AVIAN CHLAMYDIOSIS AS A ZOONOTIC DISEASE
AND RISK REDUCTION STRATEGIES

Report of the
Scientific Committee on Animal Health and Animal Welfare
Adopted 16 April 2002
1. **REQUEST FOR AN OPINION**

EU legislation contains special provisions that apply to psittacines being imported from third countries relating to freedom from psittacosis (Chlamydia psittaci).

These requirements do not apply to any other species nor are there any rules that apply to the management of psittacosis within the EU.

The Scientific Committee on Animal Health and Animal Welfare is asked to review psittacosis in the context of other zoonotic diseases and, if appropriate, to outline measures for dealing with the disease in animals (e.g. awareness, suitable tests, treatments, regulatory actions) that would result in a reduction in human morbidity.

2. **BACKGROUND**

It was decided, within the context of the request for an opinion, that this report would be restricted to chlamydiosis in birds.

Chlamydiosis has been the subject of two relatively recent reviews dealing with the disease as a zoonosis [Caul and Sillis, 1998] and specifically avian chlamydiosis [Andersen and Vanrompay, 2000] and these give more detailed accounts of the following aspects that are briefly reviewed for background information.

2.1. **Terminology**

Avian chlamydiosis is caused by the gram-negative bacterium *Chlamydophila (C.) psittaci*. *Chlamydophila* is the new genus name adopted in a reclassification that separates the family Chlamydiaceae into two genera, namely: *Chlamydia* and *Chlamydophila* [Everett et al., 1999a]. Other species in the genus *Chlamydophila* are *C. felis* (usually associated with cats), *C. abortus* (sheep, goats and cattle) and *C. caviae* (guinea pigs). Avian chlamydiosis was previously named psittacosis, or parrot fever, as the disease was originally recognised in psittacine birds and in humans in contact with these birds. In 1941, the term “ornithosis” was introduced to refer to chlamydial disease in, or contracted from, domestic poultry and wild birds other than psittacine birds [Meyer, 1941]. These diseases in birds are now all considered to be similar, and the term avian chlamydiosis is preferred [Andersen et al., 1997]. ‘Psittacosis’ still tends to be used to describe the disease in humans.

2.2. **Aetiology**

*C. psittaci* is comprised of eight known serovars (Table 1). The serovars can be identified readily by the indirect fluorescent antibody (IFA) test using serovar-specific monoclonal antibodies [Andersen, 1991], by polymerase chain reaction (PCR)/restriction fragment length polymorphism [Sayada et al., 1995; Vanrompay et al., 1997a] or by PCR/nucleotide sequence analysis [Everett et al., 1999a]. At least six distinct serovars (A to F) of *C. psittaci* are considered endemic in birds (Table 1). Each serovar appears to be associated, though not exclusively, with a different group or order of birds, from which it is most commonly isolated. Some of these serovars have a close relationship with a given host, and may induce a carrier status; this has been known for some years with pigeons and psittacine birds and it seems likely that it occurs with other birds. However, the association of a serovar with a given avian host does not
implies that it is endemic in that population; for example, in turkeys the most prevalent serovars most likely represent introductions from feral birds [Andersen et al., 1997].

Epidemiological studies indicate that the serovars are distributed worldwide. The avian serovars are distinct from those usually associated with chlamydiosis in mammals. However, the avian strains can infect humans and other mammals, and may cause severe disease and even death. Secondary spread of the disease between humans has been known to occur but is not considered a significant problem [Olson and Treuting, 1944; Meyer and Eddie, 1951; Broholm et al., 1977; Pether, 1981; Satalowich et al., 1993; Andersen and Vanrompay, 2000].

Table 1. *C. psittaci* serovars [Andersen and Vanrompay, 2000].

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Representative strain</th>
<th>Host association</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>VS1</td>
<td>Psittacine</td>
</tr>
<tr>
<td>B</td>
<td>CP3</td>
<td>Pigeon, dove</td>
</tr>
<tr>
<td>C</td>
<td>GR9</td>
<td>Duck, goose</td>
</tr>
<tr>
<td>D</td>
<td>NJ1</td>
<td>Turkey</td>
</tr>
<tr>
<td>E</td>
<td>MN</td>
<td>Pigeon, turkey</td>
</tr>
<tr>
<td>F</td>
<td>VS225</td>
<td>Psittacine</td>
</tr>
<tr>
<td>WC</td>
<td>WC</td>
<td>Cattle</td>
</tr>
<tr>
<td>M56</td>
<td>M56</td>
<td>Muskrat, snowshoe hare</td>
</tr>
</tbody>
</table>

2.3. **Avian hosts**

*C. psittaci* is known to infect most species of pet birds, poultry (here taken to mean domestic fowl, turkeys, ducks, geese and closely related domestic species) and wild birds [Vanrompay et al., 1995]. The reported infection rates vary greatly. Persistent infections, which may continue for months or years, are thought to be common.

The prevalence of *C. psittaci* infections amongst different pet or zoo bird species is not well documented. Surveillance of birds from professional breeders and pet shops undertaken in Giessen, Germany during 1984 to 2000 [E.F. Kaleta, unpublished data] showed isolation rates ranging from about 6% in African grey parrots to nearly 15% in cockatoos and “other passeriformes” (Table 2).

*C. psittaci* is known to be widespread in wild birds and some 376 species have been reported positive by stained smears or isolation [Taday, 1998]. Psittacine birds and pigeons have the highest infection rates. A number of studies have found over 10% of birds positive by isolation and over 30% positive by serology [Andersen and Vanrompay, 2000]. In particular, wild birds that have close associations with man, such as the common tits (*Parus* spp), have been shown to be infected with *C. psittaci* [Holzinger-Umlauf et al., 1997]. The strains isolated from wild birds are not thought to be normally pathogenic for these hosts, but the same strains can be highly virulent for domestic fowl and
humans. In wild birds, *C. psittaci* strains tend to produce persistent infections with periods of shedding [Roberts and Grimes, 1978; Brand, 1989].

Among poultry, major outbreaks have also occurred on turkey and duck farms and have often led to infection of humans; a few outbreaks have also been reported in farmed geese. Although chickens appear to be more resistant, natural infections have been reported in breeder flocks, broilers and layers (Durfee et al., 1975; Sadowski and Minta, 1979; Bracewell and Bevan, 1982; Farmer et al., 1982; Barr et al., 1986; Malkinson et al., 1987; Hedberg et al., 1989; Newmann, 1989; Arzey and Arzey, 1990; Arzey et al., 1990; Newman et al., 1992; Hinton et al., 1993; Hafez and Sting, 1997; Vanrompay et al., 1997b).

<table>
<thead>
<tr>
<th>Bird species or bird group</th>
<th>Number of samples tested*</th>
<th>Negative**</th>
<th>Positive**</th>
<th>% Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budgerigars</td>
<td>1066</td>
<td>996</td>
<td>70</td>
<td>6.57</td>
</tr>
<tr>
<td>Cockatiels</td>
<td>331</td>
<td>305</td>
<td>26</td>
<td>7.85</td>
</tr>
<tr>
<td>African grey parrots</td>
<td>310</td>
<td>291</td>
<td>19</td>
<td>6.13</td>
</tr>
<tr>
<td>Amazons</td>
<td>592</td>
<td>514</td>
<td>78</td>
<td>13.18</td>
</tr>
<tr>
<td>Macaws</td>
<td>152</td>
<td>137</td>
<td>15</td>
<td>9.87</td>
</tr>
<tr>
<td>Cockatoos</td>
<td>101</td>
<td>86</td>
<td>15</td>
<td>14.85</td>
</tr>
<tr>
<td>Other Psittacines</td>
<td>988</td>
<td>903</td>
<td>85</td>
<td>8.60</td>
</tr>
<tr>
<td>Pigeons</td>
<td>281</td>
<td>260</td>
<td>21</td>
<td>7.47</td>
</tr>
<tr>
<td>Canaries</td>
<td>138</td>
<td>128</td>
<td>10</td>
<td>7.25</td>
</tr>
<tr>
<td>Other Passeriformes</td>
<td>101</td>
<td>86</td>
<td>15</td>
<td>14.85</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4061</strong></td>
<td><strong>3707</strong></td>
<td><strong>354</strong></td>
<td><strong>8.72</strong></td>
</tr>
</tbody>
</table>

* Spleen, liver, swabs, faecal samples

** Tests in buffalo green monkey [BGM] cell cultures and *C. psittaci* detection either by Gimenez staining or immunofluorescence using a monoclonal antibody directed against the major outer membrane protein [MOMP].

2.4. **Disease in birds**

In birds *C. psittaci* produces a systemic infection, which varies according to the strain and the host. Typical clinical signs in a susceptible host infected with a highly virulent strain include respiratory signs, mucopurulent nasal and conjunctival discharge, diarrhoea, polyuria and dullness. Yellow-green droppings are common. Strains of low virulence will produce clinical signs that
are similar but less severe and less extensive. Asymptomatic infections can occur with strains of both low and high virulence.

The incubation period in birds infected naturally varies with the virulence of the *C. psittaci* strain for the host species, the number of organisms inhaled or ingested, and the age of birds, as the young are most susceptible. It may range from 3 days to several weeks, months or even years. In experimentally infected turkeys, virulent strains may elicit clinical signs in 5-10 days, while incubation periods of 2-8 weeks have been reported for strains of lower virulence. An incubation period of up to 1.5 years for long-time carriers and even 7 years has been suggested for budgerigars, while the incubation period in pigeons is unknown [Grimes, 1994; Gerlach, 1999].

2.5. Spread between birds

Transmission is primarily from one infected bird to another susceptible bird in close proximity. *C. psittaci* is excreted in the faeces and nasal discharges. Faecal shedding can occur intermittently and can be activated by stress factors, including shipping, crowding, chilling, breeding and even treatment/handling. Insufficient information is available as to the periods during which birds with clinical disease or carriers can transmit the organism [Gerlach, 1999], but shedding may continue for several months. There is evidence that periods of true latency (i.e. non-multiplication) also may occur [Monnickendam and Pearce, 1983]. The most important routes of transmission of *C. psittaci* in nature are the inhalation and ingestion of contaminated material [Burkhart and Page, 1971]. Direct transmission through aerosolisation of respiratory exudate or faeces is considered common during outbreaks in poultry [Grimes, 1994].

The various feeding behaviours and habits of host species that may result in exposure and transmission have been reviewed by Brand [1989]. Avian species including domestic ducks and turkeys sharing aquatic or moist soil habitats where wild aquatic birds shed high concentrations of organisms may acquire the infection via the common standing water, while granivorous birds (pigeons, doves, pheasants and house sparrows) may be infected by dust inhalation in faecal contaminated barnyards and grain storage sites. The consumption of infected carcasses may transmit *C. psittaci* to host species that are predators or scavengers of other birds. Interspecies transmission in arboreal host birds that feed above ground may be related to their gregarious behaviour and mutual close contact.

*C. psittaci* can be also transmitted in the nest. In many species, such as columbiformes, cormorants, egrets, and herons, transmission from parent to young may occur through feeding, by regurgitation, while the contamination of the nesting site with infective exudates or faeces may be important in other species such as snow geese, gulls and shorebirds [Brand, 1989]. Also the transmission of *C. psittaci* by arthropod vectors would be facilitated in the nest environment, but its occurrence has not been assessed in the wild.

Vertical transmission has been demonstrated in chickens, ducks, parakeets, seagulls and snow geese [Vanrompay et al., 1995]. The occurrence appears to be fairly low. However, vertical transmission also has the potential that biological products produced in eggs may be contaminated with *C. psittaci*. This could be a problem in the production of live vaccines.
*C. psittaci* can be introduced into susceptible pet birds or poultry through the wild bird population. Available evidence based on serotyping *C. psittaci* strains from poultry and wild birds does indeed indicate a pattern of transmission from wild birds to domestic poultry [Andersen, 1991; 1997]. Contaminated feed or equipment can also be a source of infection, and feed should therefore be protected from wild birds.

Careful cleaning of equipment is important as the organism can survive in faeces and bedding for up to thirty days. Cleaning and disinfection with most detergents and disinfectants will inactivate *C. psittaci*, as the bacterium has a high lipid content. Effective disinfectants include 1:1000 dilution of quaternary ammonium compounds, 70% isopropyl alcohol, 0.5% peracetic acid, 1:100 dilution of household bleach and chlorophenols.

### 2.6 Diagnosis

The diagnosis of *C. psittaci* infections in birds can be a problem because of the occurrence of persistent infections in non-shedding clinically healthy birds. Isolation is considered conclusive and chlamydial-specific gene detection by polymerase chain reaction (PCR) regarded as an acceptable diagnostic alternative. Antibody titres are proof of a current or past infection, but do not prove an active infection unless a four-fold increase in the humoral antibody titre is shown with paired sera taken two weeks apart, together with clinical signs. A tentative diagnosis of avian chlamydiosis can be made in a flock that includes birds with clinical signs in addition to a large proportion of birds with high antibody titres.

#### 2.6.1 Isolation

Isolation of *C. psittaci* is currently regarded as the standard method for the determination of active infections of birds. It is important that fresh samples are taken for isolation. A special buffer containing sucrose, phosphate and glutamase (SPG) is used for transporting, storing and freezing samples [Spencer and Johnson, 1983].

It is recommended that, where practicable, pharyngeal swabs, cloacal swabs and faeces samples are taken from live birds and preferably sampling should be repeated over a number of days to increase the likelihood of detecting intermittent excreters [Andersen, 1996].

*C. psittaci* can be isolated in embryonated fowls’ eggs, but more commonly cell lines are used. Buffalo green monkey (BGM) cells are considered the most sensitive for isolating *C. psittaci*, but Vero and L929 cells are often used and the organism will grow in other cell lines [Vanrompay et al., 1992; Andersen, 1998]. Standard methods of inoculation and incubation are used, with antibiotics that do not inhibit chlamydial multiplication. The presence of the organism in the inoculated cells is usually confirmed by staining or immunofluorescence techniques.

#### 2.6.2 Polymerase chain reaction (PCR)

A number of reports on the use of PCR techniques to detect *C. psittaci* have appeared in the literature [Hewinson et al., 1991; 1997; Messmer et al., 1997; Moroney et al., 1998; Olsen et al., 1998; Everett et al., 1999b; McElnea and Cross, 1999] and several different strategies have been used (Table 3). These
tests are reported as able to detect *C. psittaci* DNA in samples of tissues, faeces and choanal and cloacal swabs and are sensitive, rapid and have performed better than traditional tissue staining methods and culture when employed for specimens that have not been taken properly or mishandled [Moroney et al., 1998]. Several laboratories offer routine PCR tests for the diagnosis of infections.

**Table 3. Strategies used in PCR tests for detecting *C. psittaci***

<table>
<thead>
<tr>
<th>Authors</th>
<th>Target gene</th>
<th>Matrix</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hewinson et al., 1997</td>
<td>OmpA</td>
<td>faecal swabs, tissue</td>
<td>PCR followed by hybridisation with a radioactive probe</td>
</tr>
<tr>
<td>Messmer et al., 1997</td>
<td>16S rRNA</td>
<td>faeces, tissue, cloacal swabs</td>
<td>nested PCR</td>
</tr>
<tr>
<td>Moroney et al., 1998</td>
<td>16S rRNA</td>
<td>fresh droppings</td>
<td>nested PCR</td>
</tr>
<tr>
<td>Olsen et al., 1998</td>
<td>OmpA</td>
<td>faeces</td>
<td>PCR followed by DNA sequencing</td>
</tr>
<tr>
<td>McElnea and Cross, 1999</td>
<td>OmpB</td>
<td>cloaca, conjunctiva</td>
<td>PCR followed by dot-blot hybridisation</td>
</tr>
</tbody>
</table>

PCR tests for other *Chlamydial* species, especially those relevant to human medicine, have been well validated [Schepetiuk et al., 1997; Dean et al., 1998; Mahony et al., 2000; Dawell et al., 2001] and have become the tests of choice due to the much greater sensitivity than that of other tests. Validation studies conducted in human medicine have clearly demonstrated that PCR (or nucleic acid amplification tests) is superior to other diagnostic techniques in terms of sensitivity. The values of relative sensitivity compared to PCR were: cell culture, 60-80 %, immunohistology, 50-80 %, microimmunofluorescence, 62-75 %, enzyme immunoassays, 62-75 % [data from Newhall et al., 1999; Stary, 2000].

The dilemma for the PCR tests used in human medicine, and by analogy those used for *C. psittaci*, is that the superior performance of PCR cannot be easily verified by an independent method (because there is no method as sensitive as PCR).

At present, data validating the PCR tests in comparison with *C. psittaci* isolation, such as those reported by Hewinson et al. [1997], Messmer et al. [1997] and McElnea and Cross [1999] suggest that these tests can be especially suited to the detection of *C. psittaci* in avian samples; however, more work in this area is desirable for corroborating this evidence and standardising techniques among laboratories. In the absence of conclusive validating data, PCR still seems to be the test of choice based on its simplicity, sensitivity and comparability with validated tests used for other chlamydial species.

As with *C. psittaci* isolation, for PCR tests samples from live birds should be taken over a number of days to increase the likelihood of detecting intermittent excreters. It is recommended that samples are taken on three consecutive days, stored in appropriate transport medium and processed at the same time.
2.6.3. Enzyme linked immunosorbent assay (ELISA) for antigen detection

An ELISA kit test has been used for detecting antigen in human infections. Since it detects lipopolysaccharide it will detect all species of *Chlamydophila*. This test can be used in birds, but tends to lack sensitivity [Vanrompay et al., 1994; Arizmendi and Grimes, 1995].

2.6.4. Serological tests

The complement fixation (CF) test remains the most widely used test for detecting antibodies to *C. psittaci* despite its complexity and the need to overcome the anticomplementary effect of most avian sera (usually by the addition of normal chicken serum). Diagnosis of an active infection in individual birds can be made by demonstrating a four-fold rise in CF titre (in paired sera).

Other tests such as the elementary body agglutination test [Grimes et al., 1994] and the latex agglutination test [Arizmendi and Grimes, 1993] have been developed, but lack proper validation. Conventional ELISA tests have been developed for detecting antibodies to *C. psittaci* in birds [Evans et al., 1983; Ruppanner et al., 1984], but Andersen [1998] reports a lack of specificity, probably because of cross reaction with gram-negative bacteria.

A blocking ELISA kit is available commercially and has been reported to be highly sensitive [Gerlach, 1999].

2.7. Vaccination

No commercial vaccine is available for avian chlamydiosis. Recently, an experimental plasmid DNA vaccine containing the gene coding for the major outer membrane protein of chlamydiae was shown to give protection in turkeys [Vanrompay et al., 1999a; 1999b]. Both the level of protection afforded by a vaccine and the cost will determine whether immunisation of birds is practicable for the prevention of avian chlamydiosis.

2.8. Treatment of infected birds

Antibiotic treatment of birds is the usual response to known infections. Tetracyclines are usually considered the drugs of choice although quinolones (enrofloxacin) or macrolides (azithromycin) have also been used. Chlortetracycline (CTC) is given on food at levels of 500-5,000 ppm depending on the bird species to be treated and type of food [Gerlach, 1999]. One of the main problems is that birds are often reluctant to eat food treated with tetracyclines and achieving sufficiently high blood levels may take some time. Intramuscular injection of oxytetracycline has been used for larger birds, but possible side effects include severe muscle necrosis at the site of injection [Gerlach, 1999]. With all tetracycline treatments, problems may ensue due to the elimination of the normal gut flora.

Doxycycline has also been used for injecting and in food (1000 mg/kg) with some reported success [Gerlach, 1999]. Doxycycline medicated drinking water (200-800 mg/litre, depending on the species and environmental conditions) has also proved effective [Flammer, 2000]. Doxycycline is more stable in water than food, the drug is generally well accepted by birds, the above mentioned dose results in blood concentrations of >1µg/ml (this level is considered enough
to inhibit *C. psittaci* replication and to permit the host immune system to eliminate the infection), the drug is inexpensive [Flammer, 2000].

Enrofloxacin presented in food at 250-1,000 ppm has been used to treat caged birds infected with *C. psittaci* [Gerlach, 1999]. Further evaluation of this treatment is required.

One of the problems associated with the treatment of birds is that *C. psittaci* may still be present after treatment. Recent work has been undertaken to assess whether or not this is due to acquired antibiotic resistance [P. Theis, personal communication]. Nineteen *C. psittaci* isolates obtained from faeces or pharyngeal fluids of psittacines after the birds had received a course of tetracyclines were used to determine the minimum inhibitory concentrations (MIC) of growth in BGM cells of various antibiotics. The results obtained were:

- Chlortetracycline: 1.0-10.0 µg/ml
- Doxycycline: 1.0-10.0 µg/ml
- Enrofloxacin: 0.5-1.0 µg/ml
- Difloxacin: 0.5-1.0 µg/ml

The MIC range seen for chlortetracycline and doxycycline is similar to the ranges expected for *C. psittaci* isolates from untreated birds. These results are evidence that no antibiotic resistance has developed in these isolates. The most probable explanation is that the original treatment was ineffective because the persistent *C. psittaci* organisms were not replicating during treatment so the tetracyclines had little effect.

### 2.9. Human infections with *C. psittaci*

From 1988 to 1998, 813 cases of chlamydial infections of humans (psittacosis) were reported to the Centers for Disease Control and Prevention, Atlanta, USA (CDC) [CDC, 1998]. Most resulted from exposure to infected pet birds, usually psittacine birds such as cockatiels, parakeets, parrots, budgerigars and macaws; however, human infections have also often been linked to contacts with non-psittacine pet birds (finches, canaries, pigeons, doves, and mynah birds).

In Germany, where human psittacosis is a notifiable disease, 790 cases were reported from 1995 to 2000. In the same period, 2217 cases in birds were registered by the regional veterinary authorities (source: Robert Koch Institute, Berlin).

In Denmark, where human psittacosis is a notifiable disease, 57 cases were notified from 1.9.1995 to 31.12.1998 [Faber et al., 1999] and 30 cases in 1999 [Christiansen and Samuelsson, 2000].

In an area in Italy, 76 cases of psittacosis were reported in patients admitted to 12 hospitals between October 1981 and February 1985 [Maffei et al., 1987].

In Sweden, 336 cases were reported from 1973 to 1977 [WHO, 1976; 1977].

The number of cases in the United Kingdom was 587 from 1977 to 1979 [Harris, 1983], but over 300 yearly in 1980-83 [Isaacs, 1984], probably due to
the increased awareness of chlamydiosis as a zoonosis following outbreaks of the disease in the personnel and veterinarians working at or visiting duck-processing plants [Andrews et al., 1981; Palmer et al., 1981]. As to the veterinary data collected in Great Britain, C. psittaci infections were diagnosed in 281 birds of different species from 1975 to 1980 [Harris, 1983], and C. psittaci was isolated in 159 samples out of 1034 examined from 1981 to 1984 [Bevan and Bracewell, 1986].

The disease in humans varies from a flu-like syndrome to a severe systemic disease with pneumonia and possibly encephalitis. The disease is rarely fatal in patients treated promptly and correctly. Therefore, awareness of the danger and early diagnosis are important. Infected humans typically develop headache, chill, malaise and myalgia, with or without signs of respiratory involvement. Pulmonary involvement is common, but auscultatory findings may appear to be normal [Johnston et al., 1999].

Care should be taken in handling infected birds, as most infections occur through inhalation of contaminated airborne particulates (dried faeces or respiratory secretions). Other possible ways of infection include mouth-to-beak contact and the handling of plumage or tissues from infected birds. Although psittacine birds are the major source of human infection, outbreaks due to exposure to non-psittacine birds also occur. The more common of these are due to exposure to pigeons, both wild and domestic, and to ducks and turkeys raised commercially. Avian chlamydiosis in humans should be considered an occupational disease with commercial pet birds handlers, veterinarians and poultry workers exposed to the greatest risk. Outbreaks have occurred in humans following exposure to ducks and turkeys during slaughter and processing of infected birds [Caul and Sillis, 1998]. However, transmission to consumers has never been reported. Transmission from human to human is rare but can occur [Olson and Treuting, 1944; Meyer and Eddie, 1951; Broholm et al., 1977; Pether, 1981; Viciana et al., 1993]. Transmission from humans to birds has not been documented.

3. EU legislation

EU legislation for the importation of birds other than poultry from third countries is encompassed in Commission Decision 2000/666/EC [CEC, 2000], which is primarily concerned with restricting the introduction of Newcastle disease or avian influenza. Essentially this decision requires:

- The birds must come from a holding in the country of origin where they have been kept for at least 21 days prior to export.

- The birds are transported in cages or crates that contain only one species of bird or one species per compartment if compartmentalised.

- The birds are moved to designated licensed quarantine premises where they are held for 30 days and subjected to at least two veterinary inspections [usually beginning and end] before release.

The Decision has one mention of C. psittaci, which is referred to in the “request for opinion”; Article 5 reads: “If during quarantine as provided for in Article 3 it is suspected or confirmed that psittaciformes are infected with C. psittaci all birds of the
4. **CONCLUSIONS AND RECOMMENDATIONS**

Due to the widespread distribution of *C. psittaci* and because infection is primarily a public health threat to people in close contact with pet birds (both psittacine and non-psittacine), pigeons, turkeys and ducks, control and prevention requirements aimed at reducing human infections should focus on those four bird groups. Chlamydiosis is a significant cause of poor welfare in poultry and should be taken into account in evaluating systems for rearing poultry. The importation of infected birds into EU member states from third countries should be particularly controlled. Especially the control of illegal importation of pet birds - mainly trapped wild psittacine birds - potentially infected, should be reinforced.

4.1. **Imported birds other than poultry**

There is no doubt that *C. psittaci* is regularly introduced into EU member states by imported birds and that the conditions of quarantine and transport encourage the spread from infected to susceptible birds. Current quarantine conditions may dramatically increase the numbers of infected birds in a given consignment and the dissemination of these birds throughout the importing country and the EU will increase the *C. psittaci* burden in the caged bird population. Thus, despite the fact that *C. psittaci* infections appear to be common in pet birds already present in EU member states, the continual introduction by imported birds maintains the high prevalence of infected birds and increases the risk of human infections. Adequate control measures applied during quarantine would considerably alleviate this problem although it is unlikely that procedures short of banning importations of these birds or slaughter of all birds on the detection of *C. psittaci* will entirely eliminate the risk of infected birds passing through quarantine. Additionally, there would seem little point in restricting any control measures solely to psittacine birds.

Whenever wild-caught birds are brought into captivity, their welfare will be poor. One consequence of the effects of confinement is increased susceptibility to latest or newly transmitted pathogens, perhaps including *C. psittaci*, with consequent even poorer welfare and often death. Hence the confinement of wild birds should be avoided except where there are good scientific reasons for doing so and conditions can be provided which minimise poor health or other poor welfare.

The risk of chlamydiosis is greater with some imported birds than with others. Birds which have been reared in captivity and whose health status is known pose less risk. Wherever birds are imported, the risk of chlamydiosis should be evaluated.

4.2. **Recommended procedures for the importation of birds other than poultry**

In terms of the control and reduction of the prevalence of *C. psittaci* in these birds, gathering the birds to a single site in the country of origin and holding them for at least 21 days prior to export as required in Decision 2000/666/EC is not beneficial. This procedure is highly likely to result in the spread of *C.
psittaci from infected birds to susceptible birds in contact at the holding stations.

The spread of \textit{C. psittaci} in quarantine could be greatly reduced if, as required before, during and after a journey, birds were divided into three groups consisting of 1) psittacine birds; 2) non-psittacine birds, other than Columbiformes spp.; 3) Columbiformes spp., and complete separation (including air space) of the three groups was maintained throughout quarantine.

On arrival at quarantine premises there should be complete (air space) separation of healthy and sick birds and separation of sick birds from those remaining healthy during quarantine as they arise.

Clinically diseased birds should be tested individually for \textit{C. psittaci} by PCR test, preferably sampled using pharyngeal, choanal or conjunctival swabs, but for small, delicate birds faeces or faecal swabs may be used, as recommended in 2.6.2.

All clinically diseased, PCR positive birds must be separated from healthy birds transferred to clean disinfected cages and treated for at least 30 days by a method approved by the competent authority. Quarantine of these birds must be extended by 2 months. At the end of the extended quarantine period the birds should be retested by PCR. [Remark: treatment of sick birds prevents further \textit{C. psittaci} excretion but it is not known whether the birds ever become truly \textit{C. psittaci} free. Research is needed.] If the birds remain positive despite treatment either: a) quarantine is extended for another 2 months and the birds receive an alternative treatment [e.g. enrofloxacin or difloxacin if the original treatment was with CTC]; or b) the birds are destroyed.

As tetracycline drugs are effective only against actively metabolising microorganisms (i.e. during growth or fission), and not in treating latently or persistently infected birds in which the \textit{Chlamydia} is located inertly in macrophages (Gerlach, 1999), a prophylactic treatment of healthy birds is not recommended. In addition, the increased risk of development of antibiotic resistance of the intestinal bacterial flora should also be considered as a negative consequence/effect of prophylactic use of antibiotics. However, birds that have shared the same air space as birds becoming sick during quarantine and shown to be PCR positive to \textit{C. psittaci} must be individually tested for \textit{C. psittaci} by PCR. All PCR positive clinically healthy birds must be separated from PCR negative, healthy birds and quarantine prolonged for 2 months with treatment as above and retesting at the end of the quarantine period. If the birds remain positive either: a) quarantine is extended for another 2 months; or b) the birds are destroyed. Birds that are PCR negative after treatment are released from quarantine.

4.3. Trade in pet birds

In the wider context of reducing human infections with \textit{C. psittaci}, practicable restrictions should be placed on the sale of infected birds. Only healthy, PCR negative birds should be sold to pet shops and by retailers to pet bird owners. This could be achieved to some extent by making a certificate of no excretion, determined by PCR testing and signed by an authorised veterinarian, obligatory
for trading. The certificate should confirm that in the last 12 months the bird(s) had been sampled individually using pharyngeal swabs (but for small, delicate birds faeces or faecal swabs may be used) and tested for *C. psittaci* by PCR with a negative result. Not least the certificate would have the advantage of increasing public awareness of the dangers of *C. psittaci* in pet birds.

### 4.4. Racing pigeons and show birds

The high incidence of *C. psittaci* infections of racing pigeons is probably primarily a result of placing the birds in close proximity during transport to release sites for races. Infections of racing pigeons could be greatly reduced if a requirement for participation in races was a certificate signed by an authorised veterinarian that within the last 12 months the bird had been sampled individually by pharyngeal swab and tested for *C. psittaci* by PCR with a negative result. Similar testing and certification of show birds should also be required for participation in shows.

### 4.5. Commercially raised turkeys and ducks

The potential for humans to become infected as a consequence of contact with infected commercial turkeys and ducks on farms or during processing should not be underestimated. Many of the measures that would reduce the likelihood of poultry from becoming infected may be implemented currently for control of other diseases, but rearing of turkeys and especially ducks is frequently under traditional conditions with little awareness of the possibility of *C. psittaci* infections. This is particularly true for commercial ducks, which in many parts of the EU are reared or fattened on open range (see Report on welfare aspects of the production of Foie Gras in Ducks and Geese, 1998). Prevention of infections of poultry farm workers, poultry processing plant workers and others coming into contact with infected poultry would be greatly increased by the following practices:

- Birds reared under all-in-all-out confinement conditions, with cleaning and disinfection (*C. psittaci* specific disinfection) between flocks, addressing the environmental contamination problem and breaking the cycle of possible transmission by carrier birds.

- Exclusion of wild birds and rodents from the poultry houses. Protection of feed from rodents and wild birds.

- As a basis for taking preventive measures, monitoring for *C. psittaci* based on targeted sampling using PCR to detect excretion and antibody titre determination (ELISA) starting at the age of 4 weeks (i.e. after the loss of maternal antibodies) and then at three-week intervals.

- Implementation of preventive measures, e.g. gloves, masks, information and medical examinations of the workers, in turkey and duck slaughterhouses and plants.

### 4.6. Evaluation and validation of diagnostic tests

As mentioned above in section 2.6.2., there is an urgent need for the proper evaluation and validation of diagnostic tests for both pharyngeal swabs and faecal materials. The suggested use of the PCR test for the detection of active
excreters of C. psittaci in these recommendations assumes that such validation will occur in the near future. The alternative of C. psittaci isolation is considered impracticable due to the time involved, the need for high quality samples and the hazard to laboratory personnel.

5. RECOMMENDATIONS FOR FUTURE RESEARCH

- Development of European standards for laboratory diagnosis of C. psittaci infections in both birds and humans. This should include a comparison of PCR versus other tests (culture, ELISA, CF etc.) to detect C. psittaci infection in birds. As well as assessing the sensitivity and specificity of the tests, their accuracy and repeatability should be evaluated in interlaboratory trials.

- Identification of molecular factors of pathogenicity to improve the general understanding of the pathogenesis of avian chlamydioses. In particular the possibility of identification of virulence markers in avian C. psittaci strains for determining potential differences in pathogenicity for humans, domestic poultry and pet birds (psittacine and non-psittacine) should be investigated.

- Development of methods of genotyping, e.g. using DNA microarrays, to enable diagnosticians to assess the pathogenic potential of individual chlamydial isolates and distinguish between virulent and non-virulent strains.

- The epidemiology of C. psittaci in both humans and birds is poorly understood and requires further investigations. Transmission of C. psittaci via eggs in parrots and other species should be evaluated. The prevalence of C. psittaci in passerine birds, both captive bred (e.g. canaries, zebra finches, etc.) and wild caught (numerous species of Asian, African and South American finches) should be investigated.

- Evaluation of methods to control C. psittaci in different types of birds and bird holdings

- Research into the potential use of vaccines against C. psittaci.

- Evaluation of C. psittaci shedding in birds (clinically ill, healthy-PCR positive and treated birds).

6. EXECUTIVE SUMMARY

Avian chlamydiosis, at one time termed psittacosis, is an infection of birds caused by the gram negative bacterium Chlamydophila psittaci [C. psittaci]. C. psittaci is known to infect most species of pet birds, show birds, domestic poultry and wild birds. The disease produced in birds consists usually of respiratory signs, diarrhoea and dullness although strains of low virulence may produce few signs. Some birds may become persistently infected, with intermittent excretion, especially when the
birds are stressed. There is currently no vaccine available for *C. psittaci* but birds may be treated with several different antibiotics with some success.

*C. psittaci* causes a zoonosis and the disease in humans varies from a flu-like illness to a severe systemic disease with pneumonia and possibly encephalitis, it can be fatal, but not if treated promptly. Historically human infections have been considered to be the result of contact with pet birds, especially psittacine species. However, human infections have been recorded following contact with infected pigeons, doves, ducks and turkeys raised commercially and wild birds. Probably several hundred cases of human infections with *C. psittaci* occur each year in the EU, but under reporting is likely. Transmission between humans is extremely rare, but has been recorded.

Despite the fact that *C. psittaci* infections appear to be common in pet birds already present in EU member states, the continual introduction by imported caged birds maintains the high prevalence of infected birds and increases the risk of human infections. Measures should therefore be taken to reduce the numbers of infected birds leaving quarantine. These should include:

a. Dividing birds into three groups consisting of 1) psittacine birds; 2) non-psittacine birds, other than Columbiformes spp.; 3) Columbiformes spp., and maintaining complete separation (including air space) of the three groups throughout quarantine.

b. There should be complete (air space) separation of healthy and sick birds in quarantine.

c. Sick birds should be tested individually for *C. psittaci* by PCR test. Healthy birds that have shared the same air space with sick, PCR positive birds should also be tested.

PCR positive birds must be transferred to clean, disinfected cages and treated for at least 30 days by a method approved by the competent authority. Quarantine of these birds must be extended by 2 months. At the end of the extended quarantine period the birds should be retested by PCR. If the birds remain positive despite treatment either quarantine is extended for another 2 months and the birds receive an alternative treatment; or the birds are destroyed. Birds that are PCR negative after treatment are released.

Prophylactic treatment of healthy birds is not recommended but should be evaluated on a case by case basis.

It is also recommended that pet birds should only be sold if they are certified by an authorised veterinarian that in the last 12 months the bird(s) had been sampled and tested for *C. psittaci* by PCR with a negative result.

There is a high prevalence of *C. psittaci* infections in racing pigeons. Since spread amongst these occurs primarily while they are held in close proximity when transported to races, a requirement for participation should be that the birds are certified by an authorised veterinarian that in the last 12 months they had been sampled and tested for *C. psittaci* by PCR with a negative result. Similar certification should be required for birds taking part in shows.
The potential for *C. psittaci* to spread from infected commercial turkeys and ducks to humans in contact on farms or in poultry slaughterhouses is high. The prevalence of infection of these birds can be greatly reduced by preventing wild bird access to poultry and food and rearing the birds under all-in-all-out conditions. The *C. psittaci* status of at risk poultry should be assessed regularly by targeted sampling and PCR or serological testing. Implementation of preventive measures, e.g. wearing gloves and masks, information and medical examinations should be made available to workers in turkey and duck slaughterhouses and plants. However, transmission through meat to consumers has never been reported.

7. REFERENCES


**8. ACKNOWLEDGEMENTS**

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