys have a similar rate of infection to ducks, it is perhaps surprising that there have been no reports of human ornithosis derived from turkeys in Britain. Andrews, Major & Palmer (1981) in fact have shown that workers at chicken and turkey plants had rates of reaction on the CF test similar to those of the general population, while workers at duck plants had significantly higher rates.

Psittacine birds have long been believed to present the biggest health hazard to man, so it is also a little surprising to find their positive rate to have been lower than that of the pigeons and doves, especially in view of our finding that psittacines gave the highest rate of isolation of *C. psittaci* (Bevan & Bracewell, 1986).

Alternative serological methods to the CF test are being examined at this and other laboratories. These include an enzyme-linked immunoassay which is more sensitive than the CF test and could prove especially useful for ducks (Evans *et al.* 1983) and turkeys (Durand *et al.* 1983). However, these new methods still need further field evaluation and, until their specificity and general suitability have been proved, it is preferable to use the CF test for routine testing for chlamydiosis in birds.

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REFERENCES

ANDREWES, C. H. & MILLS, K. C. (1943). Psittacosis (ornithosis) in English pigeons. *Lancet* i, 292–294.

ANDREWS, B. E., MAJOR, R. & PALMER, S. R. (1981). Ornithosis in poultry workers. *Lancet* i, 632–634.

BEVAN, B. J. & BRACEWELL, C. D. (1986). Chlamydiosis in birds in Great Britain. 2. Isolations of *Chlamydia psittaci* from birds sampled between 1976 and 1984. *Journal of Hygiene* **96**, (see accompanying paper for page nos.)

BEVAN, B. J., CULLEN, G. A. & READ, W. M. F. (1978). Isolation of *Chlamydia psittaci* from avian sources using growth in cell culture. *Avian Pathology* **7**, 203–211.

BRACEWELL, C. D. & BEVAN, B. J. (1982). Chlamydia infection in ducks: preliminary communication. *Journal of the Royal Society of Medicine* **75**, 249–252.

DE GRUCHY, P. H. (1983). Chlamydiosis in collared doves. *Veterinary Record* **113**, 327.

DURAND, M. P., LIMOUZIN, C., SCHRICKE, E. & DAYON, J. F. (1983). Contribution de la méthode ELISA au diagnostic sérologique de la chlamydiose aviaire. Application au cas de la rhinotrachéite infectieuse de la dinde en Bretagne. *Bulletin de l'Académie Vétérinaire de France* **56**, 355–364.

EVANS, R. T., CHALMERS, W. S. K., WOOLCOCK, P. R., FARMER, H. & TAYLOR-ROBINSON, D. (1983). An enzyme-linked immunosorbent assay (ELISA) for the detection of chlamydial antibody in duck sera. *Avian Pathology* **12**, 117–124.

FUKUSHI, H., ITOH, K., OGAWA, Y., HAYASHI, Y., KUZUYA, M., HIRAI, K. & SHIMAKURA, S. (1983). Isolation and serological survey of *Chlamydia psittaci* in feral pigeons in Japan. *Japanese Journal of Veterinary Science* **45**, 847–848.

GOUGH, R. E. & BEVAN, B. J. (1983). Isolation and identification of *Chlamydia psittaci* from collared doves (*Streptopelia decaocto*). *Veterinary Record* **112**, 552.

HUGHES, D. L. (1947). Ornithosis (psittacosis) in a pigeon flock. *Journal of Comparative Pathology & Therapeutics* **57**, 67–76.

MEYER, K. F. (1965). Ornithosis. In *Disease of Poultry*, 5th ed. (ed. H. E. Biester & L. H. Schwarte), pp. 675–770. Ames, Iowa: Iowa State University Press.

MILON, A., GERAL, M. F., PELLERIN, J. L., THIESE, I. & LAUTIE, R. (1983). Enquête sur le portage et l'excrétion de *Chlamydia psittaci* par les pigeons semi-domestiques (*Columba livia*) de l'agglomération Toulousaine. *Revue de Médecine Vétérinaire* **134**, 559–565.

MINISTRY OF AGRICULTURE, FISHERIES & FOOD (1981). Epidemiological notes: Ornithosis in poultry workers. *Veterinary Record* **108**, 155.

PAGE, L. A. (1978). Avian Chlamydiosis (Ornithosis). In *Diseases of Poultry*, 7th ed. (ed. M. S. Hofstad), pp. 337–366. Ames, Iowa: Iowa State University Press.

PALMER, S. R., ANDREWS, B. E. & MAJOR, R. (1981). A common-source outbreak of ornithosis in veterinary surgeons. *Lancet* ii, 798–799.

VOGEL, K., LÜTHGEN, W., MÜLLER, H., SCHRAG, L. & VOGEL, M. (eds.) (1983). Chlamydien-Infektion (Ornithose). In *Die Taube: Taubenkrankheiten*. pp. 111–131. Berlin: VEB Deutscher Landwirtschaftsverlag.