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## 2.1 Bacterial Diseases

### 2.1.1 Tuberculosis

#### 2.1.1.1 History

Tuberculosis (Mycobacteriosis) is an ancient or ‘heritage’ disease which was observed even before the Neolithic age and in Egyptian mummies. In 1882, Robert Koch first isolated *Mycobacterium*, stained with alkaline methylene blue and vesuvin and established its etiologic relationship with tuberculosis. Koch described that the same human or bovine type *Mycobacterium* may cause avian tuberculosis. Strauss and Gamaleia (1891) and Maffucci (1892) cited in Cobbett (1917) illustrated that the etiological *Mycobacterium* of avian tuberculosis was different from human or bovine type. Hinshaw (1933) and Ackermann et al. (1974) reported occurrence of tuberculosis in Amazon parrot (*Amazona farinosa*). Coyle et al. (1992) proposed a new Mycobacterial species (*Mycobacterium genavense*) isolated from human patients with AIDS. Simultaneously in 1993, *Mycobacterium genavense* was reported from pet birds in Europe (Hoop et al. 1993).

#### 2.1.1.2 Etiology

*Mycobacterium avium* subsp. *avium* and *M. genavense* are the most common cause of tuberculosis in pet birds. *M. genavense* is common in psittacine birds, whereas, *M. avium* subsp. *avium* mostly infects the birds kept in aviaries. *M. tuberculosis*, *M. bovis*, *M. intracellulae*, *M. gordonae*, *M. simiae*, *M. intermedium*, *M. peregrinum*, *M. terrae*, *M. avium* subsp. *paratuberculosis*, *M. trivial*, *M. fortuitum*, *M. diernhoferi*, *M. chelonae*, *M. smegmatis*, *M. flavescens*, *M. scrofulaceum*, *M. celatum*, *M. nonchromogenicum* and *M. marinum* are also associated etiology. *M. avium* subsp. *hominissuis* was detected in a female blue-fronted Amazon parrot (*Amazona aestiva*).

*Mycobacterium* is gram positive, straight or slightly curved rod with occasional coccobacillary, club and branched form. In the tissues, it measures 1–4  $\mu\text{m}$  in length and 0.2–0.3  $\mu\text{m}$  in width. It occurs singly, in pair or in bundle. It is difficult to demonstrate their gram positive nature due to high lipid content of the cell wall. The stains are relatively impermeable to the bacterial cell. They can be easily stained by Ziehl-Neelsen (ZN) or acid fast staining technique.

The genus *Mycobacterium* (Actinomycetes family) contains more than 100 species. Some are pathogenic for man and animals which grow slowly in artificial media in laboratory (slow growers) than the fast growers (*M. smegmatis*). Previously, slow growing *Mycobacterium* was sub divided into three types based on their host specificity i.e. human type, bovine type and avian type. Recent classification reveals that slow growing *Mycobacterium* is composed of several species. The most common species are *Mycobacterium tuberculosis* complex and *M. avium-intracellulare* complex (MAC). *Mycobacterium tuberculosis* complex is comprised of *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. microti*, *M. africanum* and *M. canettii*. MAC is composed of two major species—*M. avium* and *M. intracellulare*. *M. avium* is subdivided into four subspecies (ssp.) i.e. ssp. *avium* (Maa), ssp. *paratuberculosis* (Map), ssp. *silvaticum* and recently added ssp. *hominissuis*. MAC is considered as ‘atypical mycobacteria’ and members of this group are highly resistant against environmental changes such as high and low temperatures, dryness, extreme pH and common disinfectants. There are total 28 serotypes of MAC and the serotypes 1–6, 8–11 and 21 belonged to *M. avium* ssp. *avium*.

*Mycobacterium genavense* forms a deep branch of Mycobacterial phylogenetic tree with other members such as *M. interjectum* and *M. simiae*. This group is characterized by slow growth albeit contains the signature molecule of fast growers (short helix 18). *M. genavense* can be distinguished from other slow growers by their fastidious growth and preference for liquid medium. *M. genavense* was first reported from human AIDS patients with disseminated infections (Hirschel et al. 1990).

### 2.1.1.3 Host Susceptibility

Among psittacine birds, grey-cheeked parakeets (*Brotogeris pyrropterus*), amazons (*Amazona* spp.), budgerigars (*Melopsittacus undulatus*) and pinous parrots (*Pionus* spp.) are the most commonly affected species with tuberculosis. Other psittacines such as green-winged macaws (*Ara chloroptera*), cockatoos (*Cacatua* spp.), conures (*Aratinga auricapillus*, *Cyanoliseus patagonus*) and red-crowned parakeet (*Cyanoramphus novaezelandiae*) are also detected to be infected with *M. genavense* and *M. tuberculosis*. *M. avium* subsp. *avium* is reported to cause infection in cockatiels (*Nymphicus hollandicus*). The non-cultivable *Mycobacterium* is detected in blue and yellow macaw (*Ara ararauna*) and grey-cheeked parakeet (*Brotogeris pyrropterus*).

The common parrots (African grey parrot, Senegal parrot) are not considered as natural host of *M. tuberculosis* although infection in African grey parrot is reported which was transmitted from human. *M. bovis* can produce natural infection in

parrots and the budgerigars are experimentally infected with *M. bovis* producing clinical syndrome within 70 days.

Among non-psittacine group of birds, canary (*Serinus canaria*), gouldian finch (*Chloebeia gouldiae*) and zebra finch (*Poephila guttata castanotis*) are commonly infected with *M. genavense*. Synergistic infection of *M. genavense* and avian polyoma virus was detected in European goldfinch (*Carduelis carduelis*). Occasionally canaries are also infected with *M. tuberculosis*.

Avian tuberculosis is a disease of adult birds although occasionally detected in young (<1 year old) canaries.

#### 2.1.1.4 Transmission

MAC is transmitted in birds chiefly by ingestion, inhalation and rarely through arthropods. *M. avium* ssp. *avium* is excreted through faeces of infected birds and survive in soil (up to 4 years), sawdust (8 months at 37 °C) and water for a long period. Bird to bird transmission in aviaries may occur through infected faeces or rarely by cannibalism. Occasionally skin abrasion acts as a route of mycobacterial infection in pet birds.

Ingestion is considered as a potential route of transmission of *M. genavense* infection in pet birds. The environment specially drinking water is an identified source of *M. genavense* infection. Lung involvement in pet birds suggests inhalation as an additional route of transmission. Birds may act as reservoir of *M. genavense*. Bird to bird transmission of *M. genavense* is rare although the possibility could not be excluded entirely. Immunosuppression plays a role in transmission of *M. genavense* in human but whether the same condition facilitates the transmission in pet birds is obscure.

Transmission of *M. tuberculosis* in pet birds (green-winged macaws, blue-fronted amazon) from human is observed due to close contact with owners suffering from tuberculosis and feeding the birds with *pre-chewed food*.

#### 2.1.1.5 Pathogenesis

##### *M. avium* subsp. *avium*

MAC enters the host chiefly through ingestion route of transmission and become present in the intestine. The waxy cell wall of the bacteria protects it from gastric acids and enzymes. Several pathogen associated molecular patterns (PAMPs) are expressed by virulent *Mycobacterium* which can recruit ‘microbicidal’ macrophages through toll like receptor (TLR) mediated signaling. During *M. tuberculosis* infection in human, these PAMPs in the bacterial surface are masked with a lipid, namely phthiocerol dimycocoserate (PDIM). The PAMPs are not recognized by the host immune system and the bacteria can avoid the reactive nitrogen species (RNS) generated by ‘microbicidal’ macrophages.

MAC (*M. avium* subsp. *avium*) does not contain PDIM in their surface but use a different strategy (still unexplored) to resist RNS. MAC is benefited with these RNS as commensal present in the gut are sensitive to it. Commensal mediated competitive inhibition is thus excluded and probably MAC enters M-cells like host

adopted *Salmonella* to invade the underlying blood monocytes. The M-cells are specialized cells of the follicle associated epithelium and the region is relatively free from commensal mediated competitive inhibition. The invasion of monocytes is followed by bacterimia and subsequent haematogenous spread to liver, spleen and other organs.

MAC enters the macrophages (histiocytes) of periarteriolar lymphoid sheath (PALS) zone in spleen within 10 days post infection in birds. *Mycobacterium* has several virulence factors which promote their survival within macrophages using different strategies such as acid resistance, avoidance of acidification etc. MAC (*M. avium* subsp. *avium*) specifically restricts vacuole maturation and prevents the fusion of phagosome and lysosome for their survival within macrophages. Haematogenous spread of the organism leads to infection of bone marrow, lungs, air sacs, gonads and rarely, kidney and pancreas. The organs become enlarged due to accumulation of macrophages within organ parenchyma.

Granuloma formation is an attempt of host tissue to localize the infection, although *Mycobacterium* exploits it for their multiplication and further dissemination. The growth of *Mycobacterium* occurs in the macrophages present in a granuloma. The infected macrophages undergo apoptosis and leave the encased bacteria which are engulfed by newly recruited macrophages. This process of apoptosis and re-phagocytosis within a granuloma is regulated by a mycobacterial secretion system (ESX-1/ESAT6) detected in *M. tuberculosis* but absent in *M. avium* subsp. *avium*. Typical tuberculous granulomas in different organs are not frequent in *M. avium* subsp. *avium* infection, although observed in lungs and periocular region of parrots. Granulomas in different organs (liver, kidney, intestine, muscle and subcutaneous tissues) are observed in red-crowned parakeet (*Cyanoramphus novaezelandiae*) and green-winged macaw (*Ara chloroptera*) infected with *M. tuberculosis*.

### ***Mycobacterium genevense***

*M. genevense* infection in pet birds mostly occurs through the oral route like MAC. There is every possibility that they follow the same pattern of pathogenesis although still unexplored. *M. genevense* causes non-tuberculous form of mycobacteriosis in pet birds albeit occurrence of granulomas are observed in glottis of amazon parrot (*Amazona ochracephala*), aorta of cockatiel (*Nymphicus hollandicus*), small intestine of canary-winged parakeet (*Brotogeris versicolurus*) and brain of spectacled amazon parrot (*Amazona albifrons*).

#### **2.1.1.6 Clinical Symptoms**

Incubation period of mycobacteriosis in pet birds is 6 months to 4 years. Clinical syndrome in psittacine birds varies widely. In acute form, sudden death without any symptom is common. In chronic form, constant loss of weight along with diarrhoea (Fig. 2.1), frequent micturition with excessively large quantity and low specific gravity of urine, depression, laboured breathing, distension of abdomen and poor feathering primarily suggests about mycobacteriosis. The condition fails to respond to common antibiotics.

**Fig. 2.1** Macaw suffering from mycobacteriosis  
(Courtesy Mousam Das, Animal Resources Development Department, Government of West Bengal, India)



Cutaneous masses are sometimes observed in skin and conjunctiva. Inflammation of feather follicles (folliculitis) is occasionally observed which includes perifollicular swelling, erythema, pruritus and pain, restlessness, shivering and feather damaging behaviour.

#### **2.1.1.7 Lesion**

The liver and spleen are enlarged, mottled and whitish. Miliary abscess in liver are observed in budgerigars experimentally inoculated with *M. avium* subsp. *avium*. The intestine becomes tubular, thickened and tan coloured. Typical tuberculous granulomas in different organs are not frequent in *M. avium* subsp. *avium* infection, albeit observed in lungs and periocular region of parrots, pericardium of gang gang cockatoo (*Callocephalon fimbriatum*) and cervical esophagus of blue-fronted parrots (*Amazona aestiva*) occluding the lumen. Involvement of lung is rare in pet birds affected with mycobacteriosis. Some post mortem findings in canary (*Serinus canarius*), eurasian goldfinch (*Carduelis carduelis*) and the red siskin (*Spinus cucullatus*) reported the occurrence of lung lesions. In a rare case of *M. genevense* infection in an amazon parrot (*Amazona albifrons*), perivascular cuffs of macrophages in the grey and white matter of the brain and spinal cord, gliosis and mild vacuolation of white matter were observed.

### 2.1.1.8 Diagnosis

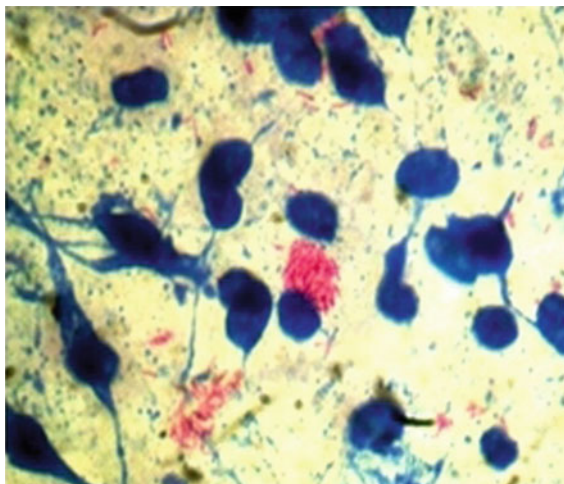
#### Clinical Specimens

Blood/sera, cloacal swabs, tracheal swabs, biopsies from organs can be collected as ante-mortem samples for diagnosis of mycobacteriosis in the laboratory. Post-mortem samples include vital organs such as liver, spleen, intestine, heart, lung and bone marrow. All the specimens should be immediately sent to the laboratory following the standard regulations for sending biohazardous substances. If there is delay in sending, refrigeration of the samples should be done to prevent the growth of contaminants. Addition of 0.5% boric acid may preserve the samples for 1 week.

#### Diagnostic Techniques

- (a) Clinical signs and history of direct contact with owner and other birds suffering from tuberculosis give a tentative diagnosis
- (b) *Haematological parameters*: Following haematological changes can be correlated with tuberculosis in pet birds although these changes are non-specific and are observed in other inflammatory and chronic infections also.
  - Moderate to marked increases in white blood cell numbers (heterophilia, monocytosis, lymphocytosis)
  - Decreased packed cell volume (PCV) (except during early stage of infection)
  - Increased enzyme concentration (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase)
- (c) *Demonstration of acid-fast organisms*: Demonstration of the organisms can be done in the smears prepared from ante-mortem samples by acid-fast (or ZN) staining. *Mycobacterium* appears as red coloured slender rods singly or in bundle (Fig. 2.2). Fluorescent acid fast stain may be used for better detection. In post mortem specimens, cytoplasm of infected cells is laden with acid-fast organisms.
- (d) *Gross and histopathology*: Enlarged liver, thickened intestinal loop, increased opacity in endosteal bone in the humerus, tibia, ulna, femur in advanced cases is suggestive for tuberculosis. Presence of visible granuloma is not a constant feature although may be detected in lungs. In decomposed carcasses bone marrow is the best specimen. Histopathological findings such as presence of acid-fast bacilli in inflammatory cells can also tentatively diagnose mycobacteriosis.
- (e) *Isolation of organism*: This is considered as gold standard method for confirmatory diagnosis of *Mycobacterium*. To isolate the organism, the tissue sample must be processed in proper way. Tissue samples are homogenized in pestle and mortar after keeping in the solution of hypochlorite (1:1000) for

**Fig. 2.2** Presence of *Mycobacterium* in Z-N stained impression smear of lung tissues (Courtesy Premanshu Dandapat, Eastern Regional Station, Indian Veterinary Research Institute, Kolkata, India)



4–16 h. It is decontaminated by the addition of acid (5% oxalic acid), alkali (2–4% sodium hydroxide) or detergent (0.375–0.75% hexadecylpyridinium-chloride, HPC). The acid or alkali mixture is neutralized, centrifuged and the sediment is used for culture. *M. avium* subsp. *avium* can be isolated in Dorset egg medium, Lowenstein-Jensen (LJ), Herrold's and Middlebrook's (7H10, 7H11) medium containing pyruvate. Glycerol and 'mycobactin' is also added as growth enhancer. Mycobactin extracted from the environmental Mycobacteria, acts as siderophore helping in acquisition of iron. Mycobactin is produced by all cultivable Mycobacteria except *M. avium* subsp. *avium* and *M. avium* subsp. *paratuberculosis*. Incubation period is 8 week at 40 °C. Even it can grow at 42–43 °C. It produces smooth or rough type of colonies. In liquid culture a radiometric method using <sup>14</sup>C labelled substrate can be used for rapid detection (BACTEC system).

*M. genavense* isolation is difficult although can be done on special media with prolonged incubation for 2–9 months. Conventionally, after decontamination of the samples with 2(N) NaOH, the samples can be inoculated in Herrold's egg yolk medium with and without mycobactin J and Sula's liquid medium and incubated at 37 °C. The growth is periodically checked in every two weeks. The successful isolation of *M. genavense* from blue headed parrot (*Pionus menstruus*) was observed in Herrold's egg yolk medium without mycobactin J after 270 days of incubation. In specialized laboratories, newly developed liquid culture systems [manual mycobacteria growth indicator tube (M-MGIT), BACTEC system) are used for confirmatory isolation of *M. genavense*.

- (f) *Immunological/serological tests*: Use of tuberculin test with purified protein derivative of *M. avium* subsp. *avium* correlates poorly with clinical disease in psittacine birds. ELISA was experimentally developed with *M. fortuitum*, *M. vaccae*, and *M. avium* antigens for detection of antibodies against *M. avium*

subsp. *avium* in birds. However ELISA produces variable results in different species of birds. Immunological or serological tests for detection of mycobacteriosis in pet birds are not routinely followed.

- (g) **Molecular biology:** Polymerase chain reaction (PCR) can detect *Mycobacterium* from fresh samples, faeces and paraffin-blocked tissues. The species-specific PCR targets *IS901* and *hsp65* genes for detection of *M. avium* subsp. *avium* and *M. genavense*, respectively. Differentiation of both the species can be done by sequencing of the 16SrRNA gene. A nested polymerase chain reaction (PCR) from the consensus sequences of the *hsp65* gene, followed by analysis with restriction enzymes can also differentiate *M. avium* and *M. genavense*. Real-time TaqMan PCR assay is developed to detect *hsp65* gene of *M. genavense* and MAC subsp. Other recent technologies such as GenoType assay and DNA microarrays can be used for diagnosis of avian tuberculosis. For detection of genetic diversity among the strains of *M. avium* subsp. *avium*, mycobacterial interspersed repetitive units—variable-number tandem-repeat markers (MIRU-VNTR) typing can be successfully used.

### 2.1.1.9 Zoonosis

*M. avium* subsp. *avium* is considered as a potential zoonotic risk in the immunocompromised persons albeit majority of human infection is caused by another member of *M. avium* group (*M. avium* subsp. *hominissuis*). *M. genavense* is associated with gastrointestinal or pulmonary mycobacteriosis in immunosuppressed patients associated with AIDS. Other species of mycobacteria associated with pet bird causes opportunistic and sporadic infections in human.

The cases of reverse zoonosis (anthroponosis) are also reported where *M. tuberculosis* was transmitted from the infected owners to their pet birds (green-winged macaws, blue-fronted amazon).

### 2.1.1.10 Treatment and Control Strategy

Successful treatment of pet birds is reported with various combinations of anti-tuberculous drugs at highest tolerable dose for a prolonged period (9 months or more). Single anti-tuberculous drug is not preferred due to possibility of resistance development. Emergence of multidrug-resistant tuberculosis (MDR-TB; resistant to isoniazid and rifampicin) and extremely drug-resistant tuberculosis (XDR-TB; in addition to being multidrug-resistant the bacteria are resistant to fluoroquinolone and 1 of 3 antibiotics such as capreomycin, kanamycin and amikacin) is a global problem now a days. In most of the cases, dose is fixed on the basis of human paediatric studies because pharmacokinetic properties of anti-tuberculous drugs in pet birds are still unknown. The anti-tuberculous drugs and antibiotics used against *M. avium* infections in pet birds are isoniazid, rifampin, rifabutin, ethambutol, clofazimine, ciprofloxacin, enrofloxacin, streptomycin and amikacin.

Successful therapy of *M. genavense* infections with combinations of moxifloxacin, clarythromycin, ethambutol and amikacin in humans has been reported but there is no specific drug recommended for *M. genavense* infection. Treatment with



clarithromycin, rifampin, and ethambutol against *M. marinum* infection in a blue-fronted Amazon parrot was not successful.

Experimentally, different combinations of anti-tuberculous drugs and antibiotics such as isoniazid (30 mg/kg) + ethambutol (30 mg/kg) + rifampin (45 mg/kg) for 12–18 months, clofazimine (6 mg/kg) + ethambutol (30 mg/kg) + rifampin (45 mg/kg) for 9–18 months, ciprofloxacin (80 mg/kg) + ethambutol (30 mg/kg) + rifampin (45 mg/kg) for 9–12 months was used successfully against confirmed cases of tuberculosis in different pet birds (grey-cheeked parakeet, double yellow-headed Amazon, lilac-crowned Amazon). Although combination of azithromycin (43 mg/kg), rifampin (45 mg/kg), and ethambutol (30 mg/kg) administered orally once daily for 180 days in ring-neck doves (*Streptopelia risoria*) naturally infected with *M. avium* subsp. *avium* failed to eradicate the infection. Further, treatment with combination of clarithromycin (61 mg/kg bw), moxifloxacin (25 mg/kg bw) and ethambutol (60 mg/kg bw) administered in budgerigars experimentally infected with *M. avium* subsp. *avium* by crop gavage every 12 h for 18 weeks significantly improved the situation but failed to recover completely. Combination of minocycline (10 mg/kg p.o. b.i.d.) and clarithromycin (10 mg/kg p.o. s.i.d.) significantly reduced oral plaques in blue penguins (*Eudyptula minor*) naturally infected with *M. intracellulare*.

Due to zoonotic potential specially for children and elderly persons and immunocompromised patients, prolonged treatment and poor success rate, difficulty of drug administration to avian patients maintaining proper doses, natural and acquired antimicrobial resistance, poor owner compliance and moreover, lack of a proper treatment schedule, the debate exists regarding advice of treating pet birds against tuberculosis. Nevertheless, euthanasia is the preferred measure in the prevention of tuberculosis in pet birds in relation to human health. For prevention of further transmission, removal of all organic matter and debris from cages, washing the cages and surroundings properly with disinfectants and maintenance of biosecurity measures are required. Chlorohexidine and quaternary ammonium compounds can act as mycobacteriostatic disinfectants.

## 2.1.2 Salmonellosis

### 2.1.2.1 History

D.E. Salmon (1885) first isolated *Salmonella* from the infected pigs. It was considered as a cause of ‘hog cholera’ until the discovery of the etiological virus. The nomenclature of the bacteria (‘*Salmonella*’) was done in memory of Salmon. In 1889, Klein (United Kingdom) first isolated *Salmonella* Gallinarum from chickens with ‘fowl typhoid’. Loeffler first described *Salmonella* Typhimurium from a natural outbreak of typhoid like infection in mice. Among the pet birds, Salmonellosis was first described in ducks (Vandervort 1954; Keymer 1958), and parakeets (Kaye et al. 1961; Madewell and McChesney 1975).

### 2.1.2.2 Etiology

The genus *Salmonella* is classified under the family Enterobacteriaceae that belongs to the order Enterobacteriales. There are two major species under the genus *Salmonella* i.e. *S. enterica* (>2440 serovars) and *S. bongori* (20 serovars). A third proposed species is *S. subterranea*, yet to be recognized as a true species. *Salmonella enterica* is considered as type species of the genus at present. *S. enterica* has 6 subspecies (ssp.): *salamae*, *arizonae*, *diarizonae*, *houtenae*, *indica*, *enterica*. Most of the pathogenic *Salmonella* are designated as ‘serovar’ under the *S. enterica* ssp. *enterica*. Virulent serovars are: Typhi, Typhimurium, Dublin, Choleraesuis, Pullorum, Gallinarum, Abortusovis.

*Salmonella* Typhimurium (var. *copenhagen*) is the most frequently isolated serovar from different outbreaks in psittacine birds. In passerine birds (perching/song birds), *S. Typhimurium* phage types DT40, DT41, DT56, DT160 are adapted. It acts as either a primary pathogen or it causes sub-clinical infection in young and immunocompromised birds. If the density of flock is high and the quantity of available feed is low, most of the birds become debilitated and are susceptible to *S. Typhimurium* infection.

Other sub-species and serovars of *Salmonella* isolated from pet birds include *S. houtenae*, *S. arizonae*, *S. Rissen*, *S. Enteritidis*, *S. Pullorum*, *S. Gallinarum*, *S. Newport*, *S. Panama*, *S. Rublislaw*, *S. Aberdeen*, *S. Thompson* and *S. Wasenaar*. Among them, *S. Gallinarum* can infect canaries, ring dove, pheasants, peacocks and peafowl. A novel *Salmonella* serovar (*S. Pajala*) was isolated from Peregrine falcon (*Falco peregrinus*) nestlings.

### 2.1.2.3 Host Susceptibility

Clinical outbreaks of salmonellosis are frequently detected in passerine and psittacine birds. Among passerine birds, finches (Fringillidae) and sparrows (Passeridae) seem to be particularly susceptible to *Salmonella* spp. infection. Salmonellosis is reported from canary (*Serinus canaria*), eurasian siskin (*Carduelis spinus*), zebra finch (*Taeniopygia guttata*), bengalese finch (*Lonchura striata domestica*) and picoplat (*Sporophila intermedia*). Fatal outbreaks with high mortality were reported in psittacine birds such as lorries and lorikeets (*Trichoglossus*, *Lorius*, *Eos* spp.), budgerigars (*Melopsittacus undulatus*), parakeets (*Psephotus* spp., *Psittacula* spp.), and sulphur crested cockatoo (*Cacatua galerita galerita*).

The birds with caecum [e.g. macaw (*Ara* sp.), amazon parrot (*Amazona* sp.)] can asymptotically carry *Salmonella* spp. like poultry. Both free-ranging and captive blue-fronted amazon parrots (*Amazona aestiva*) are detected to carry *Salmonella* spp. Although, lilac-crowned amazon parrots (*Amazona finschi Schlater*) was found died due to *S. Enteritidis* infection. Moreover, *S. Typhimurium* is detected as a primary pathogen causing death of blue and gold macaws (*Ara ararauna*).

Occasionally, raptors or hunter birds (e.g. falcon, red kite), game birds [e.g. red-legged partridge (*Alectoris rufa*)], free-ranging sparrow (*Passer domesticus*), gull (Laridae), wild birds such as temminck’s seedeater (*Sporophila falcirostris*), chestnut-capped blackbird (*Chrysomus ruficapillus*), brown-headed cowbirds

(*Molothrus ater*), white-throated sparrows (*Zonotrichia albicollis*) can also harbour *Salmonella* spp.

#### 2.1.2.4 Transmission

Infected pet birds, rodents, reptiles, wild birds, contaminated water, feed and eggs act as source of *Salmonella* spp. When the pet birds are gathered in an exhibition the healthy birds come in direct contact to the infected birds. The rodents, reptiles and wild birds having access to the open-air aviary can contaminate the place. The bacteria can survive for extensive periods on wood and dust and can live for 28 months in avian faeces. The contaminated places become a constant source of infection. In a pet shop, iguana (*Iguana iguana*) was identified as a source of *Salmonella* spp. infection in a cockatoo.

Ingestion of contaminated feed and drinking water is the major route of transmission in pet birds. Sometimes, the infection is also transmitted by owners or attendants through their contaminated hands, feet and clothes. Trans-ovarian transmission is common in poultry, although, is not frequently observed in pet birds.

#### 2.1.2.5 Pathogenesis

##### *Salmonella* Typhimurium

Following oral route of transmission, *Salmonella* is deposited in the intestine, where they invade enterocytes. The bacteria can invade the epithelial cells throughout the intestine, although, caeca and ileocaecal junction are the preferred site. In the intestine, low pH, peristalsis, intestinal mucus, lysozyme in secretions and moreover normal microbial flora try to destabilize the bacterial colonization. Normal microflora in adults prevents the *Salmonella* colonization by occupying their receptors, known as 'competitive exclusion'. Young are more susceptible as their intestinal microfloral range is not fully developed.

Bacterial fimbriae, lipopolysaccharide (LPS), pathogenicity island (SP-1-T3SS associated proteins) help in adherence with the enterocytes. The interaction between the T3SS proteins (SipA, SipC) with the actin cytoskeleton of the enterocytes causes cytoskeletal rearrangements to generate an uneven surface (membrane ruffle). The organisms are trapped within the ruffled membrane and are internalized by the enterocytes. Within the enterocytes, the bacteria reside in a membrane bound vacuole i.e. *Salmonella* containing vacuoles (SCV). As the SCV matures, it migrates from the luminal border of the enterocyte to the basal membrane. In the basal membrane, the SCV enter the macrophages associated with Peyer's patches in the sub mucosal space. The SCV never fuses with lysosome within macrophages and thus avoid phago-lysosome fusion which is necessary to kill the bacteria. Additional factors such as SP-2-T3SS (SipC protein), SP-3 associated proteins also help in intracellular survival. The formation of *Salmonella* induced fibrils (SIF) help in bacterial replication in an unknown way. It is evident that major portion of the infection is cleared by the macrophages, only certain part can survive leading to chronic infection or carrier state with persistent faecal shedding. Sometimes,

invasion of *Salmonella* takes place beyond the intestine which causes bacterimia, survival and replication of the bacteria in reticulo-endothelial cells of liver and spleen. In passerine birds (canary, finch and starling), esophagus and crop are the preferred site for bacterial colonization after bacterimia.

#### 2.1.2.6 Clinical Symptoms

Acute and chronic form of salmonellosis is detected in pet birds. In acute form, huge mortality without any prior cardinal signs was observed in a flock of canaries. A *S. Typhimurium* infected macaw (*Ara ararauna*) showed depression, anorexia, delay in the emptying of crops (ingluvies), laboured breathing and diarrhoea for 3–4 days before death. Greenish-yellow diarrhoea was observed in adult budgerigars (*Melopsittacus undulatus*) infected with *S. Gallinarum*.

The chronic form shows numerous general symptoms such as anorexia, diarrhoea, dyspnoea, lethargy, cachexia, ruffled feathers, subcutaneous granuloma, crop stasis, conjunctivitis, arthritis and panophthalmitis. Adult budgerigars with chronic salmonellosis showed loss of condition, unwillingness to fly, inability to perch, and gathering in the bottom of the cage (Fig. 2.3). Drop in egg production and increased embryonic mortality rate was observed. In pigeons, in addition to the general syndrome, polyuria, arthritis of the joint, nervous symptoms and dermatitis followed by death was detected.

Stress associated with environment, season, change of diet and housing, transport, breeding, concurrent infection, and introduction of new birds without quarantine are the major predisposing factors for salmonellosis in pet birds.

**Fig. 2.3** Sick budgerigar  
(Courtesy LafeberVet)



### 2.1.2.7 Lesion

In acute form of salmonellosis in pet birds, no specific gross lesion is observed. In chronic infection, congestion of lung and intestine, splenomegaly, hepatomegaly, liver with necrotic foci is observed (Fig. 2.4). The passerines birds infected with *S. Typhimurium* shows the granulomatous lesions like pseudotuberculosis. Esophagitis and ingluvitis with necrotic plaques in esophagus and crop, respectively, are consistently detected in wild or free-range passerine birds. In European goldfinch (*Carduelis carduelis*), instead of esophagus lesions are sometimes developed in the subcutaneous tissues. In canaries, congestion of vital organs and necrotic foci on the liver with a nodular and miliary appearance is detected. Intestinal content become dark in colour due to haemorrhagic diasthesis. In lorries and lorikeets, petechial hemorrhages on the serosal surface of the proventriculus, ventriculus, and cardiac muscle along with atrial dilation are observed. Sometimes, bacterial emboli occur in liver, spleen, lung, kidney, and proventriculus. In *S. Typhimurium* infected macaws, pectoral muscle atrophy, fibrinous exudate on intestinal mucosa, white-greyish nodules on intestinal serosa, myocardium, lungs and ingluvies mucosa are commonly observed. In young budgerigars, fibrin deposit is observed as a white, thick layer on the pericardium. Petechial haemorrhages are detected on the surfaces of pericardium, myocardium, gizzard, duodenum and ileo-caecum. In garden birds infected with *S. Typhimurium*, esophageal ulcers, granulomata in soft tissues, hepatomegaly and splenomegaly are observed.



**Fig. 2.4** Splenomegaly in Gouldian finch due to salmonellosis (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)

Microscopic inspection of histopathological sections shows necrosis of parenchymatous organs specially the liver with granulocyte infiltration and fibrin deposition. In young and adult budgerigars, the blood vessel walls become hyalinized in appearance with various-sized microthrombi. The chronic cases are characterized by formation of a granuloma. A typical granuloma consists a necrotic centre which is surrounded by granulocytes and macrophages containing *Salmonella* spp. Multinucleated giant cells are found in chronic infection. In passerine birds, epithelial surface of the esophagus is ulcerated and it forms a thick layer of necrotic cellular debris composed of degenerated and intact leukocytes with gram-negative bacteria. Infiltration of heterophils, lymphocytes and plasma cells occur into the underlying sub-mucosa.

### 2.1.2.8 Diagnosis

#### Clinical Specimens

Clinical samples include faeces or cloacal swabs, blood/serum of live birds and affected tissues, such as liver, spleen, heart, intestine/caeca, lung, esophagus/crop, brain and kidney in 10% buffered formalin. Before collection of cloacal swabs, pericloacal asepsis with iodized alcohol is performed. Blood samples are collected from jugular, wing or ulnar vein. The environmental samples, such as pooled faeces, litter and dust from the cages, feed and drinking water should be examined to know about an outbreak, if any. Specimens should be collected before antibiotic treatment of the birds. After death, the collection should be done immediately from fresh carcasses. For 'pre-enrichment', swabs should be collected in buffered peptone water. Pre-enrichment in buffered peptone water helps in survival of *Salmonella* from freezing, heating and desiccation. The cold chain (4–5 °C) should be maintained during transportation of the samples to the laboratory.

#### Diagnostic Techniques

- (a) Clinical signs and lesions after necropsy, history of direct contact with infected birds give a tentative diagnosis
- (b) *Direct Examination*: An impression smear prepared from clinical samples such as cloacal swab/faeces/tissues, is stained by Gram's Method. *Salmonella* appears as gram negative small rods with no distinct characteristics. The tissue samples of heart, lung, liver, spleen, kidney, and intestine are fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 mm, and stained with hematoxylin-eosin and periodic acid-Schiff for direct examination of the bacteria.
- (c) *Isolation of bacteria from clinical samples*: Clinical samples require pre-enrichment and enrichment for growth. For pre-enrichment, the samples collected in buffered peptone water are kept at 40 °C for 24 h. The pre-enriched clinical samples are transferred into enrichment medium such as selenite or tetrathionate broth and are incubated at 40 °C for another 24 h.

From the supernatant, the samples are plated in brilliant green agar (BGA) or xylose-lysine-deoxycolate agar (XLD) and are re-incubated for another 24 h at 40 °C. Convex, pale red, translucent colonies in BGA and red coloured colonies with black centres in XLD agar are presumably diagnosed as *Salmonella* spp. *S. Pullorum* produces small, paler colonies than other salmonellae in BGA. The suspected colonies can be confirmed by different biochemical tests such as catalase, oxidase, IMViC, TSI, carbohydrate fermentation profile and lysine decarboxylase test.

(d) *Serological tests:*

- (i) Rapid whole blood/serum agglutination test: It can be used for rapid detection of *Salmonella* spp. with crystal violet stained or unstained *Salmonella* polyvalent 'O'-antigen. Equal amount of suspected whole blood or serum is gently mixed with the antigen on a white tile. Tile is agitated gently for 2 min and is observed for reading. In a positive case clumping of antigen is visible within 2 min. The antigen is commercially available or it can be obtained from Veterinary institutes in different countries.
- (ii) Tube agglutination test
- (iii) Immunodiffusion
- (iv) Immunofluorescence
- (v) ELISA

(e) *Molecular Biology:* For rapid and reliable detection of *Salmonella*, conventional PCR based diagnostic techniques targeting *invA* or other genes are used. Genus and serovar (*S. Enteritidis* and *S. Typhimurium*) specific real-time PCR system have also developed. For phylogenetic analysis of the *Salmonella* isolates, pulsed field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) can be used.

### 2.1.2.9 Zoonosis

Zoonotic transmission of *Salmonella* spp. to human from parakeet kept as a pet was documented. A salmonellosis outbreak associated with dissection of an owl was reported among the elementary school children. The possibility of children infection (below 5 years age) is more in those families who rear a pet bird (odd ratio: 2.7) or a lizard (odd ratio: 3). In open air aviaries and children's zoos, the transmission of *Salmonella* spp. was reported between wild birds, pet birds and human. Special care for designing such aviaries should be adopted.

### 2.1.2.10 Treatment and Control Strategy

Antibiotics against *Salmonella* spp. in infected pet birds can be administered after doing the sensitivity of the bacterial isolates. Antimicrobial resistance of *Salmonella* spp. is a global concern at present. Successful treatment of infected canaries with 10% (w/v) enrofloxacin solution provided as 200 mg/l in drinking water for 5–7 days was observed. Treatment with kanamycin, gentamicin, trimethoprim/sulfamethoxazole

suspension along with anti-diarrhoeals such as daolin and pectin combination is recommended.

General control and prevention strategies such as isolation of diseased birds from the rest of the flock, cleaning and disinfection of cages, water and feed utensils with 10% (v/v) solution of sodium hypochlorite or commercial disinfectants are recommended. If the feedstuffs are suspected it should be replaced with new batch immediately.

### 2.1.3 Chlamydiosis

#### 2.1.3.1 History

Halberstaedter and von Prowazek (1907), a Czech zoologist, first described *Chlamydia*, although it was actively infecting people for centuries before its discovery. First description of *Chlamydia* associated trachoma in human was found in the Ebers papyrus dated around 1550 B.C. In modern times, Ritter (1879) first described *Chlamydia psittaci* infection in human acquired from parrots. During 1890–1930, numerous outbreaks of human psittacosis occurred in Europe, North and South America, associated with parrots and other pet birds. In 1929–30, pandemic psittacosis outbreaks in human were reported due to import of infected psittacine birds from South America to Europe and North America.

In 1930, Levinthal, Coles, and Lillie, independently described the properties of the pathogen, and accordingly *Chlamydia* was known as Levinthal-Coles-Lillie (LCL) agent. Moulder (1962) first revealed the structural and chemical composition of *C. psittaci*. Hatch (1975) demonstrated the requirement of adenosine-triphosphate (ATP) supplementation for growth of *Chlamydia*.

In literatures, first description of chlamydiosis in parakeets was reported from Germany (Strauch and Rott 1958). Further study after few years also revealed the presence of *Chlamydia* in parrots and other parakeets in Germany (Schmittiel 1966).

Human psittacosis outbreaks specially in persons associated with poultry, turkey and duck industry were reported from United States and European countries during 1980–90.

#### 2.1.3.2 Etiology

All the Chlamydiae are placed under the order Chlamydiales, and family Chlamydiaceae. Based on cluster analysis of 16S and 23S rRNA genes, the family Chlamydiaceae was divided into two genera i.e. *Chlamydia* and *Chlamydophila*. Recent genome comparison study of the two genera proposed to unite the *Chlamydia* in a single genus. The latest edition of the Bergey's manual of systematic bacteriology also described the single genus of *Chlamydia*. Pathogenic species under the genus *Chlamydia* are *C. psittaci*, *C. trachomatis*, *C. suis*, *C. muridarum*, *C. abortus*, *C. caviae*, *C. felis*, *C. pecorum* and *C. pneumoniae*. Among the pathogenic species, *C. psittaci* is mostly associated with avian chlamydiosis (psittacosis) in pet birds, ornithosis in poultry, and zoonotic infection in human. The 16S rRNA gene based phylogenetic study indicates the presence of a distinct cluster



**Table 2.1** Serovars and genotypes of *C. psittaci* and their hosts

Serovar	Host	Genotypes	Host
A	<i>Psittaciformes</i> (cockatoos, parrots, parakeets, lorries)	A	<i>Psittaciformes</i> (cockatoos, parrots, parakeets, lorries)
B	<i>Columbiformes</i> (pigeons and doves)	B	<i>Columbiformes</i> (pigeons and doves)
C	<i>Anseriformes</i> (ducks and geese)	C	<i>Anseriformes</i> (ducks and geese)
D	Turkeys	D	Turkeys
E	Pigeons, turkeys, ratites	E	Pigeons, turkeys, ratites
F	Psittacines	F	Psittacines and turkeys
		E/B	Ducks, turkeys, pigeon
		WC	cattle
		M56	rodents

of *C. psittaci* strains which are associated with chlamydiosis in Psittaciformes (cockatoos, parrots, parakeets, lorries etc.) and Columbiformes (pigeons) birds. Although, other Chlamydial species such as *C. abortus*, *C. trachomatis* and *C. pecorum* are occasionally detected in brown skua, parrots, parakeets, and pigeons.

Three new avian species of *Chlamydia*, namely *C. ibidis* sp. nov., *C. avium* sp. nov., and *C. gallinacea* sp. nov. are proposed. Among them, *C. ibidis* and *C. avium* are isolated from feral sacred ibis (*Threskiornis aethiopicus*), psittacines, and pigeons.

Earlier, avian isolates of *C. psittaci* was divided into six serovars (serotypes) which can infect different species of birds (A–F, Table 2.1). Based on major outer membrane protein (*ompA*) sequence, *C. psittaci* is currently divided into 15 genotypes. Among them, nine genotypes (A–F, E/B, M56, WC) are associated with different species of birds and mammals (Table 2.1).

### 2.1.3.3 Host Susceptibility

Avian strains of *C. psittaci* are detected in more than 460 species of birds under 30 orders. The pet birds belonged to the order Psittaciformes (cockatoos, parrots, parakeets, lorries etc.) and Columbiformes (pigeons) are most susceptible to *C. psittaci* infection. In parrots, the worldwide prevalence of *C. psittaci* varies from 16–81%. The infection in pet birds is reported from Europe, Brazil, Africa, USA, Iran and India. Free ranging Galapagos doves (*Zenaida galapagoensis*) and rock doves (*Columba livia*) in Spain; monk parakeets (*Myiopsitta monachus*), Amazon parrots, red-tailed Amazon (*Amazona brasiliensis*) in Brazil; ring necked parakeet (*Psittacula krameri*), Alexandrine parakeet (*Psittacula eupatria*), African grey parrot (*Psittacus erithacus*), Timneh grey parrot (*Psittacus erithacus timneh*) in Iran were reported to be infected with *C. psittaci*. In India, *C. psittaci* was isolated from pigeons (*Columba livia*), parrots (*Psittacula krameri*) and crows (*Corvus splendens*). Infection of Passeriformes birds is not common, although, canaries were detected to be infected with *C. psittaci* in Croatia.

*C. psittaci* was also detected in healthy asymptomatic birds such as in *Ara macao*, and *Amazona ochrocephala* in Costa Rica, and free-living Hyacinth macaw (*Anodorhynchus hyacinthinus*) and blue-fronted parrot (*Amazona aestiva*) in Brazil. The syndrome was not expressed either due to infection with low virulent strain or resistance of some bird species.

The seroprvalence studies revealed the presence of *C. psittaci* antibodies in macaws (*Ara macao*, *Ara ambigua*), hyacinth macaws (*Anodorhynchus hyacinthinus*), budgerigars (*Melopsittacus undulatus*), lovebirds (*Agapornis* sp.), cockatiels (*Nymphicus hollandicus*), Alexandrine parakeets (*Psittacula eupatria*), Eurasian siskins (*Carduelis spinus*), oriental skylarks (*Alauda arvensis*), and black-tailed grosbeaks (*Coccothraustes migratorius*) in different countries. The presence of *C. psittaci* antibodies indicates the exposure of the birds to the organism. In a study in China, highest seroprevalence was observed in cockatiel which was followed by Alexandrine parakeets, lovebirds, and budgerigars. It seems that lovebirds and budgerigars among the psittacine birds are relatively resistant against *C. psittaci* infection, although, the reason is unexplored.

Among the wild predator birds, white-tailed sea eagle (*Haliaeetus albicilla*) and the peregrine falcon (*Falco peregrinus*) are detected to be infected with *C. psittaci*.

#### 2.1.3.4 Transmission

Inhalation of contaminated dust, airborne particles from the feathers and ingestion are major ways of *C. psittaci* transmission in the birds. Direct contact during close proximity with the infected birds also helps in transmission. Throughout the breeding season, specially during incubation of eggs, male psittacine birds prefer to feed the females by regurgitation. In this process the feeds are often mixed with secretions of the crop, pharynx and nasal cavity. Transmission of *C. psittaci* is observed from parent birds to their nestlings during feeding.

Asymptomatic carrier birds infected with *C. psittaci*, excrete the organisms through faeces, nasal and lacrimal discharge, oropharyngeal mucus, crop milk and other secretions. Shedding is increased during coexisting infections and stress conditions such as shipment, breeding, crowding, chilling and nutritional deficiencies. When the excreted faecal material dries, the organisms are aerosolized. Elementary bodies (infectious form) of *C. psittaci* survive in the dried faeces for several months, in the contaminated feed for up to two months, on glass for 15 days, and in straw for 20 days.

Mechanical transmission of *C. psittaci* by biting arthropods such as flies, mites and lice are observed. Vertical transmission is infrequently observed in parakeets, seagulls, snow geese and poultry.

Zoonotic transmission of *C. psittaci* in human occurs mostly through inhalation of contaminated dust, feathers and aerosolized excretions. Direct contact with infected pet birds or their cages, utensils, beddings contaminated with discharges can transmit the bacteria. Sometimes, biting of the infected birds also helps in transmission. Person to person spread is rarely reported although possible through inhalation.

### 2.1.3.5 Pathogenesis

*Chlamydia* follows a unique life cycle with tri-phasic developmental stages. The infectious form (elementary body, EB) is extremely small (250–350 nm in diameter), pear to spherical shaped particle with electron dense irregular nucleoid. It has rigid cell wall with disulphide cross linkage among the cysteine rich amino acids of outer membrane proteins (OMP). This form can survive for a prolonged period in the environment. After transmission, infection starts with the attachment of elementary bodies to the host cell membrane. In birds, apical surface of columnar epithelial cells in intestine acts as preferred site for attachment of *C. psittaci*. Primary attachment of EB takes place by electrostatic interactions, most likely with glycosaminoglycan (GAG) moieties on the host cell surface. This reversible binding is followed by receptor mediated irreversible attachment. Protein disulfide isomerase (PDI), present in the host cell membranes and causing disulphide reduction, helps in attachment of EBs.

Chlamydial major outer membrane protein (MOMP) mostly acts as adhesin to bind with the host cellular receptor. Following receptor mediated attachment, the EB enters the cell via endocytosis [microfilament dependent/independent process (clathrin mediated)]. Some Chlamydial strains enter the host cells through cholesterol-rich lipid raft domains. Among different pathways, *C. psittaci* strains prefer to use clathrin-coated vesicles for cellular entry. *C. psittaci* elementary bodies contain rosette like long projections (Matsumoto's projection) on their surface which acts as type three secretion system (T3SS). *C. psittaci*-T3SS helps in introduction of Chlamydial proteins into the host cell cytoplasm. These T3SS-injected proteins interact with host cellular proteins and cause modulation of host cell function.

After cellular entry, vesicles containing EBs escape the lysosomes in the host cell cytoplasm and reach near the nucleus within 8–12 h after entry. In *C. psittaci* infection, IncB proteins (T3SS-effector protein) interact with host cell proteins (dynein motor proteins) for intracellular transport of vesicles with EBs into the nuclear zone. The EB is converted into reticulate bodies (RB) in this nuclear zone. The EB loses its electron dense DNA core and its cell wall loses its rigidity due to break of disulphide bridges. The reticulate bodies are non-infectious form, larger in size (500–2000 nm diameter) and metabolically active. RBs multiply by binary fission and start genus specific protein synthesis. The structural reorientation started and RBs are transformed into intermediate bodies (IB, 300–1000 nm in diameter). A central electron-dense core with radially arranged nucleoid fibres surrounding the core is observed in the IBs. The IBs are converted into progeny EBs within a vesicle after 30 h of the entry of initial EBs. Chlamydial micro colony with 100–500 EBs within the vesicle is called 'inclusion body' and it is generated after 48–50 h. The inclusion bodies move to the golgi apparatus region with the help of host dynein proteins. EB is released to attack new cells by rupturing the vesicles and the cycle is repeated. The signal for release of EBs is yet unknown but it is associated with host cellular apoptosis. Suppression of host cellular apoptosis can induce persistent Chlamydial infection.

Intracellular survival of EBs depends on escape from the lysosomal breakdown process. The EBs can induce delayed maturation of lysosomes as an escape mechanism. The intracellular inclusion bodies are covered with a mesh of host cytoskeletal filaments which prevent the exit of the content and consequent activation of the host immune system. Close attachment of *C. psittaci* inclusions with the mitochondria helps in acquisition of ATP because they cannot synthesize it. Moreover, intracellular survival of the inclusions depends on acquisition of lipids such as sphingomyelin, phosphatidylinositol and phosphatidylcholine. Golgi apparatus of the host cells act as major source of lipids for the inclusions and often the golgi apparatus are fragmented to provide the lipid.

Some non-replicating reticulate bodies persist within the host cytoplasm and produce latent infection. The growth cycle of *Chlamydia* within the body of the host is disrupted due to nutritional deprivation, treatment with antibiotic and activated immune system. In disrupted growth cycle, reticulate bodies are converted into enlarged pleiotrophic 'aberrant' RBs. The aberrant RB contains chromosome but the genes associated with growth (genes encoding membrane proteins, transcription regulators, cell division factors, EB-RB differentiation factors) are not expressed. Further, the genes encoding chlamydia protein associated with death domains (CADD) are down regulated which causes suppression of host cell apoptosis and persistence of infection. Interaction of host cellular protein (G3BP1) and chlamydial IncA (T3SS-effector protein) also suppress host cellular apoptosis. When the inducers of the disrupted growth cycle (antibiotic, immune system products) are removed, the aberrant RB is again converted into normal RB and they can complete the growth cycle.

### 2.1.3.6 Clinical Symptoms

In birds, chlamydiosis has an incubation period of 3–10 days. Clinical symptoms are not specific. General syndrome such as loss of condition, anorexia, fever, diarrhoea, respiratory problems, nasal and ocular discharges are observed. Expression of syndrome and associated mortality (up to 80%) depends on virulence of *C. psittaci* strains, age, species, nutritional and immune status of the pet birds. Occasionally, sub-clinical *C. psittaci* infection without visible syndrome is observed in birds. During stress conditions, the sub-clinical infection is activated with increased shedding of *C. psittaci*.

### 2.1.3.7 Lesion

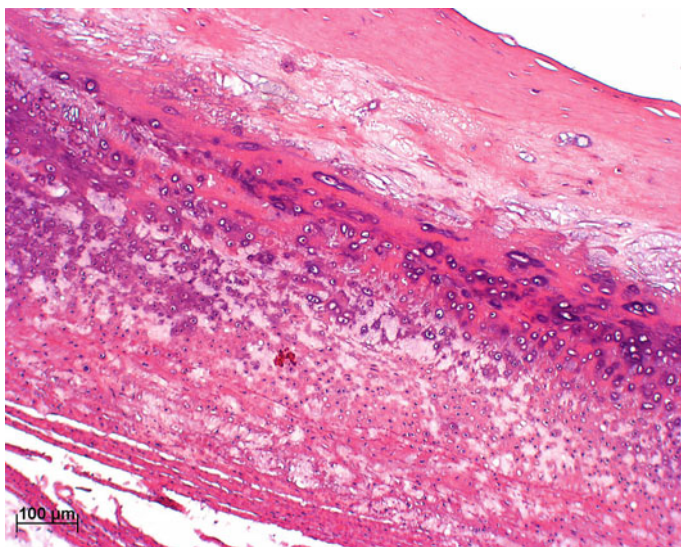
The pet birds with avian chlamydiosis do not show any pathognomonic gross lesion. Conjunctivitis, lateral nasal adenitis, sinusitis, fibrinous airsacculitis, lung congestion, fibrinous pneumonia, pericarditis with presence of fibrinous cover, peritonitis, hepatitis with multifocal necrosis and splenitis are observed (Figs. 2.5 and 2.6). In pigeons, conjunctivitis, swollen eyelids, rhinitis, presence of fibrinous exudates over peritoneum, air sac and pericardium, enlarged, soft and dark coloured liver and spleen are observed. The budgerigars, infected with *C. psittaci* and Reovirus, showed distinct cachexia, hepatomegaly and splenomegaly. The livers become enlarged, mottled and tan-brown in colour. Other lesions include uric acid



**Fig. 2.5** Pericarditis with presence of fibrinous cover in a bird with avian chlamydiosis (*Courtesy Prof. Richard Hoop, University of Zurich, Switzerland*)



**Fig. 2.6** Hepatitis with multifocal necrosis in a bird with avian chlamydiosis (*Courtesy Prof. Richard Hoop, University of Zurich, Switzerland*)



**Fig. 2.7** Histopathological section of the blood vessel of a bird with atherosclerosis (Courtesy Prof. Richard Hoop, University of Zurich, Switzerland)

deposit in kidney, conjunctivitis and air-sacculitis. Carrier birds with asymptomatic infection do not show any gross lesion.

Atherosclerosis is considered as a well defined ailment specially in aged pet birds. African gray parrots, macaws and Amazon parrots are most susceptible to this condition. Sudden death without prior symptom is the cardinal sign of atherosclerosis. Like human, the risk factors for atherosclerosis include high cholesterol and triglyceride concentrations, sex, age, species, obesity and inactivity, and moreover, *C. psittaci* infection. Arteriosclerotic plaques are observed between the intima and internal elastic lamina of the blood vessels in many species of birds (Fig. 2.7). The plaques are composed of fibrous tissues and are observed as pale yellowish areas at the thickened portion of intima. In severe cases, the plaques become circumferential lesion which cause narrowing of the lumen and reduced blood flow.

### 2.1.3.8 Diagnosis

#### Clinical Specimens

From live birds, pharyngeal/choanal slit swabs, conjunctival swabs and nasal swabs can be collected aseptically as ante-mortem samples. Faeces or cloacal swabs are less preferred because shedding of *Chlamydia* is not consistent. Post mortem samples collected from the dead birds include lungs, spleen, liver and air sacs.

Chlamydiae are relatively labile organisms and special precautions are required for their detection. Samples should be maintained in cold chamber and processed



immediately after collection. The tissue samples can be preserved at  $-80^{\circ}\text{C}$  for prolonged period. DNA extracted from the tissue samples can be stored in stabilization buffer. For successful isolation of *Chlamydia*, the clinical samples should be collected in special *Chlamydia* transport medium such as 2SP (0.2 M sucrose phosphate medium containing 10  $\mu\text{g/ml}$  of gentamicin, 25 U of nystatin and 25  $\mu\text{g/ml}$  of vancomycin) and SPG (75 g of sucrose, 0.52 g of  $\text{KH}_2\text{PO}_4$ , 1.22 g of  $\text{Na}_2\text{HPO}_4$ , 0.72 g of glutamic acid and water in 1 litre, pH 7.4–7.6) supplemented with bovine serum albumin, streptomycin, vancomycin and nystatin. Broad spectrum antibiotics like tetracycline, chloramphenicol, macrolides, sulphonamides, penicillin should not be added as they have anti-chlamydial effect.

### Diagnostic Techniques

- (a) *Direct examination*: Smears prepared from collected faecal samples, conjunctiva or impression smears of tissue samples can be stained with Macchiavello, Castaneda, Giemsa, Giménez, modified Gimenez (PVK stain), Stamp, modified Z-N, and methylene blue for demonstration of Chlamydial inclusion bodies. Giemsa stain is more useful in the smears prepared from conjunctival scrapings. The inclusion bodies appear purple/blue with Giemsa, Castaneda and methylene blue stain and red with Macchiavello, Giménez, Stamp, and modified Z-N stains.
- (b) *Isolation of Chlamydia from clinical samples*: Isolation of *Chlamydia* can be done in the yolk sacs of embryonated hen eggs, laboratory animals and cell culture. Fertile chicken eggs (6–8 days old) are inoculated through the yolk sac route. The embryo dying three or more days after incubation is examined for chlamydial inclusions. Mice are ideal laboratory animal for isolation of *Chlamydia*. The mice usually die within ten days of intranasal, intracerebral or intraperitoneal inoculation and the EBs can be isolated from viscera and peritoneal exudates. Cell lines treated with a metabolic inhibitor (cycloheximide at 2  $\mu\text{g/ml}$ ) can be used for isolation of Chlamydiae. McCoy, HeLa, monkey kidney cells, L-929, Buffalo Green Monkey (BGM) cells, mouse fibroblast cells, fish and lizard cells are used. The inoculated cells should be incubated at  $35\text{--}37^{\circ}\text{C}$  for 48–72 h and the intracytoplasmic inclusion bodies are detected by staining (Giemsa) or fluorescein conjugated monoclonal antibody. Isolation by cell culture is still considered as gold standard method for detection of *Chlamydia*. However, it requires biosafety level-3 (BSL3) laboratory with expertise.  
The clinical samples should be decontaminated by antibiotics like gentamicin (50  $\mu\text{g/ml}$ ), vancomycin (75  $\mu\text{g/ml}$ ) and nystatin (500 unit/ml) before inoculation into eggs, animals or cell lines. Transport medium (2SP) can be used as buffer.
- (c) *Detection of C. psittaci antigen*: ELISA based antigen detection kits are available for detection of *C. trachomatis* infection in human. The same kit can

be used for detection of *C. psittaci* because the two species share common antigen. However, minimum 600 elementary bodies are needed in the samples for detection.

- (d) *Serological tests*: Serological tests can be used as supplementary diagnostic tests along with detection of antigen or isolation. Presence of antibodies in the host cannot confirm active infection. Sometimes, false negative results are produced if the samples are collected before development of antibody or during treatment with antibiotics. The serological methods such as micro immunofluorescence (MIF) test, ELISA, CFT, elementary body agglutination (EBA) tests are used for detection of anti-*Chlamydia* antibodies. MIF is more sensitive and can detect all types of immunoglobulins in the sera. ELISA based tests using whole organism, LPS, lipoglycoprotein of *Chlamydia* as antigen are sensitive but less specific for detection of *C. psittaci*. Whereas, ELISA with recombinant major outer membrane protein (MOMP) of *C. psittaci* as antigen, can more specifically detect *C. psittaci*. CFT can detect anti-*Chlamydia* Ig G only, not Ig M. Further, CFT is tedious, time consuming, less sensitive test and the antigens (complement fixing) are not commercially available. The EBA test can detect anti-*Chlamydia* Ig M only, and as a consequence, infection in early stage can only be diagnosed.
- (e) *Molecular biology*: PCR is a specific, sensitive, and rapid technique to detect *C. psittaci*. Successful application of PCR depends on quality of extracted DNA from the clinical samples. Guanidine-detergent lysing solution should be used for lysis of eukaryotic host cells and *Chlamydia* for extraction of DNA. The 16S rRNA gene is conserved in the genus *Chlamydia* and is a suitable target gene for detection of *Chlamydia* up to species level. Major outer membrane protein (*ompA*) is used as a target gene in nested PCR, although, variations exist in the MOMP gene sequence among *C. psittaci* strains. SYBR green-based real time PCR targeting *ompA*, 23S rRNA gene, inclusion membrane protein A gene (*incA*), molecular cysteine-rich protein gene (*envB*) of *C. psittaci* and microarray-based detection assays are also developed for detection of *Chlamydia*.

### 2.1.3.9 Zoonosis

Human psittacosis cases are reported in Europe, USA, South America, Japan and Australia. Other than the persons who rear the birds in their home, occupational risk groups such as veterinarians, pet shop workers, avian quarantine workers, poultry processing plant workers, bird breeders, and farm workers are most susceptible. Even psittacosis outbreak was detected among custom officers in some countries due to their exposure to imported parakeets in the airport. Incubation period in human is 5–14 days. Clinical syndrome in human includes fever, chills, headache, pneumonia, renal disorders, and miscarriages in pregnant women. All the vital organs are affected with the progression of infection and endocarditis, hepatitis, myocarditis, arthritis and encephalitis are reported. Ocular infection with follicular kerato-conjunctivitis is also observed.



### 2.1.3.10 Treatment and Control Strategy

Doxycycline, tetracycline and enrofloxacin were successfully used in budgerigars and psittacine birds to cure avian chlamydiosis. Doxycycline is the drug of choice for the birds and the treatment should be continued for 45 days. It may induce toxicity in some bird species and produce signs of depression, inactivity, anorexia, greenish or yellowish urine. Use of the drug in those birds should be stopped immediately and supportive symptomatic treatment should be started. Recommended dose of doxycycline in feed is 300 mg/kg feed for 45 days. In drinking water, 400 mg of doxycycline hyclate/litre of water will maintain therapeutic concentration in psittacine birds. Administration of the drug through the feed or drinking water is suitable for aviaries. For pet bird owners, oral administration of the capsule in individual bird is appropriate. Recommended oral dose of the drug is 40–50 mg/kg body weight in every 24 h for cockatiels, Senegal parrots, blue-fronted, orange-winged Amazon parrots; 25 mg/kg body weight in every 24 h for African gray parrots, blue and gold macaws, green-winged macaws; and 25–50 mg/kg body weight in every 24 h for other psittacine birds. Injectable doxycycline is administered at doses of 75–100 mg/kg body weight, intramuscularly (pectoral muscle), in every 5–7 days for the first 30 days and subsequently in every 5 days for the rest of the treatment period. Long acting oxytetracycline can be injected sub-cutaneously at the dose of 75 mg/kg body weight in every 3 days in cockatoos, blue-fronted and orange-winged Amazon parrots, and blue and gold macaws. The oxytetracycline injection causes irritation at the site. If tetracycline is orally administered or used in feed, dietary calcium sources (mineral block, oyster shell, supplemented pellets) should be reduced.

To control the psittacosis infection in aviaries, general precautionary measures, such as quarantine of newly introduced birds for 30 days and periodical testing for *C. psittaci* infection, separation of birds after return from bird shows or fairs, rodent control, control of exposure to wild birds, regular disinfection of the cages and utensils, proper ventilation to reduce aerosol load within the unit should be followed. Use of prophylactic antibiotic is not recommended as it may produce resistant bacteria. Recommended disinfectants for *C. psittaci* infection are 1:1,000 dilution of quaternary ammonium compounds, 70% isopropyl alcohol, 1% lysol, and chlorophenols. Use of vacuum cleaner in the aviaries is not preferred as it will aerosolize infectious particles.

No vaccine is commercially available for the pet birds against *C. psittaci* infection. Experimental DNA vaccination in budgerigars with plasmid DNA expressing MOMP of *C. psittaci* was found effective.

## 2.1.4 Campylobacteriosis

### 2.1.4.1 History

In 1886, Escherich observed *Campylobacter* like organisms in stool samples of children with diarrhoea. In 1913, McFaydean and Stockman first isolated and identified *Campylobacter* spp. in foetal tissues of aborted sheep. Confirmatory tests

were also carried out by Smith in 1918 when similar organisms were isolated from aborted bovine fetuses. In this period, the bacteria were known as *Vibrio foetus*. In 1963, due to certain differentiating characteristics, the bacteria were separated from Vibrionaceae family and the new genus *Campylobacter* ('curved rod') under Campylobacteriaceae family was proposed.

### 2.1.4.2 Etiology

*Campylobacter* spp. is gram negative, comma shaped rods specially in infected tissues and young cultures. When two bacterial cells are found together in a microscopic field, occasionally it looks like 'S' or 'wing of gull' ('flying seagull'). They are motile by single unipolar/bipolar unsheathed flagella. Motility is darting or corkscrew type, best observed by dark field microscopy.

Campylobacteriaceae family contains four genera namely *Campylobacter*, *Arcobacter*, *Sulfurospirillum* and *Thiovulum*. Currently there are 18 species and 6 sub species of the genus *Campylobacter*. Important species and sub species of *Campylobacter* are—*Campylobacter jejuni* ssp. *jejuni*, *C. jejuni* ssp. *doyeli*, *C. coli*, *C. lari*, *C. fetus* ssp. *venerealis*, *C. fetus* ssp. *fetus*. Thermotolerant *Campylobacter* (*C. jejuni* ssp. *jejuni*, *C. coli*, *C. lari*, some strains of *C. upsaliensis*) is isolated from pet birds with or without clinical syndrome. The thermotolerant species requires higher temperature for their growth (42 °C) which is provided by the pet birds due to high body temperature. Other than thermotolerant *Campylobacter* species, *C. fetus* and *C. intestinalis* have also isolated from parrots.

### 2.1.4.3 Host Susceptibility

The pet birds such as tropical finches (juvenile Estrildidae), canaries, pigeons, parakeets [except red-crowned parakeet (*Cyanoramphus novaezelandiae*), dusky-headed parakeet (*Aratinga weddellii*), orange winged parrot (*Amazona amazonica*), red bellied macaws (*Ara manilata*)], emu, ostriches, waterfowls (mallard duck, shoveler duck, green-winged teal duck) are detected to harbour *Campylobacter* spp. In a study in Peruvian Amazon, parrots (*Ara*, *Brotogeris* and *Pionites*) were detected to be infected with *Campylobacter* spp.

Wild and free-living birds, for instance, sparrows, crows, waders, black-headed gull (*Larus ridibundus*), sparrow hawk (*Accipter nisus*), jackdaw (*Corvus monedula*), hooded crow (*Corvus cornix*), dunnoek (*Prunella modularis*), yellowhammer (*Emberiza citrinella*), white wagtail (*Motacilla alba*), dunlin (*Calidris alpina*), curlew sandpiper (*Calidris ferruginea*), bald ibis (*Geronticus eremita*), little stint (*Calidris minuta*), broad-billed sandpiper (*Limicola falcinellus*), ruff (*Philomachus pugnax*), wood sandpiper (*Tringa glareola*), long-eared owl (*Asio otus*), starling (*Sturnus vulgaris*), reed warbler (*Acrocephalus scirpaceus*), winter wren (*Troglodytes troglodytes*), redwing (*Turdus iliacus*), blackbird (*Turdus merula*), song thrush (*Turdus philomelos*), fieldfare (*Turdus pilaris*), blackbirds (*Turdus merula*), thrush (*Turdus viscivorus*) can act as reservoir of *C. jejuni* in nature. Certain clonal lineages of *C. jejuni* and species of wild birds are positively associated. Among the raptors (birds of prey), only hawks were detected to carry *C. jejuni* in their gut.

Possession of *Campylobacter* spp. in birds depends on feeding habits. Gulls and crows have higher possession rate than the pigeons due to their preference for sewages. The shoveler ducks (*Spatula clypeata*) have higher carriage rate than green-winged teal duck (*Anas acuta*) because they prefer bottom sediments of aquatic environments containing molluscs as a feed.

#### 2.1.4.4 Transmission

Direct and indirect contact with infected birds and vectors (house flies, beetles, cockroaches, mealworms) are major ways of *C. jejuni* transmission in pet birds. *C. jejuni* is sensitive to oxygen and cannot grow below 31–32 °C temperature. So, they cannot survive in feed and drinking water for a prolonged period. Presence of *C. jejuni* in drinking water acts as an indicator for faecal contamination from wild birds or livestock. Sometimes, *Campylobacter* spp. can make a symbiosis with aquatic protozoa and survive in the environmental water.

In human, major source of *C. jejuni* is contaminated poultry and its products, pork (with intact skin), beef, mutton and raw milk. Consumption of undercooked meat, milk or their products and handling poultry are the key ways of transmission. Direct or indirect contact with infected pet birds may play a role in zoonotic transmission of *C. jejuni*, although, not recorded in scientific literatures.

#### 2.1.4.5 Pathogenesis

In poultry, after transmission by faecal-oral route, *C. jejuni* colonizes at the mucous layer of caecal and cloacal crypts. The colonization is mediated by adhesin proteins like CadF (*Campylobacter* adhesin to fibronectin), PEB (Periplasmic/membrane-associated protein), CapA (*Campylobacter* adhesion protein A), JlpA (*jejuni* lipoprotein A), CiaB (*Campylobacter* invasin antigen B), flagella, and lipopolysaccharide (LPS). Occasional invasion of the intestinal epithelium takes place. No gross or microscopic lesions and clinical signs are produced in poultry during *C. jejuni* colonization or invasion. Similar type of *C. jejuni* colonization takes place in psittacines and canaries and they mostly act as asymptomatic carriers. Severe clinical signs and lesions are produced in tropical finches, especially in juvenile Estrildidae. The precise mechanism of *C. jejuni* infection in pet birds is still unexplored.

#### 2.1.4.6 Clinical Symptoms

No clinical signs and lesions are detected in canaries, psittacines, free-living (migrating passerines) and wild birds, and they act as asymptomatic reservoir of *C. jejuni*. In finches [juvenile Estrildidae, Gouldian finch (*Chloebia gouldiae*)], symptoms include sitting posture with its head under the wings, yellow droppings due to undigested starch (amylum), lethargy, and retarded moulting. High rate of mortality is observed among fledglings. In young ostriches, green coloured urination is the predominant sign.

Recent study indicates the possible synergistic role of *C. jejuni* in proventricular dilatation disease (PDD) in parrots caused by avian bornavirus.

### 2.1.4.7 Lesion

In tropical finches infected with *C. jejuni*, distinct cachexia, congestion in gastrointestinal tract, and presence of yellow coloured amylum or undigested seeds in gastrointestinal tract are the lesions. In sub-acute cases, hepatitis with focal necrosis and mucoïd haemorrhagic enteritis is observed.

### 2.1.4.8 Diagnosis

#### Clinical Specimens

Fresh droppings (without urine) and cloacal swabs can be collected as clinical specimens. Post mortem samples include intestine or intestinal contents and liver.

#### Diagnostic Techniques

- (a) *Direct Examination*: A smear can be prepared from clinical samples and stained by dilute carbol fuchsin (DCF). *Campylobacter* spp. appears as pink coloured small curved rod arranged in a pair. Occasionally, the bacteria produce characteristic 'S' or 'wing of gull' appearance. The bacteria can also be demonstrated by wet mounts of collected droppings by phase contrast or dark field microscopy. Darting motility of the organisms is suggestive for *Campylobacter* spp.
- (b) *Isolation of bacteria from clinical samples*: The selective media for *Campylobacter* isolation is broadly categorized into two types: charcoal based and blood based. Charcoal and blood components remove toxic derivatives of oxygen from the media. Examples of selective media are: modified charcoal, cefoperazone, deoxycholate agar (mCCDA), Karmali agar or CSM (charcoal-selective medium), Preston agar, Skirrow agar, Butzler agar and Campy-cefex agar. Commonly used non-selective media for isolation of *Campylobacter* spp. are blood agar with or without 0.1% sodium thioglycolate and antimicrobials (cephalosporins, trimethoprim, polymyxin, vancomycin, bacitracin, actidione, colistin, nystatin). Optimum growth condition for thermotolerant *Campylobacter* spp. (*C. jejuni*, *C. coli*) are 42 °C temperature for 24–48 h, pH 6.8, and 3–5% CO<sub>2</sub> with 3–15% O<sub>2</sub>. *C. jejuni* produces non-haemolytic, finely granular, irregular margin, flat, greyish colonies. On charcoal based media, the colonies may produce 'metallic sheen'. Thermotolerant *Campylobacter* spp. can be confirmed up to species level by staining, colony characteristics, and biochemical properties. Hippurate test can primarily differentiate *C. jejuni* and *C. coli*, but, it should be further confirmed by other tests.
- (c) *Detection of Campylobacter antigen*: ELISA based kits are available for detection of *Campylobacter* antigen from human stool samples. They can be used for detection of *Campylobacter* antigen from droppings collected from the suspected pet birds, although yet not evaluated.

- (d) *Molecular biology*: PCR can be used in combination with cultural technique for rapid detection of *C. jejuni*. A multiplex PCR is developed for detection of thermotolerant *Campylobacter* spp. such as *C. jejuni* (23SRNA gene); *C. coli*, *C. lari*, and *C. upsaliensis* (*glyA*).

#### 2.1.4.9 Zoonosis

Infection with *C. jejuni* in human causes watery or bloody diarrhoea, elevated body temperature, abdominal pain, nausea, and vomition. Septicaemia develops in a few diarrhoeic cases (0.15%) which may cause enlargement of the liver and spleen, endocarditis, arthritis, and meningitis. Rarely, a complicated auto-immune response is developed as a sequel, known as Guillain-Barré syndrome. It is a demyelating disorder which results muscle weakness and neuromuscular paralysis.

#### 2.1.4.10 Treatment and Control Strategy

In mild infection, treatment with antibiotics is not recommended due to possibility of antibiotic resistance development. In severe cases, several antibiotics such as clindamycin, gentamicin, tetracyclines, erythromycin, cephalothin, and fluoroquinolones (nalidixic acid) can be used under the supervision of a qualified veterinarian. Choice of antibiotic depends on sensitivity of the *C. jejuni* isolates, availability in suitable form, and species of the birds.

In aviaries or personal collection, implementation of biosecurity practices such as regular cleaning and use of fly repellents in the cages is effective to prevent the introduction of *Campylobacter* spp. No vaccine against *Campylobacter* spp. is currently available for birds.

### 2.1.5 Lyme Disease

#### 2.1.5.1 History

Lyme disease is a tick-borne, multi-system disorder of human and animals and it is characterized by swelling of joints, pain, lameness, fever, lethargy, anorexia, nephropathy with renal failure, myocarditis, cardiac arrest and CNS involvement. The etiological agent is maintained in tick and several birds and animals. Clinical description of Lyme disease was first documented by Arvid Afzelius, a Swedish dermatologist. The disease was first identified in 1975 (or 1976), among the people suffering with suspected juvenile rheumatoid arthritis in the area of Lyme, Connecticut, United States. Hence it is known as 'Lyme disease' or 'Lyme Borreliosis'. The causative agent, *Borrelia burgdorferi* sensu lato (s.l.) was identified in 1982.

#### 2.1.5.2 Etiology

*Borrelia* spirochete is a gram negative, spiral organism with linear chromosome. The life cycle of *Borrelia* requires arthropod vectors and mammalian hosts. It belongs to the family Spirochetaceae under the order Spirochaetales. *Borrelia* spirochetes comprise three distinct species groups i.e. Lyme borreliosis group

**Table 2.2** Distribution of *B. burgdorferi* s.l

Genospecies	Distribution	Reservoir host
<i>B. burgdorferi</i> sensu stricto	USA, Europe (Germany, Poland, Denmark, UK), Asia (Japan)	Rodent, birds
<i>B. afzelii</i>	Europe (Denmark, Germany, Poland, UK), Scandinavia, Asia (Japan)	Rodents
<i>B. garinii</i>	Western Europe, Asia (Japan)	Birds (blackbirds, song thrushes), rodents
<i>B. valaisiana</i>	Ireland, UK, Denmark, Germany, Poland, Netherlands, Scandinavia, Switzerland, Italy	Birds (pheasants)
<i>B. lusitaniae</i>	Europe	Lizard

(*Borrelia burgdorferi* sensu lato; hard tick transmission), relapsing fever group (*B. duttonii*, *B. hermsii*; soft ticks transmission) and a third group phylogenetically similar with relapsing fever group but transmitted by hard ticks (*B. theileri*, *B. lonestari*, *B. miyamotoi*).

Lyme disease in human and animals (dogs, horses) is mostly caused by *Borrelia burgdorferi* sensu lato (s.l.). *Borrelia miyamotoi* is recently detected to produce Lyme disease like syndrome in human.

*Borrelia burgdorferi* s.l. can be divided into 15 genomic groups or genospecies (*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. japonica*, ‘*B. andersonii*’, *B. tanukii*, *B. turdi*, *B. valaisiana*, *B. lusitaniae*, ‘*B. bissettii*’, *B. sinica*, ‘*B. californiensis*’, *B. spielmanii*, *B. americana*, *B. carolinensis*). The pathogenic genospecies [*B. burgdorferi* sensu stricto (outer surface protein A-OspA type 1), *B. afzelii* (OspA type 2), *B. garinii* (OspA types 3–8), *B. valaisiana*, *B. lusitaniae*] and their distribution in different continents are described in Table 2.2.

### 2.1.5.3 Host Susceptibility

*Borrelia burgdorferi* is maintained in nature through a cycle. In the cycle, hard ticks (*Ixodes* spp, *Haemaphysalis* spp.) and small mammals (birds, rodents) act as vector and reservoir host, respectively (Table 2.2). The serum complement of the reservoir hosts determines host preference of *B. burgdorferi*. The bird associated genospecies are resistant to the bird complement but susceptible to the rodent complement.

*Ixodes scapularis* and *I. pacificus* in USA and Canada, *I. ricinus* in Europe and *I. persulcatus* in Asia (Japan) act as major vectors of *Borrelia burgdorferi* s.l. Occasionally, other species of ticks such as *I. uriae*, *I. affinis*, *I. dammini*, *I. frontalis*, *I. angustus* Neumann, *I. spinipalpis* Hadwen and Nuttall, *I. auritulus* Neumann, *I. pacificus* Cooley and Kohls are also associated. All of these ticks cannot parasitize human to transmit the spirochete, but, they can act as maintenance host (e.g. *I. affinis*, *I. dentatus*). *Ixodes persulcatus* in Japan and *I. scapularis* in USA was detected to act as vector of *Borrelia miyamotoi*.

Different stages of *Ixodes* ticks (larva, nymph and adult) can attach with three different hosts to take the blood meal and after engorgement they drop off the host in the environment. Immatured ticks (larva or nymph) prefer to stay in moist areas

such as vegetative mat of the forest floor or meadow. Ground-feeding birds (passerines, game birds, sea birds), rodents, lizards act as preferred hosts of the immature ticks (Table 2.3). Although, in comparison to rodents, tick infestation in migratory passerine birds is 20–30 times less, but the birds can transmit the infection for long distances. Sometimes, reservoir birds generate mutant and more virulent strains of *Borrelia*. Passerine birds in mixed coniferous (evergreen) forest were more infected with *B. burgdorferi* s.l. than the birds in alder swamp forest. Experimentally, Mallard ducks (*Anas platyrhynchos platyrhynchos*) are susceptible to *B. burgdorferi* infection and the ducks shed the organism in the droppings. They may transmit the infection without the help of tick vectors. The study indicated that psittacine birds such as yellow naped amazon parrots (*Amazona auropalliata*) are generally not infected with *B. burgdorferi* s.l. More studies are needed to explore their resistance status against *Borrelia* infection.

The ticks normally attach with eyelid, head, neck and ventral feather of passerine birds during blood meal (Fig. 2.8). The immature ticks take a blood meal for 2–4 days from their preferred hosts. In adult stage, the ticks attach with the tip of the grasses to get adhere with a large mammalian host. The adult ticks take a blood meal for 5–6 days. The ticks itself have less mobility but they can be carried by their hosts specially the migratory passerine birds across the countries. The seabird tick (*I. uriae*) is observed to disseminate *Borrelia burgdorferi* s.l. from one hemisphere to another (trans-hemispheric transmission). In Canada, passerine birds move northward during spring for breeding and nesting and they disseminate ticks with the pathogens.

*B. burgdorferi* is transmitted to immature ticks from infected birds, rodents and lizards along with the blood meal. The spirochete after transmission multiplies in the gut of the ticks. When the immature ticks molt into adult stage, the numbers of *Borrelia* spirochete is decreased. During attachment of adult tick with large mammalian hosts, the multiplication of spirochete restarts and the number is increased. The expression of *B. burgdorferi* outer surface protein (osp) is also changed from ospA to ospC. The ospC helps in transmission of *Borrelia* from the mid gut to the salivary glands of ticks. Thus, *B. burgdorferi* is transmitted transstadially from larva to nymph and from nymph to adult. Rarely, within the tick population, *B. burgdorferi* is transmitted transovarially. When the adult ticks bite a new host, the spirochetes are transmitted from the salivary glands. Possibility of *B. burgdorferi* transmission by the adult ticks is more than the nymph and larvae, because the adult ticks have two blood meals in different hosts.

Sometimes, a single species of tick is infected with more than one numbers of *Borrelia* genospecies (e.g. *B. garinii* and *B. valaisiana*) due to superinfection of the already infected ticks during their consecutive blood meals. Occasionally, two different species of ticks (*I. scapularis* and *I. affinis*) may attach with the same *Borrelia burgdorferi* infected host. Co-transmission occurs between the infected and naive nymphs or larvae.

**Table 2.3** Birds as a reservoir host of *B. burgdorferi* s.l. in different countries

Tick species infected with <i>Borrelia</i>	Birds as a reservoir host	Country	Reference
–	Tree pipit ( <i>Anthus trivialis</i> )	Poland	Gryczynska et al. (2004)
	Dunnock ( <i>Prunella modularis</i> )		
	Chaffinch ( <i>Fringilla coelebs</i> )		
	Song thrush ( <i>Turdus philomelos</i> )		
	Nuthatch ( <i>Sitta europea</i> )		
	Hawfinch ( <i>Coccothraustes coccothraustes</i> )		
	Robin ( <i>Erithacus rebecula</i> )		
	Eurasian Blackbird ( <i>Turdus merula</i> )		
	Wren ( <i>Troglodytes troglodytes</i> )		
<i>Ixodes ricinus</i>	Eurasian blackbirds ( <i>Turdus merula</i> )	Italy	Mannelli et al. (2005)
<i>Ixodes pacificus</i>	Rio Grande wild turkeys ( <i>Meleagris gallopavo intermedia</i> )	California, USA	Lane et al. (2006)
<i>Ixodes ricinus</i>	Eurasian blackbird ( <i>Turdus merula</i> )	Czech Republic	Dubska et al. (2009)
	Song thrush ( <i>Turdus philomelos</i> )		
	Great tit ( <i>Parus major</i> )		
<i>Ixodes scapularis</i>	White-throated sparrow ( <i>Zonotrichia albicollis</i> )	Canada	Scott et al. (2010)
	Common yellowthroat ( <i>Geothlypis trichas</i> )		
	American robin ( <i>Turdus migratorius</i> )		
	Song sparrow ( <i>Melospiza melodia</i> )		
	Swainson's thrush ( <i>Catharus ustulatus</i> )		
	Fox sparrow ( <i>Passerella iliaca</i> )		
<i>Ixodes ricinus</i>	Eurasian blackbird ( <i>Turdus merula</i> )	Norway	Hasle (2011)
	Song thrush ( <i>Turdus philomelos</i> )		
	Redwing ( <i>Turdus iliacus</i> )		
<i>Ixodes</i> spp.	Eurasian blackbird ( <i>Turdus merula</i> )	France	Socolovschi et al. (2012)
<i>Ixodes ricinus</i>	Eurasian blackbird ( <i>Turdus merula</i> )	Spain	Palomar et al. (2012)
<i>I. frontalis</i>	European robin ( <i>Erithacus rubecula</i> )		
	Song thrush ( <i>Turdus philomelos</i> )		
	Eurasian wren ( <i>Troglodytes troglodytes</i> )		
	Eurasian jay ( <i>Garrulus glandarius</i> )		
<i>Ixodes ricinus</i>	Eurasian blackbird ( <i>Turdus merula</i> )	Portugal	Norte et al. (2013)
	Song thrush ( <i>Turdus philomelos</i> )		
	Great tit ( <i>Parus major</i> )		
	Common chaffinch ( <i>Fringilla coelebs</i> )		
<i>Ixodes affinis</i>	Carolina Wrens ( <i>Thyrothorus ludovicianus</i> )	Virginia, USA	Heller et al. (2015)
	Brown Thrashers ( <i>Toxostoma rufum</i> )		
	American Robin ( <i>Turdus migratorius</i> )		
	Eastern Towhee ( <i>Pipilo erythrophthalmus</i> )		
	Northern Cardinal ( <i>Cardinalis cardinalis</i> )		
	White-throated Sparrow ( <i>Zonotrichia albicollis</i> )		
	Swainson's Thrush ( <i>Catharus ustulatus</i> )		

(continued)



**Table 2.3** (continued)

Tick species infected with <i>Borrelia</i>	Birds as a reservoir host	Country	Reference
<i>Ixodes frontalis</i>	European robin ( <i>Erithacus rubecula</i> )	Czech Republic	Literak et al. (2015)
	Common chaffinch ( <i>Fringilla coelebs</i> )		
	Eurasian blackcap ( <i>Sylvia atricapilla</i> )		
	Eurasian blackbird ( <i>Turdus merula</i> )		
<i>Ixodes</i> spp.	Nightingale ( <i>Luscinia megarhynchos</i> )	Germany	
	Dunnoch ( <i>Prunella modularis</i> )		
	Chiffchaff ( <i>Phylloscopus collybita</i> )		
	Reed warbler ( <i>Acrocephalus scirpaceus</i> )		
<i>Ixodes uriae</i>	Kittiwake ( <i>Rissa tridactyla</i> )	France	Duneau et al. (2008)
	Puffin ( <i>Fratercula</i> spp.)		
	Guillemot ( <i>Uria</i> spp., <i>Cepphus</i> spp.)		
	Fulmar ( <i>Fulmarus</i> spp.)		



**Fig. 2.8** Greenfinch (*Carduelis chloris*) infested with ticks (Courtesy Ola Nordsteien, Jomfruland bird observatory, Norway)

**2.1.5.4 Transmission**

Migratory birds help in spreading *Borrelia* infected ticks in distant places, from which the infection can be further transmitted to human or other mammals. The migratory birds can act as carrier of previously infected tick or transovarially

infected larvae. Occasionally, transmission of *Borrelia* occurs from the infected ticks to uninfected ticks during their co-feeding from the same birds. The migratory birds not only import the infected ticks in a locality, but also, there is a possibility that local ticks get the infection during attachment with the birds. After a long journey, the birds prefer to take rest in some places for a few days. Recently, role of cottontail rabbit in this transmission cycle is also explored.

### 2.1.5.5 Clinical Symptoms

During carriage of *B. burgdorferi* most of the birds do not show any clinical symptom or lesion. Experimental inoculation of *B. burgdorferi* in Canary finches (*Serinus canaria*) produced only a brief episode of diarrhoea. Natural infestation of *B. burgdorferi* infected tick (*Ixodes auritulus*) results gasping, lameness and death in fledgling American robin (*Turdus migratorius*).

### 2.1.5.6 Diagnosis

#### Clinical Specimens

For collection of suspected ticks from the migratory birds, the birds are caught by mist nets and are observed carefully for the presence of ticks in head, neck, and beak by magnifying glasses. The ticks are collected by a blunt forcep and are placed in 70% ethanol. They should be labelled properly indicating species of bird and tick, and date of collection. For identification of bird and ticks up to the species level, expertise is needed.

From the birds, suspected for *B. burgdorferi* infection, heparinized blood and tissues from liver, spleen, kidneys can be collected after post mortem.

#### Diagnostic Techniques

- (a) *Direct Examination*: Dark field Microscopy or Giemsa stain can directly demonstrate the *Borrelia* spirochete in the blood film, liver/spleen smears. FAT can be used for direct examination of the smears.
- (b) *Isolation of bacteria from clinical samples*: Isolation of *Borrelia* is difficult due to its slow and fastidious growth and microaerophilic requirements. Modified BSK (Barbour-Stoenner-Kelly) medium is used for isolation of *B. burgdorferi* s.l. It is an enriched serum broth containing the antibiotics like kanamycin and 5-fluorouracil. The media after inoculation is incubated at 33–34 °C for 3 weeks. The collected heparinized blood sample (0.02 ml) or triturated tissue sample (0.1 ml) can be added in BSK medium (7–8 ml).
- (c) *Serological tests*: ELISA based kits for detection of total immunoglobulin, Ig G, Ig M against *B. burgdorferi* s.l is available for human. However, studies in animals (dogs), indicated that results of serological tests are inconclusive. The antibodies may be produced due to earlier exposure specially in those areas where infected tick bite is a common phenomenon. Such kind of serological studies are not conducted in birds probably due to this uncertainty.

- (d) *Molecular biology*: The whole blood samples collected from the birds can be used for DNA extraction. Whereas, from the ticks, DNA is extracted by spin column technique. PCR for consensus *flaB* gene of *Borrelia* and the spacer region between the 5S and 23S rRNA genes can be carried out to confirm *Borrelia burgdorferi* s.l.

#### 2.1.5.7 Zoonosis

Zoonotic transmission of *Borrelia burgdorferi* s.l. occurs from the bites of infected ticks. The persons during outdoor recreation, professionals such as wildlife and forest caretakers are at high risk. Migratory birds passively maintain the infection in nature. No direct transmission of *Borrelia burgdorferi* s.l. from the birds to human is evidenced. The infection in human initiates with a red coloured allergic pimple (erythema migrans), and it is followed by fever, headache, fatigue, muscle and joint pain. In severe cases, meningitis, unilateral facial nerve palsy and renal failure occur.

#### 2.1.5.8 Control Strategy

Products that kill or repel ticks (e.g. permethrin) can be used in the habitat to reduce the tick density. However, these acaricides may cause environmental hazard and they are only recommended during epidemic situation.

### 2.1.6 Others

#### 2.1.6.1 Yersiniosis

*Yersinia* spp. was first isolated by Alexandre Yersin in 1894 in HongKong. He was sent by Louis Pasteur to investigate about plague outbreak there. In Japan, S. Kitasato also independently isolated the bacteria a few days later. Previously, it was known as *Pasteurella pestis* in honour of Pasteur. Later, in memory of Yersin, the bacterium was renamed as *Yersinia pestis*. In 1976, *Yersinia pseudotuberculosis* was isolated from a sick palm dove (*Streptopelia senegalensis*) in Israel. *Yersinia enterocolitica* was first detected in budgerigars in 1980.

*Yersinia* is gram negative, short rods or coccobacilli shaped bacteria. They show bipolar staining characteristics ('safety pin appearance') when stained with Leishman's or Wright or Giemsa stain. The genus *Yersinia* is classified under the family Enterobacteriaceae that belongs to the order Enterobacteriales. *Yersinia* consists of 11 numbers of species. Among them, *Y. pseudotuberculosis* and *Y. enterocolitica* are commonly associated with psittacine and passerine bird infection.

Mynahs are most susceptible to *Yersinia* spp. infection. Yersiniosis is also reported from canaries (*Serinus canaria*), zebra finch (*Poephila guttata*), kaka (*Nestor meriondalis*), rainbow lorikeet (*Trichoglossus mollucanus*), budgerigar (*Melopsittacus undulatus*), New Zealand wood pigeons (*Hemiphaga novaeseelandiae*), blue-fronted Amazon (*Amazona aestiva*), yellow-headed Amazon (*Amazona oratrix*), Eurasian collared dove (*Streptopelia decaocto*) and cockatoo



**Fig. 2.9** Yellow coloured foci in vital organ of a weaver bird suffering with Yersiniosis (Courtesy Prof. Richard Hoop, University of Zurich, Switzerland)

(*Cacatua alba*). Rodents and wild birds are major reservoir of infection and the feed and water are often contaminated with rodent urine or faeces. Ingestion of contaminated feed and water is the key route of transmission.

High mortality and non-specific clinical signs such as ruffling of feathers, depression, diarrhoea, and biliverdin in the urine are observed in the birds. The infection is acute and mostly enteric in passerine birds. Chronic infection takes place in psittacines and pigeons, and it produces hepatitis, splenitis, pneumonia, nephritis and enteritis. The liver becomes dark, swollen and congested. Yellow coloured foci (bacterial granulomata) are found in the liver, spleen, lungs, kidneys, intestines and heart (Fig. 2.9). Microscopically, these foci are composed of necrosed hepatocytes and splenic pulp with fibrin and *Yersinia* colonies. Accumulation of iron in the liver (hepatic haemosiderosis) may act as a predisposing factor for systemic *Yersinia* spp. infection.

A smear can be prepared from the collected tissues of liver, spleen, kidney, intestine and stained by Leishman's, Wright, and Giemsa stain. *Yersinia* shows typical 'bipolar characteristics' (safety-pin appearance). *Yersinia* can be isolated in blood agar, nutrient agar, MacConkey's agar, brilliant green agar (*Y. enterocolitica*). The selective medium is CIN agar which contains antibiotics such as cefsulodin (15 mg/l), irgasin (4 mg/l) and novobiocin (2.5 mg/l). 'Cold enrichment' method can be followed for primary isolation of *Y. pseudotuberculosis* and *Y. enterocolitica* from clinical samples. The samples are kept in sterile phosphate

buffered saline (PBS) or nutrient broth at 4 °C for 3 weeks. Subculture in MacConkey's or CIN agar is done at weekly interval.

Amoxicillin in drinking water or soft food is the choice of treatment. In unresponsive cases, treatment should be carried out on the basis of antibiotic sensitivity test.

### 2.1.6.2 Mycoplasmosis

Albert Bernhard Frank (1889), a German Biologist, first coined the term *Mycoplasma* which is originated from the Greek word *mykes* (fungus) and *plasma* (formed). Earlier *Mycoplasma* was known as pleuropneumonia-like organisms (PPLO). Adler (1957) first reported isolation of PPLO from the air sac of a parakeet bird.

*Mycoplasma* is the smallest pathogenic bacteria (0.3–0.8 µm) and is pleomorphic in shape due to absence of the rigid cell wall. In stained smears, they appear as ring, globules, filaments or elementary bodies. The cell membrane is constituted of trilaminar structure enriched with phosphoprotein, lipoprotein, glycolipid, phospholipid and sterol moieties. *Mycoplasma* belongs to the class Mollicutes, order Mycoplasmatales, and family Mycoplasmataceae. The family comprises of two genera i.e. *Mycoplasma* and *Ureaplasma*. Among different species under the genus *Mycoplasma*, *M. gallisepticum*, *M. iowae*, and *M. sturni* are associated with pet bird infection.

An epidemic of Mycoplasmal conjunctivitis was noticed in house finches (*Carpodacus mexicanus*) in USA in 1994. Other birds such as budgerigars, cockatiel, canary, yellow-naped Amazon parrot, pigeons, pea-fowls (*Pavo cristatus*), fledgling cliff swallows, European starling (*Sturnus vulgaris*), chukar partridges (*Alectoris chukar*), ring-necked pheasants, purple finches (*Carpodacus purpureus*), evening grosbeaks (*Coccothraustes vespertinus*), pine grosbeaks (*Pinicola enucleator*) are also reported to be infected. Concomitant Mycoplasmal infection with other bacteria and protozoa (*Cryptosporidium* spp.) was detected in Amazon parrots and fledgling cliff swallows. Experimentally, American goldfinch (*Carduelis tristis*) carried *M. gallisepticum* for prolonged period without showing any clinical sign. House sparrows (*Passer domesticus*) are transiently infected experimentally with *M. gallisepticum* for a short period. In United States, tufted titmice (*Baeolophus bicolor*) bird was reported as non-clinical carriers of *M. gallisepticum*.

Feeders or any focal point where the birds gather, act as a source of *M. gallisepticum* infection, because the infected birds excrete their droplets in the feeder. Statistical correlation (multivariate analysis) was established between presence of tube style feeders, non-breeding period and low environmental temperature and *M. gallisepticum* infection in house finches. Vertical way is a rare possibility of Mycoplasmal transmission in birds.

Clinically the infected birds show variable symptoms ranging from serous nasal discharge, sinusitis, swollen eyes with discharge, conjunctivitis and blindness (Fig. 2.10). In fledgling cliff swallows and European starling (*Sturnus vulgaris*) infected with *M. sturni*, bilateral conjunctivitis, episcleritis, epiphora, hyperaemia of palpebrae and nictitans are observed. Gross lesions in birds include congestion of



**Fig. 2.10** Mycoplasmal conjunctivitis in a pea-fowl (Courtesy M. Scott Echols, Medical Center for Birds, California)

mucosa and accumulation of exudates in nasal sinus, trachea, bronchi, and air sacs. Air sac congestion was also detected in budgerigars experimentally challenged with *M. gallisepticum*. Histological investigation in birds reveals the presence of mucous gland hyperplasia and thickened mucous membrane of the respiratory tract with mononuclear cell infiltration. In European starling birds, ulceration of mucous membrane and absence of epithelial hyperplasia and lymphoplasmacytic infiltration was observed. In fledgling cliff swallows, lymphoplasmacytic conjunctivitis, rhinitis and infraorbital sinusitis with follicular lymphoid hyperplasia were detected.

Conjunctival swabs and head, lung, and spleen in 10% buffered formalin after post-mortem, can be collected as clinical samples from the suspected birds. The smears prepared from clinical specimens are stained with Giemsa. *M. gallisepticum* appears as coccoid organisms having 0.25–0.5  $\mu\text{m}$  in size. Contrast phase microscopy, dark phase illumination techniques can be used for their direct visualization. *M. gallisepticum* can be isolated in specialized medium supplemented with 10–15% heat-inactivated avian, swine or horse serum. Change in broth colour indicates positive growth after incubation at 37 °C for 3–5 days. Serum plate agglutination test is the rapid serological test for detection of *M. gallisepticum* antibodies in birds,



although, sometimes it produces false positive result due to cross reaction. PCR targeting 16SrRNA gene and loop-mediated isothermal amplification (LAMP) assay based on a gene within the pyruvate dehydrogenase complex (*pdhA*) are developed for rapid detection of *M. gallisepticum*.

In house finches, application of oral tylosin (1 mg/ml drinking water for 21 days) and ciprofloxacin eye drop (for 7 days) successfully treated conjunctivitis associated with *M. gallisepticum*. Doxycycline (40–50 mg/kg body weight, orally) is also recommended for Mycoplasmal infection in cockatiels and amazons.

### 2.1.6.3 *Pasteurella multocida*, *Gallibacterium* spp., *Volucribacter* spp.

Bollinger (1878) first reported the isolation of *Pasteurella* like organisms from cattle and wild animals. Louis Pasteur (1880) conducted more comprehensive studies on fowl cholera and its etiological agent. Trevisan (1887) coined the name *Pasteurella* for the bipolar organisms described earlier by Pasteur and others. Lignières (1900) proposed the specific name for each species of *Pasteurella* according to their host preference, such as *Pasteurella aviseptica* for fowls, *P. suis* for pigs, *P. bovis* for bovines, *P. ovis* for ovines and *P. lep-*  
*tiseptica* for rabbits. Rosenbusch and Merchant (1939) proposed a single species *Pasteurella multocida* and it is in use till date. Miringa (1975) described pasteurellosis in African grey parrots.

*P. multocida* is a gram-negative, non motile, non spore-forming short rod or coccobacillus bacterium. In fresh cultures and animal tissues, it produces typical bipolar staining characteristics, particularly with Leishman or methylene blue stain. *Pasteurella* belongs to the family *Pasteurellaceae*. Other avian pathogens such as *Gallibacterium* (*Pasteurella anatis*) and *Volucribacter* are also members of the same family. *P. multocida* can be classified into 6 capsular types (A–F) and 16 somatic types (1–16).

Psittacines [parrots, red-fronted conure (*Aratinga wagleri*)], passerine birds, owls, raptors and waterfowls (ducks) suffer from pasteurellosis. *P. multocida* is also isolated from eye swabs of healthy psittacine birds. Unclassified members of the *Pasteurellaceae* family were isolated from lesions in domestic goose (*Anser anser forma*), Fischer's lovebird (*Agapornis fischer*), parrots (*Amazona* spp.), macaws (*Ara macao*), rock dove (*Columba livia*), budgerigars (*Melanopsittacus undulates*), and African gray parrot (*Psittacus erithacus*).

Contaminated environment (e.g. water) is the major source of *P. multocida* infection. Mechanical transmission by blood sucking arthropods and cat-bite is possible. In cat-bite cases, dermatitis and myositis develops rapidly and it is followed by septicaemia and death. In psittacine birds *P. multocida* serotype 3 and 4 are associated with septicaemia and cutaneous lesions, respectively. In African gray parrots, *P. multocida* produced obstruction of nares and dyspnoea, due to formation of intranasal caseous and fibrinous plugs along with other bacteria. In *P. multocida* infected budgerigars, crop inflammation and apathy was observed. *Gallibacterium melopsittaci* are associated with septicaemia and salpingitis in budgerigars and

parakeets. *Volucribacter psittacidica* causes respiratory tract infections, septicaemia, crop inflammation, and diarrhoea in psittacine birds.

A smear can be prepared from collected blood sample or the nasal swabs and it is stained by Leishman or methylene blue or Gram's stain. *Pasteurella* appears as gram negative non-sporing coccobacilli with typical bipolar staining characteristics. *P. multocida* can be isolated in dextrose-starch agar, casein-sucrose-yeast (CSY) medium with 5% blood (bovine or sheep). *P. multocida* specific PCR (PM-PCR) helps in rapid and confirmatory detection from clinical samples.

Treatment of avian pasteurellosis with ampicillin (150–200 mg/kg body weight for pigeons, Amazon parrots) and tiamulin fumarate (25–50 mg/kg body weight, oral) are recommended.

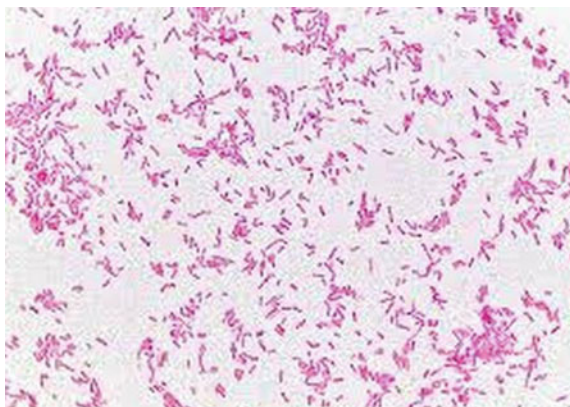
#### 2.1.6.4 *Escherichia Coli*

*Escherichia coli* was first isolated by Theodor Escherich in 1885 from the faeces of human infants. It was named in honour of the German pediatrician and its major natural habitat i.e. colon. In 1978, *E. coli* was detected in faecal samples collected from psittacine birds. Raphael and Iverson (1980) described *E. coli* associated coligranuloma in Amazon parrot along with psittacosis.

*E. coli* is gram negative, short rods, varying from coccoid shape to long filamentous forms (Fig. 2.11). They occur singly, in pair or in short chain. They are non-spore forming and mostly motile by peritrichous flagella. The genus *Escherichia* is classified under the family Enterobacteriaceae that belongs to the order Enterobacteriales. There are total 6 species under the genus *Escherichia*. Among them, *Escherichia coli* are the important pathogen.

The gastro-intestinal tract of all vertebrates including birds is the most common natural habitat of *E. coli*. The studies revealed that healthy parrots (31%), cockatoos (*Cacatua* spp., 60%) and shore birds carried *E. coli* in their intestine. In healthy passerine birds, *E. coli* are not considered as a major intestinal flora. Psittacines imported or illegally traded from other countries and shore birds act as source of *E. coli*. Majority of these psittacine *E. coli* isolates possessed antimicrobial

**Fig. 2.11** Gram stained *Escherichia coli* isolated from bird (100×)





resistance due to the exposure of the birds to the prophylactic antibiotics after their capture.

In pet birds, *E. coli* is transmitted by contaminated feed, drinking water, aerosols, and fomites. The stress conditions like transport, dietary change and extreme climate also help to establish the infection. In adult canaries and finches, *E. coli* are most common secondary pathogens associated with epizootic mortality. Non-specific clinical signs and lesions such as lethargy, rhinitis and conjunctivitis are detected. *E. coli* as a primary pathogen is reported from a hyacinth macaw (*Anodorhynchus hyacinthinus*), died due to septicaemia and enteritis with hemorrhages in different organs, and a kakapo (*Strigops habroptilus*) with exudative cloacitis. Recently, attaching-effacing *E. coli* is detected as a primary pathogen in a captive flock of budgerigars (*Melopsittacus undulatus*). Common lesions in budgerigars include hepatitis, enteritis, and attaching and effacing lesions along the intestinal tract.

In nestlings of canaries and finches, *E. coli* is considered as most important cause of diarrhoea, dehydration, cachexia and mortality. Appearance of young birds and their mothers became dirty, wet and yellowish ('sweating disease').

Isolation of *E. coli* from the clinical samples is the major diagnostic technique. Blood agar, MacConkey's agar are choice of the medium for isolation. After overnight incubation in MacConkey's agar, characteristic pink coloured colonies are transferred into eosine methylene blue (EMB) agar for detection of 'metallic sheen' (Fig. 2.12). The isolates are further confirmed by different biochemical tests. Pathogenicity of the *E. coli* isolates from clinical samples should be confirmed as they are present as normal bacterial flora within the body. Virulence of the isolates can be ascertained by ligated loop assay, cell culture cytotoxicity assay, typing and detection of toxin by serological or DNA based methods.

*E. coli* infections can be treated with ampicillin sodium, amoxicillin/clavulanate, cephalexin, oxytetracycline and spectinomycin. In unresponsive cases, antibiotic

**Fig. 2.12** Metallic sheen in EMB agar by *Escherichia coli* isolates



**Table 2.4** Other important bacterial infection of pet birds

Bacteria	Susceptible hosts	Clinical signs/gross lesions	Treatment
<i>Staphylococcus</i> spp.	Resident of skin in healthy birds such as African Grey Parrots, Budgerigars and Cockatiels. Detected as primary pathogen in hyacinth macaw, lovebird and passerine birds	In hyacinth macaws, ulcerative dermatitis and septicaemia associated endocarditis are reported. In lovebirds, septicaemia followed by blindness and CNS disorder was detected. In passerines, abscesses, dermatitis, bumble foot, conjunctivitis, sinusitis, arthritis, pneumonia, and death occurs	Amoxicillin/clavulanate, Piperacillin sodium/tazobactam Sodium, Tiamulin fumarate
<i>Enterococcus</i> spp.	Commensal flora of gastro intestinal tract	Wound infection, septicaemia and death in psittacine birds. In canaries, tracheitis, pneumonia, air sac infections, dyspnoea and loss of normal voices occur	Treatment should be carried out on the basis of sensitivity test
<i>Pseudomonas</i> spp.	Parrots and passerine birds. Contaminated feed and water are major source of infection	Upper respiratory tract infection, cellulitis and conjunctivitis in parrots. Pneumonia and aerosacculitis in passerine birds. Necrotizing hepatitis is also detected in pt birds	Enrofloxacin, amikacin sulfate. In unresponsive cases, treatment should be carried out on the basis of sensitivity test
<i>Clostridium</i> spp.	Parrots, blue and yellow macaw, lories, rainbow lorikeet, pigeon, ostrich	Necrotic enteritis, ulcerative enteritis with dilatation of the small intestine and presence of yellow foci, necrotizing hepatitis, myocarditis, and ventriculitis	Treatment should be carried out on the basis of sensitivity test
<i>Bordetella avium</i>	Psittacine, turkey, finches	'Lockjaw syndrome', upper respiratory tract infection	Tiamulin fumarate
<i>Klebsiella</i> spp.	Psittacine	Pneumonia	Ampicillin sodium

should be selected after sensitivity test of the etiological *E. coli* isolates. In nestlings, antibiotics are administered in drinking water and egg food from one day before hatching up to 6 days after hatching. Extra drinking water should be provided to prevent dehydration.

Other bacterial infections of pet birds are described in Table 2.4.

## 2.2 Viral Diseases

### 2.2.1 Newcastle Disease

#### 2.2.1.1 History

Newcastle disease in virulent form was first observed in poultry in Java (Indonesia) and NewCastle-on-Tyne (United Kingdom) in 1926. The disease was prevalent in Korea and Scotland prior to 1926 but was not documented in details. Doyle first coined the term ‘Newcastle disease’ to describe the infection in poultry according to the name of the place. The infection spread rapidly among the poultry in the subsequent years in Asian countries. In India, it was first described in poultry in 1927 in Ranikhet, Uttarakhand. Consequently, the infection is still known as ‘Ranikhet disease’ in India and neighbouring countries.

Malbrant (1942) reported an outbreak in Australian parrots and red-headed lovebirds (*Agapornis pullaria pullaria* L.) in Africa suspected to be suffered and died off Newcastle disease. Zuydam (1952) first isolated Newcastle disease virus (NDV) from parakeet (*Psittacula krameri borealis* Nearn) and osprey birds (*Pandion haliaetus*) in The Netherlands. The first major outbreak of Newcastle disease among grey parrots (*Psittacus erithacus* L.) occurred in Kenya in 1955 (Scott et al. 1956). Subsequently, in 1960, NDV was isolated from grey parrots (*Psittacus erithacus* L.) in Kenya (Scott and Winmill 1960). In 1970s, Pigeon paramyxovirus-1 (PPMV-1), a variant of NDV, was discovered in the Middle East countries.

#### 2.2.1.2 Etiology

NDV belongs to the *Avulavirus* (Avian Paramyxovirus-1) genus within the *Paramyxoviridae* family in the order *Mononegavirales*. The virus is an enveloped, single-stranded, un-segmented, negative-sense RNA virus. The virion has a genome of 15 kb in size and the genome comprises of the genes which encode nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN) and polymerase enzyme (L).

On the basis of virulence, NDV can be classified into velogenic (highly virulent, ICPI > 1.5), mesogenic (intermediate, ICPI > 0.7 but ≤ 1.5) and lentogenic strains (non-pathogenic, ICPI ≤ 0.7). Intracerebral pathogenicity index (ICPI) is OIE recommended in vivo test for determination of NDV virulence. The strains differ in virulence also differ in amino acid sequence at the cleavage site of precursor fusion protein (F0). Velogenic strains have more than two basic amino acids (arginine or lysine at positions 113–116) and phenylalanine at the position 117. On the basis of fusion protein (F) and polymerase enzyme (L) nucleotide sequence, NDV is classified into two major classes (class I and II) with a single genotype under class I and 18 genotypes under class II. Distribution of NDV genotypes and sub-genotypes in different species of birds is described in Table 2.5. The virulence of class I NDV isolates is low (except one isolate from Ireland) and they are mostly maintained by

**Table 2.5** Distribution, hosts, virulence of different NDV class, genotypes and sub-genotypes

NDV class	NDV genotype/sub-genotype	Virulence	Host	Country
Class I	Genotype I, sub-genotype 1a	Lentogen	Domestic ducks (Anatidae spp.), chickens (Gallus gallus domesticus), geese	China
	Genotype I, sub-genotype 1b	Lentogen	Chickens, ducks, black swan, peafowl, egret, heron	China
	Genotype I, sub-genotype 1c	Lentogen	Ducks, swans, geese, shorebirds	United States, Europe, China
Class II	Genotype I, sub-genotype Ia	Lentogen	Chickens	Australia, China, Colombia, Malaysia, and South Korea
	Genotype I, sub-genotype Ib	Lentogen	Wild and domestic waterfowls	China, Japan, Luxembourg, Madagascar, Nigeria, Russia, South Korea, Ukraine, and the United States
	Genotype I, sub-genotype Ic	Lentogen	Shorebirds, waterfowl, gulls	Japan, Mexico, Russia, Sweden, and the United States
	Genotype II	Velogen	Poultry, domestic waterfowl	United States, Africa, Asia, Europe, South America
	Genotype III	Velogen	Poultry, domestic waterfowl	Australia, Japan, UK, Taiwan, Zimbabwe, and Singapore, Pakistan, China
	Genotype IV	Velogen	Poultry, pigeons	Europe, Sudan (Africa), HongKong (Asia), Russia
	Genotype V	Velogen	Psittacine birds, poultry, ducks	Central and South America, Europe, Africa
	Genotype V, sub-genotype Va	Velogen	Double-crested cormorants, pelicans, gulls	United States
	Genotype V, sub-genotype Vb	Velogen	Poultry, caged-birds	United States, Brazil, Central America, Africa
	Genotype V, sub-genotype Vc	Velogen	Poultry, caged-birds, tree-ducks, quails	Mexico and Central America
	Genotype V, sub-genotype Vd	Velogen or mesogen	Poultry	Kenya, Uganda
	Genotype VI, sub-genotype VIa	Velogen or mesogen	Columbidae birds	Asia, Europe, Middle East, United States
	Genotype VI, sub-genotype VIb	Velogen or mesogen	Pigeons, kestrels, falcons, cockatoos, budgerigars, pheasants, swans, robin	Argentina, China, Italy, United States, Europe, South Africa

(continued)

**Table 2.5** (continued)

NDV class	NDV genotype/sub-genotype	Virulence	Host	Country
	Genotype VI, sub genotype VIc	Velogen or mesogen	Chickens, Columbidae birds	East Asia
	Genotype VI, sub genotype VI d	Velogen or mesogen	Poultry	Europe
	Genotype VI, sub genotype VIe	Velogen or mesogen	Pigeon, poultry	China
	Genotype VI, sub genotype VI f	Velogen or mesogen	Pigeon	United States
	Genotype VI, sub genotype VIg	Velogen or mesogen	Pigeon, dove	Nigeria, Russia, Ukraine
	Genotype VI, sub genotype VIh	Velogen or mesogen	Pigeon	Argentina
	Genotype VI, sub genotype VIi	Velogen or mesogen	Collared doves, pigeons	Italy, Nigeria
	Genotype VII, sub genotype VIIa	Velogen	Poultry	Western Europe
	Genotype VII, sub genotype VIIb	Velogen	Poultry, wild birds	China, Vietnam, Israel, Europe, Turkey, South Africa, Mozambique, Kazakhstan, the Far East, the Middle East, India
	Genotype VII, sub genotype VIIc	Velogen	Poultry	Czech Republic, Switzerland
	Genotype VII, sub genotype VIId	Velogen	Poultry, wild birds (crested ibis)	China, South Korea, Colombia, Israel, South Africa, Ukraine, Venezuela, Europe, Kazakhstan
	Genotype VII, sub genotype VIIe	Velogen	Chickens, domestic waterfowl	China, Japan, Taiwan, Vietnam
	Genotype VII, sub genotype VIIf	Velogen	Poultry, pigeon	China
	Genotype VII, sub genotype VIIg	Recombinant strains		
	Genotype VII, sub genotype VIIh	Velogen	Poultry, wild egret	Bali, Indonesia, Malaysia, China
	Genotype VII, sub genotype VIIi	Velogen	Chicken, koklass pheasants ( <i>Pucrasia macrolopha</i> ), peafowl	Indonesia, Israel, Pakistan, Eastern Europe
	Genotype VIII	Velogen	Chicken, turkey	Argentina, China, Malaysia, South Africa, Singapore, Italy

(continued)

**Table 2.5** (continued)

NDV class	NDV genotype/sub-genotype	Virulence	Host	Country
	Genotype IX	Velogen	Poultry, whooper swan ( <i>Cygnus cygnus</i> ), spotted necked dove ( <i>Streptopelia chinensis</i> ), green peafowl ( <i>Pavo muticus</i> ), white-cheeked starling ( <i>Sturnus cineraceus</i> ), Eurasian blackbird ( <i>Turdus merula</i> )	China
	Genotype X	Lentogen	Wild waterfowl, turkey	United States, Argentina
	Genotype XI	Velogen	Chickens, wild birds	Madagascar
	Genotype XII	Velogen	Chickens, geese	South America, China
	Genotype XIII	Velogen	Cockatoo (family Cacatuidae)	India
	Genotype XIII, subgenotype XIIIa	Velogen	Chicken, wild little tern ( <i>Sterna albifrons</i> )	Europe, Asia, Middle-East, Russia
	Genotype XIII, subgenotype XIIIb	Velogen	Chicken, Japanese Quail	India, Pakistan
	Genotype XIV, subgenotype XIVa	Velogen	Chicken	Nigeria
	Genotype XIV, subgenotype XIVb	Velogen	Chickens, turkeys, guinea fowl	Nigeria
	Genotype XV	Velogen	Chickens, geese	China
	Genotype XVI	Velogen	Chicken	Mexico, Dominican Republic
	Genotype XVII	Velogen	Poultry	West Africa
	Genotype XVIII, subgenotype XVIIIa	Velogen	Chickens, guinea fowl	Ivory Coast, Mali, and Mauritania
	Genotype XVIII, subgenotype XVIIIb	Velogen	Poultry, wild village weaver ( <i>Ploceus cucullatus</i> )	Ivory Coast, Mali, Nigeria, Togo

poultry and waterfowls. Whereas, class II NDV isolates are highly virulent and are associated with fatal outbreaks in poultry, pet birds and wild waterfowls.

### 2.2.1.3 Host Susceptibility

Velogenic or mesogenic strains of NDV were detected in psittacine birds (cockatoos, budgerigars, macaw, lory, parrot, love bird, conure, yellow-headed Amazon parrots, yellow-naped Amazon parrots), pelicans, gulls, kestrels, falcons, white crested laughing thrush, pheasants, swans, robin, peafowl, whooper swan, spotted necked dove, white-cheeked starling, Eurasian blackbird, wild little tern, wild village weaver, mynah (*Gracula religiosa*), drongo (*Dicrurus* spp.) and partridges (family Phasianidae) (Table 2.5). These wild and pet birds may act as reservoir or

spillover host for poultry. Occasional outbreaks of NDV were observed in racing pigeons and double-crested cormorants (*Phalacrocorax auritus*). The lentogenic strains are common in gulls, waterfowls, shorebirds, peafowl, egret and heron (Table 2.5) without any clinical symptoms. From neuronal tissues of parrots, lentogenic NDV strains were isolated.

Migratory birds may act as ‘global reservoir’ of NDV which transmits the infection into distant places across the continents. Generation of new variety of NDV (multi-recombinant) having the sequences from different putative parents of distant places suggests co-replication of the virus in the nature. In a natural NDV multi-recombinant strain, isolated from cockatoo in Indonesia (cockatoo/Indonesia/14698/90), parental lineage from NDV isolate of anhinga or snakebird (*Anhinga anhinga*) of United States and a NDV vaccine strain was detected.

#### 2.2.1.4 Transmission

In pet birds, horizontal transmission through direct contact with infected birds or ingestion of contaminated feed and water are the major ways of NDV transmission. Sometimes, infection of parrots occurs from live animal market during their direct contact with poultry. Use of contaminated or improperly attenuated live vaccines in poultry against NDV is another possible source of infection in nature and pet birds. Experimentally, yellow-headed Amazon parrot was infected with velogenic NDV strain by nebulization. Common houseflies (*Musca domestica*) act as mechanical vector for NDV transmission, although, yet to be validated in pet birds.

Imported exotic birds including the psittacines may act as reservoir and they excrete the virus in the faecal matter for prolonged period without showing clinical signs. Legally or illegally trafficked pet birds and migratory birds thus introduce the virus into the countries which produces a persistent risk for poultry. Moreover, the study has also shown the possibility of spreading NDV in a native wildlife population through NDV contaminated illegal wildlife trade.

#### 2.2.1.5 Pathogenesis

After transmission of the virus into the susceptible host, cellular entry requires activation of fusion protein (F0) present in the viral envelope. The F0 is activated by post-translational cleavage by the host protease enzyme. The post-translational cleavage varies with the amino acid sequence present in the cleavage site and the type of host protease enzyme. In lentogenic NDV strains, cleavage site contains monobasic amino acid sequence at the C-terminus and leucine at the N-terminus ( $^{112}\text{G-R/K-Q-G-R-L}^{117}$ ). The cleavage site of velogenic and mesogenic strains contains multibasic amino acid sequence at the C-terminus and a phenylalanine at the N-terminus ( $^{112}\text{R/G/KR-Q/K-K/R-R-F}^{117}$ ). The cleavage site of lentogenic strains is cleaved by the protease present in respiratory and intestinal tract only. Velogenic strains are cleaved by ubiquitous protease present in all vital organs. Moreover, activation of HN (HN0) protein and other viral proteins such as V, NP, P, and L also play role in pathogenesis.

### 2.2.1.6 Clinical Symptoms

Psittacine birds suffering with NDV mostly show respiratory signs (rhinitis, conjunctivitis), greenish watery diarrhea (green staining around the vent), lethargy, drooping of wing, torticollis, waving movement of head and neck and limb paralysis. Experimental inoculation of six species of pet birds (budgerigar, yellow-headed Amazon parrot, halfmoon conure, hill mynah, black-headed nun, canary) with velogenic NDV strain produced ruffled plumage, conjunctivitis, ataxia, wing tremors, paralysis of the extremities, and tremors of the head. Neurological signs are more common in parakeets.

Mortality can reach as high as 100%, but typically ranges between 20–80% depending upon the virulence of the virus strain, host species, age, and immune status.

### 2.2.1.7 Lesion

Petechial haemorrhages are often observed in viscera of pet birds suffering with Newcastle disease. In naturally infected cockatiels and parrots, diffuse spongiosis of gray and white matter, neuronal necrosis, perivascular infiltration of mononuclear cells are detected. The lamina propria of the proventriculus shows infiltration of mononuclear cells and ulceration. Accumulation of haemosiderin is detected in the cytoplasm of mononuclear cells. Depletion of lymphoid cells is observed in spleen and bursa of Fabricius.

### 2.2.1.8 Diagnosis

#### Clinical Specimens

Tracheal and cloacal swabs from live birds and the organs such as liver, brain, spleen, kidney after post mortem can be collected as clinical specimens. Collection of cloacal swabs from small birds is a complicated process which may cause injury of the vent. Fresh faeces collection is an alternative approach. During transport, the samples should be kept in isotonic phosphate buffered saline (pH 7.0–7.4) or brain heart infusion broth with antibiotics such as penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamicin (50 µg/ml), and mycostatin (1000 units/ml). The samples can be preserved at 4 °C for four days.

#### Diagnostic Techniques

- (a) Clinical signs and lesions after necropsy, history of direct contact with infected birds give a tentative diagnosis.
- (b) *Isolation of virus from clinical samples:* The clinical samples collected in isotonic phosphate buffered saline with antibiotics are centrifuged (1000 g) and the supernatant fluid is inoculated into 9–11 days old embryonated hen's eggs (specific pathogen free) by allantoic sac route or the cell lines such as chicken embryo kidney (CEK) cells, chicken embryo fibroblast (CEF) cells. The inoculated eggs are incubated at 37 °C for 4–5 days. After incubation,



eggs are kept at 4 °C. The embryo will die in positive samples and the allantoic fluids are collected to detect the haemagglutination activity of the viral isolate. The isolate is further confirmed by haemagglutination inhibition (HI) test. Virulence of the isolate should be detected by intracerebral pathogenicity index (ICPI) or amino acid sequencing of fusion protein to confirm a ND outbreak.

- (c) *Serological tests*: Virus neutralization test, plaque neutralization, hemagglutination-inhibition (HI), single radial immunodiffusion, agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA) are employed for detection of NDV antibodies. However, these tests can not differentiate the infection caused by velogenic and lentogenic viral isolates. Serological tests can give a tentative diagnosis of NDV exposure in the birds.
- (d) *Molecular biology*: Real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) can be used for detection of fusion protein, matrix protein and RNA-dependent RNA polymerase enzyme either from the viral isolate or from the collected tissues and faeces of suspected birds. It can also confirm the virulence of the isolates. In conventional reverse-transcriptase polymerase chain reaction (RT-PCR), cloning and sequencing of PCR products will confirm the pathogenic potentiality of the isolate.

### 2.2.1.9 Zoonosis

Zoonotic transmission of NDV is possible which causes acute conjunctivitis, malaise, and sinusitis in susceptible human. The flu like symptoms resolves automatically within 1–3 weeks. Direct transmission of NDV into human from pet birds is still not documented possibly due to similarity of symptoms with common flu and its auto recovery. The pet birds as reservoir of NDV become a potential hazard for poultry population where the human may act as intermediate host. Human to human transmission of NDV is not documented.

### 2.2.1.10 Treatment and Control Strategy

No effective treatment for pet birds against NDV infection is documented. To control the infection in pet birds, exposure to live bird market, wild birds, and migratory birds should be restricted. Infected birds should be kept separately and general hygiene practices should be followed to avoid the contamination of feed and drinking water.

Vaccination is an effective measure to control NDV infection in commercial and domestic poultry. Vaccination in pet birds is not recommended because it cannot eliminate the carrier birds. Vaccination with modified live virus may produce the infection in pet birds. However, experimental use of inactivated NDV vaccine produced a humoral response in wild house sparrows with doses above 0.05 ml per bird. The humoral response was produced within 4–6 weeks after experimental inoculation of the vaccine.

## 2.2.2 Avian Influenza Infection

### 2.2.2.1 History

In 1878, avian influenza (earlier known as fowl plague) was described for the first time in Italy by Perroncito. Centanni and Savonuzzi (1901) first observed the role of filterable agent (virus) as etiology of avian influenza. The definitive etiological correlation with Influenzavirus A was established later (1955). Highly pathogenic avian influenza (H5 subtype) was first reported from chickens in Scotland (1959) and common terns (*Sterna hirundo*) in 1961 from South Africa. During 1972–80, Influenzavirus A was reported from exotic birds including budgerigars (*Melopsittacus undulatus*), migratory ducks, and pelagic seabird (shearwater).

### 2.2.2.2 Etiology

Avian Influenza virus (AIV) belongs to the family Orthomyxoviridae, genus *Influenzavirus A*. The virions are sensitive to heat (56 °C for 30 min exposure), acid (pH 3.0) and lipid solvents. So they are easily destroyed under common environmental conditions. The virions are enveloped and pleomorphic, spherical to filamentous in shape. The virion surface is covered with two types of glycoprotein projections, known as haemagglutinin (HA, rod shaped trimer protein) and neuraminidase (NA, mushroom shaped tetramer protein). Other constituent viral proteins are nucleoprotein (NP), matrix proteins (M1, M2), polymerase basic (PB1, PB2), polymerase acidic (PA), and non structural proteins (NS2). The viral genome is linear, negative sense single stranded RNA and it contains eight numbers of segments. During genetic reassortment, these segments are exchanged between two viral strains to generate a mutant one (antigenic shift). The point mutation in HA and NA gene causes antigenic drift which can generate a new viral strain.

On the basis of HA and NA gene sequences, *Influenzavirus A* has 16 HA (H1–16) and 9 NA (N1–9) subtypes. Genetic reassortment between the subtypes theoretically can produce 144 types of combinations. On the basis of pathogenicity, AIV can be further differentiated into two categories i.e. highly pathogenic avian influenza (HPAI, e.g. H5 and H7 subtypes) and low pathogenic avian influenza (LPAI, e.g. H9N2). HPAI causes 90–100% mortality in birds and LPAI infection is mostly restricted within the respiratory system. Further molecular variations of HA gene can differentiate HPAI (H5N1) into several clades (first order–fourth order clade).

### 2.2.2.3 Host Susceptibility

Other than commercial and domestic poultry, birds belong to the order Anseriformes (waterfowls) and Charadriiformes (shorebirds, gulls, auks, terns, waders) are considered as major reservoir of avian influenza virus (Table 2.6). H3 and H6 subtypes are common in waterfowls (Anseriformes), and H4, H9, H11, and H13 subtypes are widespread in Charadriiformes. Among the Anseriformes, mallard (*Anas platyrhynchos*) and northern pintail ducks (*Anas acuta*) in United States and bar headed geese (*Anser indicus*) in India are considered as major reservoirs.

Natural H5N1 outbreak among the migratory waterfowls such as bar-headed geese (*Anser indicus*), great cormorants (*Phalacrocorax carbo*), Pallas's gulls (*Larus ichthyaetus*), brown-headed gulls (*Larus brunnicephalus*), ruddy shelducks (*Tadorna ferruginea*) is also detected in China.

Role of Passeriformes (canary, finch, starling, sparrow) as AIV reservoir is uncertain. A study in France with large numbers of wild passerine birds failed to detect AIV. Although, wild passerine birds such as Eurasian tree sparrow (*Passer montanus*) in China and golden crowned kinglet (*Regulus satrapa*), fox Sparrow (*Passerella iliaca*), western tanager (*Piranga ludoviciana*), northern waterthrush (*Seiurus noveboracensis*), Cassin's finch (*Carpodacus cassinii*) in United States, and flycatchers (family Muscicapidae) in Central Africa is detected to possess AIV infection. Experimentally, society finches (*Lonchura striata domestica*), zebra finches (*Taeniopygia guttata*), house sparrows (*Passer domesticus*) and starlings are found susceptible to AIV (H5N1, H7N9, H7N7). The sialic acid receptors for both avian influenza ( $\alpha$  2, 3) and human influenza ( $\alpha$  2, 6) viruses are present in house sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*). In contrast, Eurasian tree sparrows contain abundance of sialic acid receptors ( $\alpha$  2, 6) for AIV.

Detection of AIV is rare in psittacine birds and as such the psittacines are not considered as potential reservoir of AIV. Limited reports of viral isolation such as H9N2 subtype from Indian ring-necked parakeets and H5N2 subtype (Mexican lineage) from red-lored amazon parrot is available (Table 2.6). Experimentally, parakeets (*Melopsittacus undulates*) are found susceptible to human H7N9 isolate and after inoculation, development of clinical signs and shedding of the virus into water troughs is observed. On the other hand, psittacine isolate can also replicate in chicken, duck and turkeys and transmission into healthy cage mates is observed.

#### 2.2.2.4 Transmission

Water mediated transmission of AIV is possible in waterfowls and shorebirds due to their exposure to the contaminated water. Lower temperature maintained in water bodies helps in survival of AIV for prolonged period. Possibility of AIV transmission in waterfowl is more during their assembly in water bodies associated with post-breeding and pre-migratory molt. Migratory shorebirds and other Charadriiformes birds mostly transmit the infection when they congregate to feed and roost at places *en route* of migration. Thus the migratory birds become a possible source of infection in pet birds living in open air aviaries.

Possibility of water mediated transmission is low in terrestrial and passerine birds. Detection of AIV in passerines is possible, when the virus is maintained in local poultry population or the passerines share a common habitat with infected waterfowls. This kind of AIV transmission dynamics was observed during H5N1 outbreaks (2005–10) in wild birds in China, Russia and Mongolia. Consumption of infected bird carcass by raptors (bird of prey) is another possible way of transmission.

In psittacine birds, AIV infection is transmitted by direct contact with infected birds if kept together after capture or during quarantine. International trade of exotic birds can transmit the AIV infection from one continent to another.

**Table 2.6** Avian Influenza virus (AIV) subtypes documented in waterfowls, passerines, and wild birds in different countries

Bird (family)	AIV subtype	Country
Pigeons ( <i>Columba livia</i> ), starlings ( <i>Sturnus vulgaris</i> )	H9N3	Iraq
Fringillidae, Parulidae, Turdidae, Tyrannidae	H1N1	Ontario, Quebec
Passerines (Fringillidae, Timaliidae)	H6N4, H8N3, H11N3, H11N8, H12N6, H2N3, H11N6, H12N2, H1N3, H9N3, H3N1, H3N3, H6N3, H7N3, H8N6, H10N3, H11N1, H12N3, H12N4, H13N1, H2N1, H14N3, H8N1, H1N1, H14N3, H2N8, H6N8, H12N1, H12N3, H13N2, H15N4, H5N5, H7N1, H14N4, H11N5, H8N7	Slovakia
Columbidae, Corvidae, Estrillidae, Laniidae, Muscicapidae, Passeridae, Strunidae, Timaliidae, Zosteropidae	H5N1	Hong Kong
Alaudidae, Hirundinidae, Locustellidae, Motacillidae, Passeridae, Ploceidae, Pycnonotidae, Sylviidae	H5	South Africa
House sparrow ( <i>Passer domesticus</i> )	H9N2	Iran
Cuculidae, Emberizidae, Fringillidae, Hirundinidae, Motacillidae, Muscicapidae, Paridae, Passeridae, Remizidae, Sylviidae, Timaliidae, Turdidae	H10N2, H9N2, H7N5, H13N1, H2N5, H6N5, H12N2, H12N5, H13N1, H9N5, H11N3, H12N3, H10N2, H11N5, H11N2, H12N5, H13N3, H3N2, H12N1, H3N5, H9N5, H7N2, H9N2, H13N2, H10N3, H12N5, H6N5, H10N6, H7N6, H1N6	Slovakia
Anseriformes, Charadriiformes, Passeriformes, Falconiformes, Ciconiiformes, Columbiformes	H5N1	Europe
Magpies ( <i>Pica pica sericea</i> )	H5N1	South Korea
Passeridae (tree sparrow)	H5N1	Indonesia
Columbidae, Dicruridae, Emberizidae, Strunidae	H5N1	Thailand
Dicruridae, Pycnonotidae, Timaliidae	H5, H6, H9	Vietnam
Strunidae	H1N1, H7N7	Israel
Strunidae	H7N7	Victoria (Australia)
Passeridae (tree sparrow)	H5N1	China
Columbidae	H5N1	Japan
Ostrich	H7N1	South Africa
Ostrich	H5N9	South Africa

(continued)

**Table 2.6** (continued)

Bird (family)	AIV subtype	Country
Ostrich	H5N2	Zimbabwe
Emu, Casowaries	H5N9	The Netherlands
Rhea, Emu	H3N2, H4N2, H5N2, H7N1, H4N6, H5N9, H10N4, H7N3, H10N7	United States
Sparrow ( <i>Arremonops</i> spp.)	H7	Lebanon
Mediterranean Gull ( <i>Larus melanocephalus</i> )	H9N2	France
Psittacine [red-lored amazon parrot ( <i>Amazona autumnalis autumnalis</i> )]	H5N2	United States
Indian ring-necked parakeets	H9N2	HongKong
Passerines (song birds)	H1N1, H4N6, H5N1, H5N2, H7N9, H9N2	Asia, Europe, North America
Parrot	H5N2	Guangdong (South China)
Bar-headed geese ( <i>Anser indicus</i> ), great cormorants ( <i>Phalacrocorax carbo</i> ), Pallas's gulls ( <i>Larus ichthyaetus</i> ), brown-headed gulls ( <i>Larus brunnicephalus</i> ), ruddy shelducks ( <i>Tadorna ferruginea</i> )	H5N1	China
Domestic geese	H5N1	China
Sparrow	H7N7	Australia
Pigeon	H7N9	China
Ring-necked parakeet	H9N2	Japan

Further transmission of AIV from wild birds to local poultry population is possible. In HPAI infection, most of the wild birds die except in H5N1-HPAI (Guangdong lineage) infection. The virus of Guangdong lineage can persist in wild birds and is transmitted to the poultry. Sometimes, LPAI maintained in the wild birds is transmitted into poultry as observed during HPAI outbreaks in poultry in United States. The source of the virus was confirmed as LPAI from wild birds which undergo several mutations to generate HPAI strain.

### 2.2.2.5 Pathogenesis

Pathogenesis of AIV in psittacine and passerine birds is still unexplored. Experimental inoculation of HPAI (H5N1) in finches, sparrows and budgerigars indicated the existence of variations in pathogenesis of AIV from the gallinaceous poultry. Neurotropism of HPAI in nongallinaceous birds is identified as the major cause of

mortality. Experiments with migratory passerines speculated that the virus enters the central nervous system via the cerebrospinal fluid.

Localization of virus is also detected in other tissues such as heart, pancreas, spleen, nasal epithelium, and reproductive organs of nongallinaceous birds. Multi-organ failure or dysfunction is also identified as additional factor for mortality of the affected birds.

### 2.2.2.6 Clinical Symptoms

Non-specific clinical symptoms such as neurological signs (head between legs), depression, ruffled feathers, and standing at the bottom of the cage are observed in pet birds with AIV infection (Fig. 2.13). In a natural outbreak of LPAI infection (H5N2) in a red-lored amazon parrot (*Amazona autumnalis autumnalis*), lethargy, diarrhoea and dehydration are noted. Sudden onset, depression, neurological symptoms are detected in finches and budgerigars experimentally inoculated with a chicken isolate of HPAI (H5N1). In wild migratory passerine birds (blackcap, red-billed quelea) experimentally inoculated with HPAI (H5N1), sudden death, ruffled feathers, lethargy, and neurological disorders (ataxia) are observed.



**Fig. 2.13** Head between legs in a parrot (Courtesy Sanjoy Shit, Animal Resources Development Department, Government of West Bengal, India)

### 2.2.2.7 Lesion

In experimentally inoculated passerine and psittacine birds (zebra finches, house finches, budgerigars) with HPAI (H5N1), carcass dehydration, splenomegaly with mottling of parenchyma, accumulation of watery faeces in cloaca is observed. In house finches and budgerigars, vents are pasted with faeces and bile tinged urates.

In wild migratory passerine birds (blackcap, red-billed quelea) experimentally inoculated with HPAI (H5N1), lung congestion, pancreatic necrosis with multiple, white foci on pancreas are major gross lesions.

### 2.2.2.8 Diagnosis

#### Clinical Specimens

Cloacal swabs from live birds and tissue specimens from heart, pancreas, spleen, and brain after post-mortem can be collected as clinical specimens. The samples should be transported in isotonic phosphate-buffered-saline (PBS, pH 7.0–7.4) with antibiotics such as penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml), mycostatin (1000 units/ml) and protein (5% cattle serum, 0.5% bovine albumen). The specimens can be preserved at 4 °C for 4 days and at –80 °C for extended period.

#### Diagnostic Techniques

The laboratory should have at least biocontainment level 3 facilities and official clearance from the concerned authority to handle the AIV suspected samples.

- (a) *Isolation of virus from clinical samples:* The clinical samples collected in isotonic phosphate buffered saline with antibiotics are centrifuged (1000 g) and the supernatant fluid is inoculated into 9–11 days old embryonated hen's eggs (specific pathogen free) by allantoic sac route. The inoculated eggs are incubated at 37 °C for 4–5 days. After incubation, eggs are kept at 4 °C. The embryo will die in positive samples and the allantoic fluids are collected to detect the haemagglutination activity of the viral isolate. Use of Madin-Darby canine kidney (MDCK) cells in place of eggs is an alternative approach. The viral isolate is confirmed by haemagglutination inhibition test (HI), neuraminidase inhibition test (NI), immunodiffusion test and antigen-capture enzyme-linked immunosorbent assay (ELISA). Differentiation of HPAI and LPAI can be performed by chicken inoculation test, intravenous pathogenicity index (HPAI has the index > 1.2), and amino acid sequencing of haemagglutinin protein.
- (b) *Serological tests:* Hemagglutination-inhibition (HI), agar gel immunodiffusion (AGID), virus neutralization, enzyme-linked immunosorbent assay (ELISA) are employed for detection of AIV antibodies. The sera collected from the birds other than chicken sometimes agglutinate chicken red blood cells used in HI test (idiosyncrasy). To avoid idiosyncrasy, sera of the suspected birds should be adsorbed with chicken RBC before conducting the test. Immunodiffusion test (AGID) can detect the antibodies against nucleoprotein

(NP) and matrix proteins (M1, M2) of AIV which are antigenically similar in all isolates. AGID cannot differentiate between HPAI and LPAI isolates.

- (c) *Antigen detection tests*: ELISA (antigen capture) based commercial kits are available to detect the nucleoprotein (NP) of AIV. The kits are mostly intended for use in poultry, yet not validated in other species of birds.
- (d) *Molecular biology*: Real-time reverse-transcriptase polymerase chain reaction (rRT-PCR), light upon extension PCR (LUX-PCR) and conventional reverse-transcriptase-PCR can be used for detection of H5 or H7 subtype of AIV. Although false negative results are obtained in cloacal swabs and faecal samples due to presence of PCR inhibitors. To avoid the high cost and expertise needed in PCR based techniques, isothermal techniques such as rapid isothermal nucleic acid detection assay (RIDA), loop-mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification (NASBA) are used for detection of AIV. Further development in the diagnostic approach used proximity ligation assay (PLA) for detection of AIV in chicken, and DNA-microarray based technique for characterization of AIV in wild and domestic birds. In future, rapid isothermal nucleic acid detection assay-lateral flow (RIDA-LF) and immunoassay-based biosensors will be a better choice due to less dependence on instrumentation and rapid process of a good numbers of samples in less time, respectively.

### 2.2.2.9 Zoonosis

World Health Organization (WHO) has reported more than 600 human infections with HPAI (H5N1) since 2003, of which 60% infected people died. Transmission of H5N1 infection in human occurred during close contact with birds or contaminated environments. Keeping pet birds in household also increased the seroconversion of the owners during a H7N7 outbreak. Religious ceremonies, such as 'merit release' among Buddhists, in which a passerine bird is purchased, kissed and released, may increase the transmission possibility of AIV among the human. No report of human to human transmission of AIV is reported so far.

### 2.2.2.10 Treatment and Control Strategy

No effective treatment for pet birds against AIV infection is documented. To control the infection in pet birds, exposure to live bird market, wild birds, and migratory birds should be restricted. Infected birds should be kept separately and general hygiene practices should be followed to avoid the contamination of feed and drinking water. The cages or aviaries should be cleaned with formaldehyde, glutaraldehyde, beta-propiolactone, binary ethylenimine, quaternary ammonium disinfectants, sodium hypochlorite, dilute acids, and hydroxylamine after an AIV outbreak.

Vaccination against AIV in birds is a controversial issue. Due to high mutating capability of the virus, instead of control, vaccination with live virus may cause more damage to the birds. Experimentally, inactivated recombinant H5N3 vaccine was used in several types of zoo birds (Anseriformes, Charadriiformes,



Ciconiiformes, Columbiformes, Coraciiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Psittaciformes) which produced strong antibody titer against H5 subtype in all the birds except in Psittaciformes. Prime-boost strategy of vaccination (priming with H5N9 and booster with H5N3) produced strong antibody titer in Psittaciformes.

### 2.2.3 West Nile Virus Infection

#### 2.2.3.1 History

West Nile virus (WNV) was first isolated from a woman suffering with fever and other complications in Uganda (1937). In Africa, Middle East and European countries, WNV was mostly known to cause sub-clinical and self-limiting infections in horses and human during 1960s. Later in 1990s, higher frequency of WNV infection was noticed among human, farm animals, pet animals and birds of prey. In 1999, a fatal outbreak of WNV was detected among birds, horses and human in New York, USA. During 2008–10, WNV was isolated from a clinically infected sun conure (*Aratinga solstitialis*) and green-winged macaw (*Ara chloropterus*) in United States.

#### 2.2.3.2 Etiology

West Nile virus is an arthropod-borne (arbovirus), enveloped virus which belongs to family *Flaviviridae* and genus *Flavivirus*. The virion is 50 nm in diameter with icosahedral symmetry. No spikes or peplomers are present on the virion surface. Genome of the virus is positive sense single-stranded RNA. The genome (11 kbp) encodes envelope protein (E), membrane precursor protein (prM), capsid protein (C) and non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5). The structural proteins help in formation of virion and the non-structural proteins help in viral replication and evasion of host immune response.

Seven lineages of WNV have identified and lineages 1 (clade Ia and Ib) and 2 are considered as major lineages. Lineage 1 is mostly distributed in Europe (clade Ia), America (clade Ia), the Middle East (clade Ia), India, Africa (clade Ia) and Australia (clade Ib, Kunjin virus). Lineage 2 is widespread in South Africa, Madagascar and Europe. Both the lineages of WNV have neurotropism property, although, viruses belong to lineage 1 (clade Ia) are more virulent than the clade Ib and lineage 2 viruses.

#### 2.2.3.3 Host Susceptibility

WNV is identified in 326 species of birds with or without clinical symptoms. The most susceptible birds to WNV infection are crows (*Corvus* spp.), ravens (*Corvus corax*), jays (*Garrulus* spp.), magpies (*Pica* spp.), owls (*Strigiformes* spp.), and some raptors (Spanish imperial eagle, goshawk, golden eagle, sparrow hawk, gyrfalcon). The passerine birds and the mosquitoes (*Culex* spp.) are considered as major host and vector of WNV, respectively. Crows are more exposed to WNV

infection due to their communal roosting (perching) behaviour. After sunset, during the communal roosting, the mosquitoes (*Culex* spp.) mostly feed on the birds.

Migratory passerines (American Robins) can transmit the infection in distant places, whereas, resident passerines (house sparrows), crows [American crows (*Corvus brachyrhynchos*)] and other birds act as local amplifying host of WNV. WNV is detected from resident birds such as Columbiformes (*Columbina talpacoti*), Coraciiformes (*Melanerpes aurifrons*), Piciformes (*Cardinalis cardinalis*, *Molothrus aeneus*), and Passeriformes (*Myiozetetes similis*, *Sporophila torqueola*, *Thamnophilus doliatus*, *Tiaris olivaceus*, *Tyrannus melancholicus*). Seroprevalence of WNV is observed among passerines such as Northern Cardinals (*Cardinalis cardinalis*) and Carolina Wren (*Thryothorus ludovicianus*). Studies indicated that presence of non-passerine birds (enormous numbers) in a population can reduce the virus amplification and human transmission risk due to diversity of hosts (dilution effect).

Among the psittacine birds, seroprevalence of WNV is detected in budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*), cockatoos (*Cacatua* spp.), macaws (*Ara* spp.), parrots (*Amazona*, *Rhynchopsitta*, *Poictephalus*, *Psittacus* spp.), pacific parrotlets (*Forpus coelestis*), canary-winged parakeet (*Brotogeris versicolurus*), rosellas (*Platycercus* spp.), lorries and lorikeets (*Eos*, *Lorius*, *Pseudeos*, *Trichoglossus* spp.) and blue-crowned conure (*Thectocercus acuticaudata*). Red-legged partridges (*Alectoris rufa*) are resistant to natural WNV infection, although they can be experimentally infected with the virus.

Other than birds, human, horses, sheep, alpacas, dogs, cats, white-tailed deer, reindeer, squirrels, chipmunks, bats, and alligators are susceptible to WNV. Human and horses are dead-end-hosts of the virus as sufficient amount of virions are not maintained in the blood to infect the mosquitoes feeding on the hosts.

### 2.2.3.4 Transmission

Maintenance of WNV throughout the world takes place by an enzootic cycle ('rural cycle'). The susceptible birds and mosquitoes (*Culex* spp.) are two major components of the cycle. The birds maintain the virus by acting as reservoir and the mosquitoes act as vector and spread the virus into new hosts. Microfilarial infection of mosquitoes can hasten viral replication and rapid transmission of the virus (microfilarial enhancement of arboviral transmission). When the mosquitoes introduce the virus into the human habitats, the 'urban cycle' begins. In endemic zones, urban cycle begins with mortality of wild birds (summer to autumn) and the cycle ends with human and horse infection (dead-end-hosts).

In winter months, when the adult mosquitoes are mostly inactive, vertical transmission of the virus takes place to sustain in the vector population ('overwintering strategy'). Sometimes, WNV is re-introduced into the vector population through migratory birds and rarely by human transport (mosquitoes on aeroplanes).

Other than mosquito bites, WNV is rarely transmitted by oral route (ingestion of infected prey, drinking water) and direct contact in birds, cats and other vertebrates. In psittacine birds, feathers are identified as an important source of WNV. Association of testes in psittacine birds suggests the possibility of sexual transmission.

### 2.2.3.5 Pathogenesis

Following the WNV transmission through the mosquitoes in human and rodents, the virus primarily enters dendritic cells (Langerhans cells) via receptor mediated endocytosis. The cell surface proteins (DC-SIGN, integrin) act as WNV receptors. The dendritic cells carry the virus into draining lymph nodes where viral multiplication occurs. Following genomic replication and translation, the progeny virions are matured through ER-golgi secretion pathway and are released by exocytosis into the blood circulation. Transient viraemia develops and different vital organs such as liver, spleen, kidney are infected. Neuroinvasion can take place through direct infection with or without breakdown of blood-brain barrier or virus transport along peripheral neurons. Certain host proteins such as Drak2 (death-associated protein-kinase related 2), ICAM-1 (intercellular adhesion molecule), MIP (macrophage migration inhibitory factor) and MMP-9 (matrix metalloproteinase 9) help in altering blood-brain barrier permeability. Sometimes, host innate immune response (TLR3) mediated up regulation of tumor necrosis factor alpha (TNF $\alpha$ ) causes capillary leakage and increased permeability of blood-brain barrier.

In contrast, in birds, viraemia develops within 30–45 min of mosquito bite without any local virus multiplication in the lymph nodes. Positive correlation of peak viraemia and bird mortality is observed. In birds, WNV prefers to replicate primarily in spleen and mononuclear phagocytic cells and are disseminated into vital organs (liver, kidney, heart). Different lineages (1 and 2) of WNV have different tissue tropism in avian hosts. Lineage 1 virions prefer to infect liver and myocardium, whereas, lineage 2 of WNV mostly infect spleen, kidney and liver in goshawks. Depending upon the viral load in blood WNV may infect central nervous system in birds. Exact mechanism of neuroinvasion in birds is unexplored. Role of endothelial cells and immune cells in neuroinvasion is predicted. Death of the birds occurs due to WNV associated lesions and secondary infections with bacteria, fungi and parasites. Pathogenicity of WNV infections in birds is influenced by route of viral transmission, host defense, age and species of birds.

The virus can persist in different organs of birds such as spleen, kidney, eye, brain and skin. Detection of WNV in a hawk (birds of prey) during winter months, when mostly the mosquitoes are inactive and unable to transmit the infection, revealed the possibility of persistent viral infection. Experimentally, persistent WNV infection is produced in ducks, pigeons and immunocompromised mice. Effect of persistent WNV infection in health status of the birds is indistinct. Low level of WNV infection is detected in carcasses of rock pigeons (*Columba livia*) and mourning doves (*Zenaida macroura*), although, WNV is not confirmed as a cause of death. Whereas, in a kea (*Nestor notabilis* Gould), a large mountain parrot, natural persistence of WNV in central nervous system for more than 6 years is detected. This persistence is associated with death of the bird after prolonged incubation period.

### 2.2.3.6 Clinical Symptoms

Non-specific clinical signs such as depression, anorexia, dehydration and ruffled feathers are observed in birds. In complicated cases, neurological signs, for instance, convulsions, ataxia, abnormal head postures and movements, tremors,

paresis, and uncoordinated flight are detected. The neurological signs do not always correlate with the lesions in the brain (neuronal necrosis). Partial or complete blindness develops in raptors and owls. Sequelae of viral neuroinvasion are observed in long-lived birds (e.g. raptors). In raptors, feather pulp abnormalities and abnormal molt can persist up to 4 years as sequelae of WNV infection.

In naturally infected psittacine birds (rosellas, conures, lorikeets, cockatoos, caiques, parakeets), sudden death without any symptoms or non-specific signs like loss of weight, anorexia, lethargy, depression, and weakness are noted. Specific neurological signs consisted of rolling over, legs stretched backward, stumbling and disorientation.

### 2.2.3.7 Lesion

No pathognomonic lesion of WNV infection is detected in birds. In highly susceptible birds (crows) sudden death without any gross lesion is observed. In passerines, necrosis and mild inflammation in the heart, spleen, liver, kidney, mild encephalitic lesions and absence of neuronal necrosis is detected. In naturally infected psittacines (rosellas, conures, lorikeets, cockatoos, caiques, parakeets), splenomegaly, hepatomegaly, mottled pale liver with multifocal petechiae, diffuse pallor in kidneys, myocardial pallor, petechiae on the gizzard serosa are observed. In long-lived birds (raptors) due to chronic WNV infection, hemorrhages, petechiae and congestion in vital organs, splenomegaly, hepatomegaly, myocardial pallor, pale mottling in the liver, spleen, kidney, cerebral atrophy and malacia are detected. In central nervous system, gliosis, perivascular cuffing and glial nodules are major microscopic findings.

### 2.2.3.8 Diagnosis

#### Clinical Specimens

From the dead birds after post mortem, brain, heart, liver and kidney can be collected as clinical specimens for laboratory confirmation. The laboratories should have containment level 3 to handle the samples suspected for WNV infection

#### Diagnostic Techniques

- (a) *Isolation of virus from clinical samples:* WNV can be isolated in rabbit kidney (RK-13) and Vero cells or in embryonated chicken eggs. Several passages in cell line are required to observe the cytopathic effects. The virus isolates are confirmed by indirect FAT or PCR.
- (b) *Immunological tests:* The tissues collected from the suspected birds and fixed with formalin can be stained by immunohistochemical (IHC) staining for identification of WNV antigen.
- (c) *Serological tests:* Hemagglutination inhibition (HI), plaque reduction neutralization (PRN), IgM capture ELISA can be used for detection of antibodies against WNV in avian serum.
- (d) *Molecular biology:* Reverse transcriptase-nested PCR (E-protein as target) and real-time RT-PCR can be used for detection of WNV from avian tissues.

### 2.2.3.9 Zoonosis

Sporadic outbreaks of WNV occurred in human in the mediterranean region, Africa and Europe before 1994. Severe WNV outbreaks with neuroinvasion took place in human throughout the world after 1994. Global warming associated increased temperature and prolonged rainfall helps in breeding of mosquitoes and spreading of arboviral diseases such as WNV. In human, WNV transmission can occur through biting of infected mosquitoes, blood transfusion, organ transplantation, breast milk, and intrauterine route. The clinical presentation ranges from asymptomatic (80% of infections) to encephalitis/paralysis and death (1% of infections). Sometimes, flu like symptoms such as fever, headache, malaise, myalgia, fatigue, skin rash, lymphadenopathy, vomiting, and diarrhea are observed.

### 2.2.3.10 Treatment and Control Strategy

No effective treatment for pet birds against WNV infection is documented. To control the infection in pet birds, exposure to mosquitoes should be restricted.

Maternal antibodies can protect the house sparrow chicks up to 3 days post hatch. No specific vaccine against WNV is available to use in birds. In some countries, equine vaccine is used, although not licensed. Successful use of recombinant subunit WNV vaccine is reported from experimental geese. Experimental vaccination in thick-billed parrots (*Rhynchopsitta pachyrhyncha*) produced detectable antibody titer against WNV.

## 2.2.4 Usutu Virus Infection

### 2.2.4.1 History

Usutu virus (USUV) was first detected in a mosquito (*Culex neavei*) in 1959 in South Africa. The isolated virus is currently considered as a reference strain of USUV (SouthAfrica-1959). Subsequently, the virus was detected in different bird and mosquito species in Africa. In recent decade, USUV was identified in passerine birds in Austria (2001), in different birds and mosquitoes in Hungary (2005), Spain (2006), Switzerland (2006) and Italy (2009).

### 2.2.4.2 Etiology

Usutu virus belongs to the genus *Flavivirus* (Japanese encephalitis serocomplex) under the family *Flaviviridae*. Different species of *Flavivirus* such as USUV, West Nile virus (WNV), Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV) and Saint Louis encephalitis virus are originated from same ancestral virus. Nucleotide and amino acid sequencing revealed that MVEV, among Japanese encephalitis serocomplex, are the closest relative of USUV.

USUV is a small (40–60 nm), spherical, enveloped virus with positive sense single stranded RNA genome (11 Kbp). The genome has a cap at 5' end but no poly-A-tail at 3' end. The genome can encode three structural proteins (core protein, pre-membrane and envelope protein) and eight non-structural (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, NS5) proteins.

### 2.2.4.3 Host Susceptibility

Eurasian blackbirds (*Turdus merula*) mostly suffer from USUV infection. Several other families of birds (Accipitriformes, Anseriformes, Caprimulgiformes, Charadriiformes, Ciconiiformes, Columbiformes, Coraciiformes, Galliformes, Passeriformes, Piciformes, Strigiformes) of different European countries are also susceptible to USUV infection (Table 2.7). Migratory birds such as whitethroat (*Sylvia communis*), lesser whitethroat (*Sylvia curruca*), garden warbler (*Sylvia*

**Table 2.7** Distribution and susceptible hosts of Usutu virus infection

Birds	Country	Year
Eurasian blackbird ( <i>Turdus merula</i> )	Italy, Germany, Hungary, Austria	2001–11
Great grey owl ( <i>Strix nebulosa</i> )	Germany, Austria	2001–11
Chicken ( <i>Gallus gallus domesticus</i> )	UK, Italy, Switzerland	2006–09
Humboldt penguin ( <i>Spheniscus humboldti</i> )	Switzerland	2006–07
Greater flamingo ( <i>Phoenicopterus ruber</i> )	Switzerland	2006–07
Laughing kookaburra ( <i>Dacelo novaeguineae</i> )	Switzerland	2006–07
White stork ( <i>Ciconia ciconia</i> )	Austria	2006–07
Marabou stork ( <i>Leptoptilos crumeriferus</i> )	Austria	2006–07
Egyptian vulture ( <i>Neophron percnopterus</i> )	Austria	2006–07
Eurasian eagle owl ( <i>Bubo bubo</i> )	Austria	2006–07
Ural owl ( <i>Strix uralensis</i> )	Austria	2006–07
Snowy owl ( <i>Bubo scandiacus</i> )	Austria	2006–07
boreal owls ( <i>Aegolius funeri</i> )	Italy	2007
Eurasian pygmy owl ( <i>Glaucidium passerinum</i> )	Italy	2007
Northern hawk-owl ( <i>Surnia ulula</i> )	Italy	2007
Grey heron ( <i>Ardea cinerea</i> )	Germany, Italy	2010–11
Eurasian bee-eater ( <i>Merops apiaster</i> )	Germany, Italy	2010–11
House sparrow ( <i>Passer domesticus</i> )	Germany, Italy	2010–11
Eurasian green woodpecker ( <i>Picus viridis</i> )	Germany, Italy	2010–11
Common starling ( <i>Sturnus vulgaris</i> )	Germany, Italy	2010–11
Partridge ( <i>Alectoris rufa</i> )	Italy	2010–11
Long-eared owl ( <i>Asio otus</i> )	Italy	2010–11
Nightjar ( <i>Caprimulgus europaeus</i> )	Italy	2010–11
Eurasian jay ( <i>Garrulus glandarius</i> )	Italy	2010–11
Yellow-legged gull ( <i>Larus michahellis</i> )	Italy	2010–11
Eurasian magpie ( <i>Pica pica</i> )	Italy	2010–11
Eurasian collared dove ( <i>Streptopelia decaocto</i> )		
Common kingfisher ( <i>Alcedo atthis</i> )	Germany	2011
Canary ( <i>Serinus canaria domestica</i> )	Germany	2011
Song thrushes ( <i>Turdus philomelos</i> )	Spain	2012
Domestic pigeon ( <i>Columba livia domestica</i> )	Greece	2014
Great spotted woodpecker ( <i>Dendrocopos major</i> )	Belgium	2014
Bullfinch ( <i>Pyrrhula pyrrhula</i> )	Belgium	2014

borin), kestrel (*Falco tinnunculus*), marsh harrier (*Circus aeruginosus*), house martin (*Delichon urbica*), reed warbler (*Acrocephalus scirpaceus*), pied flycatcher (*Ficedula hypoleuca*), barn-swallow (*Hirundo rustica*) are detected to carry antibodies against USUV.

Most of these bird families are susceptible to both USUV and West Nile virus (WNV) infection. Evidence of both viral infection was detected in Eurasian blackbirds, Eurasian blackcaps, European robins, magpies etc.

#### 2.2.4.4 Transmission

USUV is maintained in nature by a mosquito-bird cycle in which mosquitoes act as vector and the birds act as amplifying host. The mosquitoes occasionally spread the virus into other hosts (incidental) such as human, horses and rodents. USUV is detected in several mosquitoes such as *Culex pipiens*, *Culex neavei*, *Culex perexiguus*, *Aedes albopictus*, *Aedes caspius*, *Anopheles maculipennis*, *Culex perfuscus*, *Coquillettidia aurites*, *Mansonia Africana*. Among them, *C. pipiens* and *C. neavei* are considered as competent vectors for USUV. Although the migratory birds are sometimes infected with USUV the evidence of their role in transmission of infection is still missing.

Trematode infestation in blackbirds is detected as a predisposing factor for USUV infection.

#### 2.2.4.5 Pathogenesis

After introduction of USUV into the body of the host, viraemia lasts for a short period (2 days). The tissue tropism of the virus is almost similar to WNV infection. The virus is detected in brain, heart, liver, kidney, lungs, and intestinal tissues of laboratory mice and naturally infected birds. Demyelination of neurons and formation of autophagosome are unique features of USUV infection. The process of autophagy helps in incorporation of host cellular components in viral replication.

#### 2.2.4.6 Clinical Symptoms

USUV infection in birds produces non specific clinical symptoms such as apathy, depression, anorexia, dehydration, ruffled feathers and moulting. In complicated cases, neurological signs, for instance, convulsions, ataxia, abnormal head postures and movements, tremors, paresis, torticollis and nystagmus are detected. Neurological symptoms are often followed by death.

#### 2.2.4.7 Lesion

The disease in birds is characterized by encephalitis, myocardial degeneration, and necrosis in liver and spleen. Degeneration of Purkinje cells, accumulation of glial nodules surrounding the degenerated Purkinje cells ('glial shrubbery'), perivascular cuffing are characteristic findings in brain of affected birds. Hepatomegaly, enlargement and discoloration of the kidneys, necrosis on the sheathed arteries of spleen are detected in great grey owls and boreal owls. In blackbirds, affected liver and spleen contains myriads of small (up to 1 mm) yellowish foci. Enlarged gall-bladder and intestine, hyperaemic meninges and brain are also found in blackbirds.

### 2.2.4.8 Diagnosis

#### Clinical Specimens

Blood or serum (paired sera collected in two weeks interval) from live bird and liver, spleen, lung, kidney, gizzard, and intestines are collected and fixed in 10% buffered formalin as post mortem specimens.

#### Diagnostic Techniques

- (a) *Isolation of virus from clinical samples:* USUV can be isolated in Vero, PK-15 and goose embryo fibroblast cells. The laboratories should have containment level 3 to handle the samples suspected for USUV infection.
- (b) *Immunological tests:* The tissues collected from the suspected birds and fixed with formalin can be stained by immunohistochemical (IHC) staining for identification of USUV antigen.
- (e) *Serological tests:* Detection of four fold rise in antibody titer and seroconversion from IgM to IgG in paired sera sample collected in two weeks interval indicates USUV infection in birds. USUV-IgM appears 5 days after onset of clinical symptoms. USUV antibody titer is detected by haemagglutination inhibition (HI) and plaque reduction neutralization test (PRNT). USUV-specific IgG-capture ELISA is developed for human use. However, the serological tests often produce cross reactivity with other *Flavivirus* infections such as WNV. PRNT is more specific than other serological tests but requires specialized laboratory which can handle the virus. Detection of acute infection is not possible by serological tests as the birds die before development of antibody titer.
- (f) *Molecular biology:* Reverse transcriptase-PCR can specifically detect USUV in tissues of suspected birds. Recently real-time PCR is also developed for detection of USUV in human blood and cerebrospinal fluid samples which can be adapted in avian diagnostics.

### 2.2.4.9 Zoonosis

In 1981, in Central African Republic, a man with fever and rashes was diagnosed as a first human patient of USUV infection (CAR-1981). In Italy (2009), USUV infection was detected in two different patients of meningoencephalitis and orthotropic liver transplantation. Both the patients were immunosuppressed and received blood transfusion before the infection. The common clinical symptoms were persistent fever, headache and neurological disorders. In 2012–13, a sero-surveillance program in Germany and Croatia detected low prevalence of USUV antibodies among the human population.

### 2.2.4.10 Treatment and Control Strategy

No effective treatment for pet birds against USUV infection is documented. To control the infection in pet birds, exposure to mosquitoes should be restricted. Water should not be stagnant in the vicinity of the aviaries or bird owner's houses.



*Culex pipiens*, the potent vector of USUV do not prefer to fly a long distance and lack of breeding site will significantly reduce their numbers. Use of mosquito net (window and door) and repellants help to reduce the mosquito population. N, N-diethyl-meta-toluamide (DEET) is most effective repellent against *Culex pipiens*.

No specific vaccine against USUV is available to use in birds. Vaccines against *Flavivirus* (Japanese encephalitis, yellow fever) are available for human use but no cross protection against USUV infection is reported.

## 2.2.5 Avian Borna Virus Infection

### 2.2.5.1 History

Borna disease was first detected among animals (horse, sheep) in Southeast Germany during 19th century. It was named after the German district of Borna around the town of Borna in Saxony where the infection remained endemic for prolonged period. The etiological correlation of Borna disease with a virus was established in 1920.

Proventricular dilatation disease (PDD) was first reported from macaws and conures in USA during 1977. At that time, PDD was known as ‘macaw wasting or fading syndrome’ and ‘gastric distension of macaws’ as mostly macaws were associated with the syndrome. Actual etiology of PDD remained uncertain for a considerable period. Two independent research groups from Israel and USA (Honkavuori et al. 2008; Kistler et al. 2008) identified a novel genus of the family *Bornaviridae*, provisionally named as avian bornavirus (ABV) as etiological agent of PDD. The virus was identified by molecular techniques such as panviral DNA microarray and high throughput sequencing.

### 2.2.5.2 Etiology

Avian bornavirus (ABV) belongs to the family *Bornaviridae* and order *Mononegavirales*. The virion is enveloped, spherical, and 80–100 nm in diameter. The virions replicate in the nucleus of the host cells and use the host cellular splicing machineries for generation of mRNAs. The genome is non-segmented, single stranded RNA which encodes six major viral proteins such as nucleoprotein (N), regulatory protein (X), phosphoprotein (P), matrix protein (M), membrane-bound glycoprotein (G), and RNA-dependent RNA polymerase (L).

After the discovery of Avian Bornavirus in psittacine birds, several types of Bornavirus were detected in both psittacine and non-psittacine birds. Recently it is proposed that the genus should include five species such as *Mammalian 1 bornavirus*, *Psittaciform 1 bornavirus* (avian/psittacine bornaviruses 1, 2, 3, 4, 7), *Passeriform 1 bornavirus* (canary bornaviruses C1, C2, C3, LS), *Passeriform 2 bornavirus* (estrildid finch bornavirus EF) and *Waterbird 1 bornavirus* (avian bornavirus 062CG).

Till date, 14 ABV genotypes have been detected in psittacine (ABV-1, 2, 3, 4, 5, 6, 7) and non psittacine birds (ABV-C1, ABV-C2, ABV-C3 in canaries; ABV-CG in Canada geese; ABV-EF in estrildid finch; ABV-BF in Bengalese finch).

### 2.2.5.3 Host Susceptibility

Avian borna viruses are detected in over 80 species of birds of which more than 70 species belongs to psittaciformes. The members of psittaciformes commonly infected with ABV include *Cacatuidae* (cockatoos, cockatiels) and *Psittacidae* (lovebirds, macaws, parakeets, parrots, Amazon parrots, conures) (Table 2.8). Among non-psittacine birds, canary, long-wattled umbrella bird, weaver finch, red-tailed hawk, falcon, Canada geese, swan, duck, bald eagle are found naturally infected with ABV (Table 2.8). PDD is reported from United States, Australia, Middle East, South America, South Africa and Japan.

### 2.2.5.4 Transmission

The studies suggest about faecal-oral or faecal-oral-nasal transmission of ABV between the captive birds. In a bird with PDD, ABV infected cells are detected in the intestinal villi from where they are excreted through the faeces. Sometimes ABV infected birds remain healthy and act as source of infection for other birds kept in the same aviary. It is observed that birds with high serum antibody titer against ABV or viral RNA load are prone to become clinically infected with PDD. Overcrowding in aviaries, hand-feeding of parrot chicks are detected as predisposing factors for PDD. No gender based predisposition of PDD is observed. Transmission route of ABV in wild birds is still unexplored.

### 2.2.5.5 Pathogenesis

Irrespective of transmission route, classical borna disease virus enters central nervous system. In experimentally infected rats, centrifugal spread of virus into peripheral nerves and autonomic nerve fibers and ganglia are detected. In avian borna virus infection in birds, autonomous nervous system of the upper and middle digestive tract, including the esophagus, crop, proventriculus, ventriculus, and duodenum is chiefly infected. Further spread of ABV in extraneural tissues such as smooth or heart muscle fibers, liver, kidney, spleen, pancreas, lung, gonads, thyroid, and skin is observed. In mammalian borna virus infection, extraneural spread in hepatocytes, kidney epithelial cells, and myocytes of the intestine and heart is associated with immunosuppression of the host.

### 2.2.5.6 Clinical Symptoms

The incubation period of ABV infection in birds is highly variable (10 days–years). Clinically the birds show gastrointestinal dysfunction or neurological signs or both. The symptoms of gastrointestinal dysfunction are impaction of proventriculus, dysphagia, polyuria, regurgitation, diarrhoea, presence of undigested food (seeds) in faeces, and crop stasis which leads to starvation and death. Death due to circulatory collapse or food aspiration is also found. Neurological signs are ataxia, seizure, blindness, tremor, abnormal gait, reduced proprioceptive skills, motor deficit and peripheral neuritis in sciatic, brachial and vagal nerves.

### 2.2.5.7 Lesion

No gross lesion is observed in sudden death of birds due to PDD. In majority of the birds suffering with PDD (70%), proventriculus is thin walled and distended with seeds. Rupture of proventriculus wall releases the food particles and causes

**Table 2.8** Distribution and susceptible hosts of Avian Bornavirus

Psittacine birds	Continent/Country	Non-psittacine birds	Continent/Country
Cockatiel ( <i>Nymphicus hollandicus</i> )	Asia	Canary ( <i>Serinus canaria</i> )	Spain, Germany
White cockatoo ( <i>Cacatua alba</i> ), Ducorps's Cockatoo ( <i>Cacatua ducorpsii</i> ), sulphur-crested cockatoo ( <i>Cacatua galerita</i> ), red-vented cockatoo ( <i>Cacatua haematuropygia</i> ), Salmon-crested cockatoo ( <i>Cacatua moluccensis</i> ), Little corella ( <i>Cacatua sanguinea</i> )	Asia, Europe	Long-wattled umbrella bird ( <i>Cephalopterus penduliger</i> )	Spain
Galah ( <i>Eolophus roseicapillus</i> )	Asia	Bearded barbet ( <i>Lybius dubius</i> )	Spain
Red tailed black Cockatoo ( <i>Calyptorhynchus magnificus</i> )	Asia	Honey creeper ( <i>Chlorophanes spiza</i> )	Spain
Palm Cockatoo ( <i>Probosciger atterimus</i> )	Asia	Weaver finch	Spain
Red-breasted parakeet ( <i>Psittacula alexandri</i> )	Asia	Red-tailed hawk ( <i>Buteo jamancensis</i> )	–
Eclectus parrot ( <i>Eclectus roratus</i> )	Asia, Europe	Falcon ( <i>Falco peregrinus</i> )	–
Rainbow lorikeet ( <i>Trichoglossus moluccanus</i> )	Asia	Canada geese ( <i>Branta canadensis</i> )	USA
Blue-and-yellow macaw ( <i>Ara ararauna</i> ), Golden collared macaw ( <i>Ara auricollis</i> ), green-winged macaw ( <i>Ara chloropterus</i> ), Blue-throated macaws ( <i>Ara glaucogularis</i> ), scarlet macaw ( <i>Ara macao</i> ), military macaw ( <i>Ara militaris</i> ), red shouldered macaw ( <i>Ara nobilis</i> ), red-fronted macaw ( <i>Ara rubrogenys</i> ), chestnut-fronted macaw ( <i>Ara severus</i> )	USA	Roseate spoonbills ( <i>Ajaia ajaja</i> )	–
Hyacinth macaw ( <i>Anodorhynchus hyacinthinus</i> )	USA, Europe	Toucans ( <i>Ramphastos sp.</i> )	–
Spix's macaw ( <i>Cyanopsitta spixii</i> )	USA	Bald eagle ( <i>Haliaeetus leucocephalus</i> )	–

(continued)

**Table 2.8** (continued)

Psittacine birds	Continent/Country	Non-psittacine birds	Continent/Country
Blue-crowned Conure ( <i>Aratinga acuticaudata</i> ), Peach-fronted Parakeet ( <i>Aratinga aurea</i> ), golden-capped parakeet ( <i>Aratinga auricapillus</i> ), red-masked parakeet ( <i>Psittacara erythrogenys</i> ), Finsch's Conure ( <i>Aratinga finschi</i> ), Golden parakeet ( <i>Aratinga guarouba</i> ), jenday conure ( <i>Aratinga jandaya</i> ), sun conure ( <i>Aratinga solstitialis</i> ), dusky-headed parakeet ( <i>Aratinga weddellii</i> )	USA	American geese, swans, ducks	–
Nanday Conure ( <i>Nandayus nenday</i> )	USA, Europe	Estrildid finches ( <i>Estrildidae</i> )	Germany
Burrowing parrot ( <i>Cyanoliseus patagonus</i> )	USA, Europe	Bengalese finch ( <i>Lonchura striata</i> )	–
Grey cheeked parakeet ( <i>Brotogeris pyrrhoptera</i> )	USA, Europe	Trumpeter swans ( <i>Cygnus buccinator</i> ), feral mute swans ( <i>Cygnus olor</i> )	USA
Green-cheeked conure ( <i>Pyrrhura molinae</i> ), black-capped parakeet ( <i>Pyrrhura rupicola</i> )	USA, Europe		
Thick-billed parrot ( <i>Rhynchopsitta pachyrhyncha</i> )	USA		
Blue-fronted amazon ( <i>Amazona aestiva</i> ), white-fronted amazon ( <i>Amazona albifrons</i> ), orange-winged amazon ( <i>Amazona amazonica</i> ), yellow-naped parrot ( <i>Amazona auropalliata</i> ), red-lored parrot ( <i>Amazona autumnalis</i> ), Cuban amazon ( <i>Amazona leucocephala</i> ), ellow-crowned parrot ( <i>Amazona ochrocephala</i> ), Yucatan amazon ( <i>Amazona xantholora</i> )	USA		
Pileated parrot ( <i>Pionopsitta pileata</i> )	USA		
Bronze-winged parrot ( <i>Pionus chalcopterus</i> )	USA, Europe		

(continued)

**Table 2.8** (continued)

Psittacine birds	Continent/Country	Non-psittacine birds	Continent/Country
Green-thighed parrot ( <i>Pionites leucogaster</i> )	USA		
Red-fan parrot ( <i>Derophtus accipitrinus</i> )	USA		
Pacific parrotlet ( <i>Forpus coelestis</i> )	USA		
Congo African grey parrot ( <i>Psittacus erithacus</i> )	Africa, Europe		
Red-fronted parrot ( <i>Poicephalus gularis</i> )	Africa, Europe		
Greater vasa parrot ( <i>Coracopsis vasa</i> )	Africa, Europe		
Yellow-collared Lovebird ( <i>Agapornis Personata</i> )	Africa, Europe		
Canindae macaw ( <i>Ara glaucogularis</i> ), Vinaceous Amazon ( <i>Amazona vinacea</i> )	USA		
Umbrella cockatoo ( <i>Cacatua alba</i> ), Solomons cockatoo ( <i>Cacatua ducorsii</i> ), Sulfur-crested cockatoo ( <i>Cacatua galerita</i> )	Japan		
Barred parakeet ( <i>Bolborhynchus</i> spp.), Golden parakeet ( <i>Guaruba</i> spp.), superb parrot ( <i>Polytelis</i> spp.)	Europe		

peritonitis. Sometimes, enlargement of duodenum and adrenal glands, pale area on the epicardium is observed. Microscopic lesions consist of lymphocytic infiltration along with plasma cells in the ganglia and nerve plexus (specially myenteric plexus supplying the digestive tract) of proventriculus, intestine, crop, esophagus, adrenal gland, conduction fibers of heart, central nervous system and spinal cord. Perivascular cuffing by lymphocytes are detected in cerebral cortex, cerebellum, spinal cord and in peripheral nerves such as sciatic, brachial and vagus nerves.

### 2.2.5.8 Diagnosis

#### Clinical Specimens

For *intra vitam* (ante-mortem) diagnosis of ABV infection, faeces, blood, swabs of crop and cloaca, tissue biopsies from crops can be collected. Left lateral sac of the crop (cranial portion) is preferred site for biopsy collection. The biopsy should be

elliptical and it should contain a blood vessel so that the nerve sections can be visualized. After post-mortem, brain, crop, intestine and adrenal glands are collected.

### Diagnostic Techniques

- (a) *Clinical signs, haematology*: Clinical signs are mostly associated with gastrointestinal upset and/or neurological signs. Non-regenerative anemia, leukocytosis, heterophilia, decreased total protein and albumin, increased level of muscle enzymes such as lactate dehydrogenase, creatine kinase, aspartate amino-transferase are detected in PDD infected birds.
- (b) *Radiography*: Distended proventriculus, ventriculus, crop and small intestine with ingesta and gas and prolonged gastrointestinal transit time are observed in infected birds by contrast radiography, contrast fluoroscopy and ultrasonography. Spontaneous ruptures of the dilated proventriculus are rarely observed. Although these findings are not specific for PDD. In healthy neonatal birds, distension of proventriculus and crop is also found. Contrast radiography is performed in birds by introducing barium sulfate or iodine-based contrast media (@ 10–15 ml/kg) into the crop by gavage. Barium sulfate produces better contrast but causes airway irritation. In psittacine birds, normal gastrointestinal transit time is 90 min–3 h.
- (c) *Isolation of virus from clinical samples*: Isolation of ABV can provide confirmatory diagnosis for PDD. It can be done in quail cell lines (CEC-32, QM7) and other avian cell lines. Incorporation of mammalian cell line for virus isolation is not always successful.
- (d) *Histopathology*: Histopathological investigation is another way of confirmatory diagnosis. Lymphocytic infiltration in the ganglia and nerve plexus of proventriculus, ventriculus, intestine and crop is considered as diagnostic for PDD.
- (e) *Serology*: ELISA, indirect immunofluorescence assay and Western blot are developed for detection of anti-ABV antibodies. The serological tests cannot differentiate between PDD infected birds from asymptomatic carrier of ABV.
- (f) *Molecular biology*: Reverse transcriptase-PCR (RT-PCR) for detection of L, M and N genes of ABV is developed. Quantitative real time-PCR for detection of P gene is recently developed. Brain, crop, intestine and adrenal glands collected after post-mortem and crop tissue, blood, cloacal swabs, and faeces can be used for ABV-RNA extraction. However, both false-positive (from asymptomatic bird) and false-negative results can be obtained by RT-PCR.

#### 2.2.5.9 Zoonosis

Zoonotic potentiality of ABV is not established.

#### 2.2.5.10 Treatment and Control Strategy

PDD is a highly contagious infection and it spreads rapidly from one bird to another within a flock. Decision to offer long term treatment or euthanasia of the affected

bird is crucial. Euthanasia is the best policy for management of PDD, although not preferred by most of the owners. Management of inflammation, indigestion and secondary bacterial infections are currently considered as line of treatment for PDD. Use of nonsteroidal anti-inflammatory drugs (NSAIDs, e.g. celicoxib, 20 mg/kg body weight, orally) along with antivirals (amantadine hydrochloride, 10 mg/kg po or 20 mg/kg with food) is recommended to treat PDD in birds. Use of surfactants (for reduction of gas production), metoclopramide (0.5 mg/kg body weight, intramuscularly) and B complex vitamins are suitable supportive therapy.

Diet of the PDD infected birds should be easily digestible (preferably formulated diets), and in liquid or pelleted forms because the proventriculus and ventriculus function is adversely affected in PDD. Addition of vegetables in the diet will increase intestinal motility. Toys and cage accessories should be provided to the birds to avoid ingestion of foreign bodies.

For prevention of PDD, new birds should be quarantined and checked for PDD before introduction into aviaries. Maintenance of strict biosecurity and hygienic measures should be followed. Overcrowding should be avoided in the aviaries.

## 2.2.6 Beak and Feather Disease

### 2.2.6.1 History

First description of Beak and feather disease (BFD) was observed in 1907 in an Australian journal ('The Emu') and the author described about wild red-rumped parrots (*Psephotus haematonotus*) in the Adelaide hills being unable to fly due to loss of feathers (Ashby 1907). In 1916, death of a captive sulphur-crested cockatoo (popular by its name 'Cocky Bennett') at the age of 120 years in Sydney was published in local news paper. The bird was suspected for BFD due to loss of feather and presence of elongated beak. Psittacine beak and feather disease (PBFD) was first scientifically documented in 1975 in sulfur-crested cockatoos, lovebirds, budgerigars and galahs in Australia (Pass and Perry 1984).

### 2.2.6.2 Etiology

Beak and feather disease is caused by beak and feather disease virus (BFDV) which belonged to the genus *Circovirus* and family *Circoviridae*. Circoviruses are icosahedral, non-enveloped and the smallest known autonomously replicating animal virus, measuring 15–26 nm in diameter. The viruses have an ambisense, circular, single-stranded DNA genome (2000 nt) which can encode a replicase enzyme and capsid protein. The virus possesses highest mutation rate and genetic diversity although it is antigenically conserved. No serotype variation of *Circovirus* is detected. The virus is considered as a model to study host parasite interaction due to its simple genome structure. It is also the representative of ancient viral form and *Circovirus* sequences are detected in fossils of vertebrates, invertebrates, protozoa, plant, fungi, algae and bacteria.

### 2.2.6.3 Host Susceptibility

BFD virus mostly infects psittacine birds (more than 60 species) besides other bird families such as Passeriformes, Columbiformes and Anseriformes (Table 2.9). A few susceptible bird species are enlisted as endangered or threatened by International Union for Conservation of Nature (<http://www.iucnredlist.org>). BFDV infection is considered as a significant conservation threat. The infection is more fatal in young birds (0–3 years) due to poor development of immune system and the viral load is more prevalent in parental bird species than the hybrids. Cockatoos mostly show chronic viral infection with excretion of virus through the faeces and dystrophic feathers. Sometimes, cockatoos do not show any visible symptoms. Occasionally, birds other than the common susceptible species are also infected with BFDV ('host switch over'). Recently, BFDV infection is detected in rainbow bee-eaters (*Merops ornatus*), a species of Coraciiformes, unrelated to psittacine birds.

Mostly the BFD infection is detected in Australia, New Zealand, Europe (Poland, UK, Denmark, Portugal, Germany, Italy), United States, Africa (Zambia, Zimbabwe) and Asia (Japan, China, Taiwan, Thailand). Circumstantial evidence indicated that the infection was originated in Australia. The virus was disseminated from Australia in early 1970s to European countries with the imported parrots. From Europe, the virus is further distributed to Africa, New Zealand, Japan and United States during unregulated parrot trafficking.

### 2.2.6.4 Transmission

Transmission of BFDV can take place by both horizontal and vertical means. Horizontal route via direct contact with infected birds is the major mode of transmission in both wild and captive birds. The virus is excreted in high titers in the environment through feather dust, crop secretions, and faeces of infected birds. In aviaries, the virus once spread is difficult to control due to its high infectious and persistence nature. In the fomites, BFDV can persist for several years and after a long period, the fomites may act as a source of ancestral viral genotype in the host population.

In forests, 'host switch over' is facilitated by horizontal transmission. The switch over mostly takes place in the unoccupied nests in the trees where competition exists between Psittaciformes and other birds for reproductive opportunities.

### 2.2.6.5 Pathogenesis

BFDV depends on host cell machineries for their replication and prefers the actively dividing cells such as basal follicular epithelium, lymphoid tissues, and intestinal epithelium. The virus causes necrosis of basal epithelium in the birds. The necrosis is found to be responsible for feather dystrophy and beak and claw deformities. BFDV also causes lymphoid depletion and associated immunosuppression. Secondary infection with bacteria, fungi, parasite takes place due to immunosuppression and it is responsible for 70% bird mortality as observed in ducks, pigeons, geese, black-backed gull and other avian species. More experiments are needed to explore the pathogenesis of BFDV in pet birds.



**Table 2.9** Common susceptible hosts of BFDV

Bird order	Bird species	Common name
Psittaciformes	<i>Amazona aestiva</i>	Blue-fronted Amazon
	<i>Amazona albifrons</i>	White-fronted Amazon
	<i>Amazona amazonica</i>	Orange-winged Amazon
	<i>Amazona auropalliata</i>	Yellow naped Amazon
	<i>Amazona autumnalis</i>	Red lored Amazon
	<i>Amazona vinacea</i>	Vinaceous-breasted Amazon
	<i>Amazona aestiva</i>	Turquoise-fronted Amazon
	<i>Poicephalus robustus</i>	South African Cape Parrot
	<i>Alisterus scapularis</i>	Australian king parrot
	<i>Aprosmictus erythropterus</i>	Red-winged parrot
	<i>Poicephalus senegalus</i>	Senegal Parrot
	<i>Pionites leucogaster</i>	Green-thighed parrot
	<i>Eclectus roratus</i>	Eclectus Parrot
	<i>Poicephalus rufiventris</i>	African red-bellied parrot
	<i>Poicephalus gulielmi massaicus</i>	Jardine parrot
	<i>Poicephalus rueppellii</i>	Ruppell's parrot
	<i>Pionites melanocephalus</i>	Black headed parrot
	<i>Pionus chalcopterus</i>	Bronze winged parrot
	<i>Guarouba guarouba</i>	Golden parakeet
	<i>Psittacula echo</i>	Echo Parakeet
	<i>Psittacula krameri</i>	Ring-necked parakeet
	<i>Psittacula eupatria</i>	Alexandrine parakeet
	<i>Cyanoramphus novaezelandiae</i>	Red-crowned parakeet
	<i>Psittacula krameri</i>	Rose-ringed parakeet
	<i>Cyanoramphus unicolor</i>	Antipodes parakeet
	<i>Coracopsis vasa</i>	Vasa parrot
	<i>Psittacara finschi</i>	Crimson-fronted parakeet
	<i>Cacatua alba</i>	White cockatoo
	<i>Cacatua leabeateri</i>	Major Mitchell's cockatoo
	<i>Cacatua galerita</i>	Sulphur-crested cockatoo
	<i>Calyptorhynchus banksii</i>	Red-tailed black cockatoo
	<i>Calyptorhynchus lathami</i>	Glossy black cockatoo
	<i>Cacatua ducorpsii</i>	Solomon's corella
	<i>Cacatua galerita triton</i>	Triton cockatoo
	<i>Cacatua goffiniana</i>	Tanimbar corella
	<i>Cacatua haematuropygia</i>	Philippine cockatoo
	<i>Cacatua moluccensis</i>	Moluccan cockatoo
	<i>Cacatua ophthalmica</i>	Blue-eyed cockatoo
	<i>Cacatua sulphurea</i>	Yellow-crested cockatoo
	<i>Cacatua tenuirostris</i>	Eastern long-billed corella
	<i>Nymphicus hollandicus</i>	Cockatiel

(continued)

**Table 2.9** (continued)

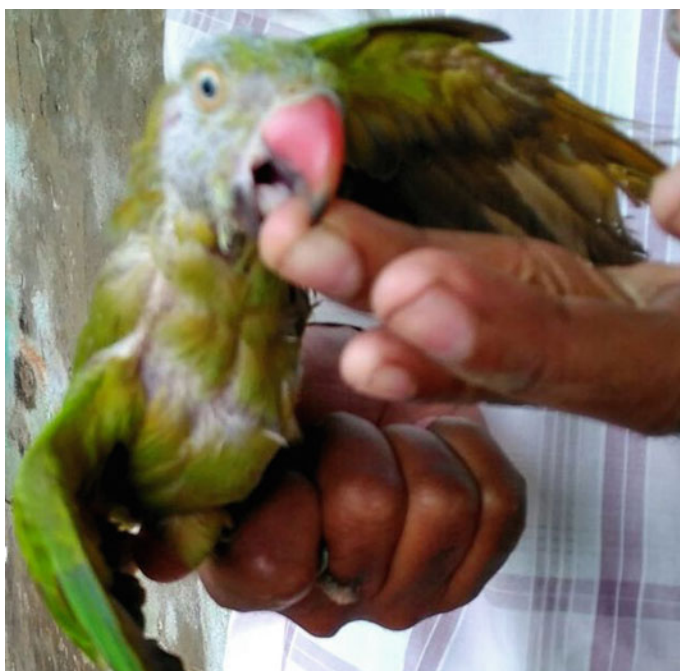
Bird order	Bird species	Common name
	<i>Melopsittacus undulatus</i>	Budgerigar
	<i>Psephotus haematogaster</i>	Blue Bonnet
	<i>Trichoglossus haematodus</i>	Rainbow lorikeet
	<i>Lorius chlorocercus</i>	Yellow bib lorikeet
	<i>Psitteuteles goldiei</i>	Goldie's lorikeet
	<i>Eos reticulate</i>	Blue-streak lorikeet
	<i>Agapornis roseicollis</i>	Lovebird
	<i>Agapornis lilianae</i>	Nyasa lovebird
	<i>Agapornis nigrigenis</i>	Black-cheeked lovebird
	<i>Agapornis roseicollis</i>	Peach-faced lovebird
	<i>Eolophus roseicapillus</i>	Galah
	<i>Cacatua tenuirostris</i>	Eastern long billed corella
	<i>Platycercus elegans</i>	Crimson rosella
	<i>Platycercus eximius</i>	Eastern rosella
	<i>Cacatua alba</i>	White cockatoo
	<i>Forpus coelestis</i>	Pacific parrotlet
	<i>Ara macao</i>	Scarlet Macaw
	<i>Ara ararauna</i>	Blue-and-yellow macaw
	<i>Ara severa</i>	Chestnut-fronted macaw
	<i>Ara nobilis</i>	Red-shouldered macaw
	<i>Ara chloropterus</i>	Red-and-green macaw
	<i>Ara militaris</i>	Military macaw
	<i>Aratinga solstitialis</i>	Conure
Passeriformes	<i>Serinus canaria</i>	Canary
Columbiformes	<i>Columbia livia</i>	Pigeon
Anseriformes	<i>Anser sp.</i>	Goose
	Hybrid of Pekin ( <i>Anas platyrhynchos domestica</i> ) and Muscovy duck ( <i>Cairina moschata</i> )	Mulard duck

### 2.2.6.6 Clinical Symptoms

BFDV infection has three clinical forms in birds such as per acute, acute and chronic. Sudden death without any symptom or mild symptom (feather dystrophy) occurs in per acute and acute forms, respectively. These forms are common in juvenile birds. In chronic form of the infection, weight loss, lethargy, anaemia, diarrhoea, shedding of developing feathers, abnormal development of new feathers is observed (Figs. 2.14, 2.15 and 2.16). Mostly, contour, tail and down feathers are lost symmetrically and they are replaced with dystrophic feathers that fail to grow. Deformities of beak and claws are not a constant feature. It depends on species of the bird and other predisposing factors. Cockatoos are more susceptible to beak and claw deformities than other psittacine birds. Beak elongation, transverse or longitudinal fractures, palatine necrosis are possible beak deformities observed (Fig. 2.17). Chronic infection is not always fatal, the birds may survive for several years. Sometimes death occurs due to secondary infection.



**Fig. 2.14** Young Budgerigar affected with Beak and feather disease (*Courtesy* M. Scott Echols, Medical Center for Birds, California)



**Fig. 2.15** Parrot affected with Beak and feather disease (*Courtesy* Barun Dev Das, Animal Resources Development Department, Government of West Bengal, India)



**Fig. 2.16** Gouldian finches affected with Beak and feather disease (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)

#### 2.2.6.7 Lesion

Gross lesions in feathers of BFDV infected birds are retention of sheaths, fracture of the proximal rachis, haemorrhage in pulp cavity, short clubbed feathers, curled feathers and circumferential constrictions. In naturally infected cockatoos, vane of feather is ragged with multiple fractures. Hooklets, barbules and barbs are poorly developed and fractured (Fig. 2.18). Hyperkeratotic sheaths are found in affected feathers which results terminal clubbing and mid-shaft constriction. In beaks of infected birds, abnormal elongation, palantine necrosis, transverse to longitudinal fractures is detected.

Histopathological examinations revealed basophilic intranuclear and/or intracytoplasmic inclusion bodies in feather epithelial cells, follicular epidermal cells and macrophages.

#### 2.2.6.8 Diagnosis

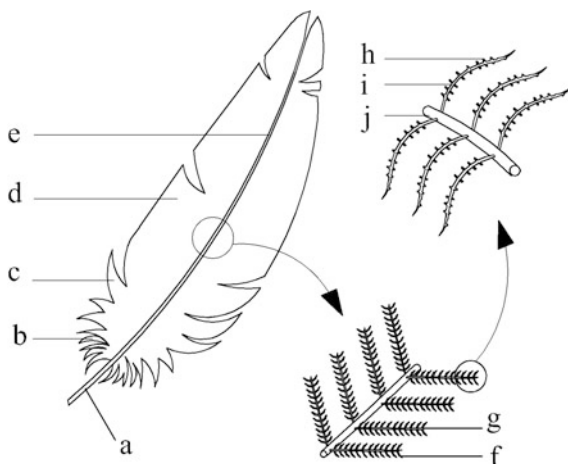
##### Clinical Specimens

Feathers (newly grown quill portion is the best specimen), blood or serum, cloacal swabs, pharyngeal swabs can be collected as clinical specimens from the suspected birds. Although, feathers produce low amount of viral DNA because most of the fully grown feathers are separated from the blood supply. The blood should be collected from vein (not toenail) to avoid environmental contamination of BFDV.



**Fig. 2.17** Fracture at tip of the beak (Courtesy LafeberVet)

**Fig. 2.18** Structure of a bird feather (schematic). *a* Shaft  
*b* barbs *c* afterfeather *d* vane  
*e* rachis *f* barb *g* barbules  
*h* hooklets *i* barbules *j* barb



## Diagnostic Techniques

- (a) *Clinical signs:* Progressive feather loss preliminarily suggests BFDV infection. Feather loss is also associated with Polyomavirus infection, trauma, bacterial folliculitis, malnutrition, endocrine abnormalities, and adverse drug reactions to penicillins and cephalosporins. Specific laboratory tests should be performed to confirm the BFDV infection.



- (b) *Histopathology*: Histopathology with light or electron microscopy is a reliable and frequently used technique for confirmation of BFD. Presence of intranuclear or intracytoplasmic or both type of inclusion body acts as primary indicator of BFDV infection. The virus is confirmed by electron microscopy or immunohistochemistry.
- (c) *Serology*: Haemagglutination inhibition (HI) and blocking ELISA are developed for detection of Avian Circovirus antibodies. The serological tests cannot confirm the active BFDV infection as the antibodies might be present due to latent infection. Agglutination of red blood cells by BFDV varies with the source. Red blood cells collected from South American birds (Amazon, Macaw) are generally not agglutinated with BFDV.
- (d) *Molecular biology*: PCR is one of the sensitive tools for rapid detection of BFDV infection in pet birds which should be coupled with clinical signs, lesion and histopathology. Conventional PCR targeting ORF C1 or ORF V1 section of the viral genome, nested-PCR, duplex shuttle PCR, and real-time PCR are developed for detection of BFDV. Whole genome sequencing is a recent progress for diagnosis of BFDV which is a cost effective, rapid and sensitive technique due to small genome size of the virus.

#### 2.2.6.9 Zoonosis

Zoonotic potentiality of BFDV is not established.

#### 2.2.6.10 Treatment and Control Strategy

Currently there is no known treatment for BFDV infection. Secondary infection should be diagnosed and treated properly. Avian gamma interferon injection (intramuscular) along with quaternary ammonium compound (as nebulizer) has shown success in treatment of BFD.

No vaccine is commercially available to control BFDV infection. Studies revealed that maternal antibodies against BFDV can protect the young birds. Surviving birds sometimes develop long lasting immunity. The only way to control the disease is through maintenance of hygiene, strict isolation or culling of infected birds.

### 2.2.7 Other Viral Infection

#### 2.2.7.1 Psittacid Herpesvirus Infection (Pacheco's Disease)

Pacheco and Bier (1930), a veterinarian from Brazil first described an outbreak of acute, fatal hepatitis in psittacine birds. This syndrome became known as 'Pacheco's disease'. Later in 1975, psittacid herpesvirus type 1 (PsHV-1) was confirmed as etiological agent. Psittacid herpesvirus type 1 (PsHV-1) is closely related with Gallid herpesvirus-1 (infectious laryngotracheitis of chicken). PsHV-1 has been classified into 4 genotypes (1–4) on the basis of variations in UL16 gene sequence. The genotypes of the virus have preference for different hosts (Table 2.10). Amazon parrots and conures (Patagonian conures) most commonly suffer with

**Table 2.10** Susceptible hosts of Psittacid herpesvirus-1 genotypes

Virus	Genotype	Susceptible hosts
Psittacid herpesvirus-1	1	Amazon parrot, cockatiels, cockatoos
	2	Amazon parrot, cockatiels, cockatoos, African grey parrots
	3	Amazon parrot, cockatiels, cockatoos, African grey parrots, macaws
	4	Amazon parrot, cockatiels, cockatoos, African grey parrots, macaws

Pacheco's disease. The infection is common in United States, United Kingdom, Spain, South Africa, Kenya and Japan.

PsHV-1 is transmitted by direct contact with the infected birds. Persistently infected birds can shed the virus through faeces and pharyngeal secretion. In most of the cases, the infected birds die suddenly without showing any syndrome. If the birds survive, non-specific clinical signs such as depression, anorexia, diarrhoea, tremor and instability are observed. Neurological disorder is followed by death. Gross lesions are not distinct. Intranuclear inclusion bodies (Cowdry type A) are observed in liver, kidney, spleen, pancreas and small intestine.

Cloacal swabs, pharyngeal swabs, faeces, newly emerged feathers (blood/pin feather) can be collected from live bird as clinical specimens. After post-mortem, liver, spleen, kidney, lung, cerebellum can be used for detection of the virus. Laboratory confirmation of PsHV-1 infection depends on isolation of virus in cell lines, demonstration of virus in clinical samples by electron microscopy, detection of viral DNA by PCR or real-time PCR. Presence of intranuclear inclusion body in tissues is inconclusive because many other viruses (*Avian Polyoma*, *Psittacine Adenovirus*) also produce the same.

Use of antiviral (Acyclovir, oral or intramuscular, intravenous injection) in early stage of infection can prevent the outbreak. An inactivated virus vaccine adjuvanted with oil is available for selected psittacine birds against Pacheco's disease. The vaccine is recommended to use subcutaneously in smaller psittacines and subcutaneously or intramuscularly in larger psittacines (more than 100 g body weight). Maintenance of strict hygiene, quarantine (30 days) of newly procured birds, and regular use of disinfectants in cages can prevent the PsHV-1 infection.

### 2.2.7.2 *Psittacine adenovirus* Infection (PsAdV)

*Psittacine adenovirus* (PsAdV) was first reported from Senegal parrots (*Poicephalus senegalus*) with acute infection. The virus was confirmed by amplification of hexon gene (L1 variable loop) by PCR (Raue et al. 2005). *Psittacine adenovirus* belongs to the family *Adenoviridae* and genus *Aviadenovirus*. *Adenovirus* is non-enveloped and has an icosahedral capsid with a diameter of 70 nm. The hexon protein is the major capsid protein and it has conserved pedestal regions (P1, P2) and the variable loops (L1–L4). *Adenovirus* infection is reported from pet birds such as budgerigars, macaws, Amazon parrots and cockatoos.

Aviadenoviruses are present in faeces, urine, tracheal and nasal secretions of infected birds. The virus is readily transmitted by horizontal mode. Direct faecal contact and aerial spread are major ways of horizontal transmission. Fomites, personnels and transport also contribute in horizontal transmission of *Aviadenovirus*. In most of the cases, the infected birds die suddenly without showing any syndrome. If the birds survive, non-specific clinical signs such as depression, anorexia, diarrhoea, ruffled feathers are observed. Gross lesions include hepatomegaly, splenomegaly, nephromegaly, dilatation of duodenum and , and congestion of lungs. The livers become enlarged, friable, haemorrhagic, pale or mottled. Basophilic intranuclear inclusion bodies are observed.

Faeces from live birds and organs (kidney, liver, intestine) after post mortem are collected from the suspected birds for confirmatory diagnosis. PsAdV can be isolated in primary chicken embryo kidney (CEK) or chicken embryo liver (CEL) cell lines. Embryonated chicken eggs can be inoculated by yolk sac, chorioallantoic membrane and allantoic cavity route. The eggs should be free of pathogen (SPF) and antibodies against Aviadenovirus. Replication of the virus can be confirmed by death of embryos and gross microscopic lesions observed in hepatocytes. Psittacine embryonated eggs are very expensive and are not readily available for diagnostic purpose. Other diagnostic methods for detection of PsAdV in clinical samples are electron microscopy due to typical morphology of the virus and demonstration of basophilic or eosinophilic, intranuclear inclusion bodies in liver and intestinal epithelium by haematoxylin and eosin staining. PsAdV in clinical samples can be confirmed by PCR.

No specific treatment for PsAdV infection is documented. Secondary bacterial infection can be treated by broad spectrum antibiotics. Aviadenoviruses are extremely resistant to the environment (heat, pH 3–9) and common disinfectants (ether, chloroform, trypsin, 50% alcohol) and they can persist for prolonged period in the cages or aviaries. Treatment with formalin, aldehydes and iodophors for more than 1 h can inactivate the virus.

### 2.2.7.3 Psittacine Poxvirus Infection

Poxvirus infection was reported for first time from lovebirds (*Agapornis personata*, *A. roseicollis*) in Germany (Kraft and Teufel 1971). Psittacine poxvirus belongs to the genus *Avipoxvirus*, family *Poxviridae* and subfamily *Chordopoxvirinae*. The viral genome consists of double stranded DNA (230–300 kbp). Several psittacine and passerine birds, specially Amazon and pious parrots, lovebirds and canaries are susceptible to Psittacine Poxvirus infection. Transmission of Poxvirus from infected owners or caretakers is possible into the birds having skin injuries. Mechanical transmission is also possible by mosquitoes and mites. In a contaminated cage or aviary, infected aerosols generated from dried scabs and feathers may act as source of infection.

The infection in psittacine birds is characterized by ocular discharge, rhinitis, conjunctivitis and ulcerations on the eyelid. Typical crusty lesions develop on the eyelid margins, lateral and medial canthi of the eyes and occasionally on face and feet (Figs. 2.19 and 2.20). Persistent cutaneous lesion for a period of 13 months is





**Fig. 2.19** Conjunctivitis and scabby lesion in a canary infected with Poxvirus (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)

**Fig. 2.20** Poxvirus lesions in a pigeon (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)



detected in yellow-shafted flicker. Diphtheretic lesions develop in some birds which is associated with dyspnea and higher mortality rate. Diphtheritic form produces necrotic lesions in the trachea, larynx and oral cavity. Gross lesions include necrosis of heart, liver, air sac, lungs, peritoneum, and accumulation of necrotic debris on the surface of the alimentary tract. Intracytoplasmic inclusion bodies (Bollinger bodies) are observed in the mucosa of sinus, trachea, crop, esophagus and throat.

Presence of typical cutaneous lesions primarily suggests about Poxvirus infection. Vesicular fluid from cutaneous lesions, faeces and pharyngeal swabs can be collected as clinical specimens. The infection is confirmed by electron microscopy, detection of Bollinger body in tissue samples and Psittacine Poxvirus specific-PCR.

No specific treatment and vaccine is available to control Psittacine Poxvirus infection. Infected birds should be separated from the healthy group. Cages, fomites and utensils should be properly disinfected because the virus persists for prolonged period in the dried scabs and the aerosols generated from the infected scabs.

#### 2.2.7.4 Avian Polyoma Virus Infection

Among the pet birds, *Polyomavirus* was detected for first time in young budgerigars (*Melopsittacus undulatus*) in 1980. The virus was named as budgerigar fledgling disease polyomavirus which was renamed later as Avian Polyomavirus. The virus belongs to the family *Papovaviridae*. The virion is icosahedral, non-enveloped with a diameter of 45–50 nm. The viral genome is a circular double-stranded DNA and



**Fig. 2.21** Filoplume feather (schematic)



**Fig. 2.22** Feather disorders in society finches due to Avian Polyomavirus infection (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)

**Table 2.11** Age related syndrome of Avian Polyoma virus infection

Age of the bird	Clinical symptom
3–6 weeks	Death without symptom
5–16 weeks	Depression, anorexia, crop stasis, regurgitation and ecchymosis in subcutaneous tissues
16 and 21 weeks	Feather deformities followed by death
More than 24 weeks	Viraemia without clinical symptoms

has two regions-early and late. The early region encodes tumour protein and the late region encodes four structural proteins (VP1, VP2, VP3, VP4).

Other than budgerigars, lovebirds, canaries, finches, macaws, eclectus parrots, conures, cockatoos and Indian ringneck parakeets are also susceptible to Avian Polyoma virus infection. The infection is common in Canada, China, Australia, Germany, Slovakia and Italy. Direct contact with infected birds is the major way of transmission. Contaminated cages, fomites, utensils, nestboxes, egg incubator may also act as source of infection as the virus is highly stable in the environment.

The infection is more fatal in young birds of less than 16 weeks age. In fledgling and young budgerigars, death without symptom, or brief illness showing feather dystrophy, loss of down feathers, presence of ‘filoplumes’ (feather like projection with a thin rachis and few barbs, Figs. 2.21 and 2.22) on head and neck, abdominal distension followed by death, are observed. It is known as ‘French molt’ or ‘Budgerigar and fledgling disease’. In cockatoos, two clinical forms i.e. acute death with haemorrhages in the feather shaft and pneumonia with gasping (in young



**Fig. 2.23** Subcutaneous haemorrhages in African grey parrot due to Avian Polyomavirus infection (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)

birds) are detected. In other susceptible birds age related syndrome is prevalent (Table 2.11). Gross lesions include distension of heart with hydropericardium, swollen liver, congested kidneys, and hemorrhage in the body cavity (Fig. 2.23). Intranuclear and basophilic inclusion bodies are detected in spleen, liver and kidney.

Blood, cloacal swabs from live animals and tissues from spleen, liver and kidney can be collected as clinical specimens. The presence of Avian Polyomavirus in the clinical samples is confirmed by electron microscopy, virus neutralization test, immunofluorescent antibody staining, in situ hybridization, PCR and real-time PCR.

No specific treatment for Avian Polyomavirus is documented for pet birds. An inactivated and oil adjuvanted vaccine is commercially available. A dose of 0.25 ml is recommended for birds below 200 g of body weight and larger dose (0.5 ml) is given to the birds with more than 200 g of body weight. Primary vaccination is done at 5 weeks of age which should be followed by a booster after 2–3 weeks. Annual vaccination is recommended. Sometimes thickened skin at the vaccination site is observed as adverse reaction.

### 2.2.7.5 Psittacine Papillomavirus Infection

Papovavirus infection was detected in budgerigars (*Melopsittacus undulatus*) and splendid parakeets (*Neophema splendida*) which died suddenly without distinct clinical symptoms (Graham and Calnek 1987; Pass et al. 1987). Psittacine Papillomavirus belongs to *Papovaviridae* family. Pet birds such as Amazon parrots, African grey parrots, macaws, finches, budgerigars, canaries and parakeets are most susceptible to Psittacine Papillomavirus infection. Direct contact with infected bird is the major way of transmission. Introduction of infected bird into aviary or cage rapidly transmit Papillomavirus infection into other birds.

The infection is characterized by reddish cauliflower like growth (papilloma) in oral (larynx, crop and upper gastrointestinal tract) and cloacal mucosa. Presence of a large mass in larynx causes wheezing and change of voice. Papillomas in oral mucosa hinder swallowing and digestion which causes anorexia, chronic weight loss and vomiting. In cloacal papillomatosis, raised, coalescing mass appears at the cloaca (Fig. 2.24). Presence of fresh blood is noted in the droppings. Sometimes cloacal prolapse is detected. Gross lesion is characterized by proliferation of epithelial cells on thin fibrovascular stalks. Neoplastic growth of bile duct, pancreas and liver is also observed specially in Amazon parrots and macaws.

Presence of typical cauliflower like growth in cloaca or oral mucosa primarily suggests about Psittacine Papillomavirus infection. Tissue biopsy samples from



**Fig. 2.24** Cloacal papilloma in a blue and green macaw (Courtesy Kenneth R. Welle)





**Fig. 2.25** Collection of biopsy by gloved hand from cloacal papilloma in a macaw (Courtesy Ashley Zehnder)

cloaca can be collected by a sterile speculum, moistened cotton-tipped applicator or a gloved hand (Fig. 2.25). Histological examination of biopsy samples, in situ hybridization and PCR can confirm the infection.

Treatment of papillomas includes chemical cauterization with silver nitrate or surgical removal of the mass. In chemical cauterization, possibility of re-appearance of the papillomas is detected. Use of interferon (50,000 IU/kg, intramuscular) in some species of birds can prevent the recurrence of growth. In a few countries, autogenous vaccine is used to prevent the infection.

#### **2.2.7.6 Avian Reovirus Infection**

Among the members of family *Reoviridae*, *Orbivirus* mostly causes pet bird infection. Parrots, budgerigar, cockatiel, duck and American woodcock are susceptible to *Orbivirus* infection. Pheasants, pigeons and raptors mostly act as carriers. The carrier birds can shed the virus through faeces and contaminate the environment that can act as a source of infection. Biting insects sometimes also help in transmission of infection. In psittacine birds, conjunctivitis, swollen eyelids, enteritis, emaciation, incoordination and other neurological signs are observed. In budgerigars and cockatiels, sudden death without clinical symptom is detected. Stunted growth and feather deformity is observed in Muscovy ducks. Gross lesions include swollen liver, kidney and spleen with necrotic areas. Accumulation of fluid

in lungs and pericardium and myocarditis are often detected as gross lesions. Cloacal swabs and tissues from liver, spleen, and kidney can be collected as clinical specimens. The virus can be isolated in chick embryo liver cells or chick kidney cells. The virus is confirmed by electron microscopy, immunofluorescence staining and PCR.

2.2.7.7 Coronavirus Infection (Infectious Bronchitis)

Psittacine birds (budgerigar, Amazon parrot), pigeons, ostrich, rhea are sometimes infected with Infectious Bronchitis virus of *Coronaviridae* family. Respiratory signs, mucopharyngitis, ulcerated crop and esophagus, swollen kidney, egg peritonitis are commonly observed. In ostriches, thin walled and blood filled proventriculus is detected. Nasal swabs, pharyngeal swabs can be collected as clinical specimens in 50% glycerol. The virus can be isolated in chicken embryo liver cells and primary embryo liver cells derived from blue and yellow macaw embryos. Presence of virus can be confirmed by electron microscopy and pan-coronavirus reverse transcriptase-PCR.

2.3 Parasitic Diseases

2.3.1 Toxoplasmosis

2.3.1.1 History

Splendore (1913, Brazil) first observed *Toxoplasma* like organisms in blood smear prepared from an infected rabbit. In the same year, Nicole and Manceaux detected the same organisms at Gondi (Tunisia) and described them as *Toxoplasma gondii*. In pet birds (pigeons), Carini (1911) first observed *Toxoplasma* like parasites in smears prepared from liver and spleen in São Paulo, Brazil. Based on phenotypical detection in smears prepared from blood and tissues, *Toxoplasma* was described subsequently in different species of birds. Catar (1974) detected the organisms in mistle thrush (*Turdus viscivorus*), song thrush (*Turdus philomelos*), robin

**Table 2.12** *Toxoplasma gondii* genotypes common in animals, birds and human

Conventional genotype	ToxoDB PCR-RFLP genotypes
Type I	#10
Type II	#1
Type III	#3
Type 12 (atypical)	#4
Type BrI (atypical)	#6
Type BrII (atypical)	#11
Type BrIII (atypical)	#8
Type BrIV (atypical)	#17
Chinese 1 (atypical)	#9

(*Erithacus rubecula*), house sparrow (*Passer domesticus*) and pheasants (*Phasianus colchicus*) in Slovakia.

### 2.3.1.2 Etiology

*Toxoplasma gondii* is an obligate intracellular protozoa and a member of suborder *Eimeriina*, phylum Apicomplexa. Feral and domestic cats (*Felidae* family) are the definitive host of the parasite. It has a wide range of intermediate hosts including different species of birds. *T. gondii* has three conventional clonal lineages (type I, II, III) which has different host preference and virulence pattern. Most of the animal strains belong to type III, whereas, type I and II strains commonly infect human. Occasionally, all the typical genotypes (I, II and III) and their variants are isolated from birds such as free-range chickens, raptors etc. Progress in this area identified several other genotypes ('atypical') of *T. gondii* such as Type BrI, BrII, BrIII, BrIV, Type 12, Africa 1 and Chinese 1. A classification scheme is adopted to designate each genotype as 'Toxo DB-PCR-RFLP genotype' followed by a specific number (Table 2.12). A total of 189 ToxoDB PCR-RFLP genotypes is identified so far (2012).

### 2.3.1.3 Host Susceptibility

Clinical toxoplasmosis is detected in birds belonged to *Passeriformes*, *Psittaciformes*, *Columbiformes*, *Strigiformes*, *Galliformes* and *Anseriformes* orders (Table 2.13). Among the pet birds, passerines (canaries, finches, mynahs), pigeons and partridges are commonly infected with *Toxoplasma*. Clinical toxoplasmosis in pet birds is reported from different countries of Europe, North America, South America and Oceania (Table 2.13).

Apparently healthy wild and domestic birds belonged to different families (*Accipitriformes*, *Anseriformes*, *Galliformes*, *Gruiformes*, *Charadriiformes*, *Columbiformes*, *Strigiformes*, *Passeriformes*) can also harbour *T. gondii* without showing clinical symptoms. Examples of carrier birds include goshawk (*Accipiter gentilis*), common buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), pallid harrier (*Circus macrourus*), black vulture (*Aegypius monachus*), red-tailed hawk (*Buteo jamaicensis*), pheasant (*Phasianus colchicus*), turkey (*Meleagris gallopavo*), mallard duck (*Anas platyrhynchos*), pintail duck (*Anas acuta*), coot (*Fulica atra*), blackheaded gull (*Larus ridibundus*), common tern (*Sterna hirundo*), collared dove (*Streptopelia decaocto*), woodpigeon (*Columba palumbus*), common pigeon (*Columba livia*), ferruginous pygmy owl (*Glaucidium brasilianum*), little owl (*Athene noctua*), chaffinch (*Fringilla coelebs*), house sparrow (*Passer domesticus*), tree sparrow (*Passer montanus*), yellowhammer (*Emberiza citrinella*), starling (*Sturnus vulgaris*), black bird (*Turdus merula*), mistle thrush (*Turdus viscivorus*), song thrush (*Turdus philomelos*), robin (*Erithacus rubecula*), great tit (*Parus major*), treecreeper (*Certhia familiaris*), jackdaw (*Corvus monedula*), rook (*Corvus frugilegus*).



**Table 2.13** Clinical toxoplasmosis in pet birds in different countries

Order	Birds	Country/continent	Gross lesions
<i>Passeriformes</i>	Canary ( <i>Serinus canarius</i> ), greenfinch ( <i>Carduelis chloris</i> ), goldfinches ( <i>C. carduelis</i> ), siskins ( <i>C. spinus</i> ), bullfinches ( <i>Pyrrhula pyrrhula</i> ), linnets ( <i>C. cannabina</i> )	United States, United Kingdom, Uruguay, Australia, Italy, New Zealand	Non-suppurative inflammation of optic nerve, uveitis, choroiditis, focal necrosis and detachment of retina, atrophy of periocular tissue
	Mynah ( <i>Acridotheres</i> spp.), gold crested mynah ( <i>Ampeliceps coronatus</i> )	United States (Indiana), Netherlands	–
	Hawaiian crow ( <i>Corvus hawaiiensis</i> )	Hawaii, United states	–
	Satin bowerbird ( <i>Ptilonorhynchus violaceus</i> ), bowerbird ( <i>Sericulus chrysocephalus</i> ), red-whiskered bulbul ( <i>Pycnonotus jocosus</i> )	Australia	–
<i>Psittaciformes</i>	Budgerigars ( <i>Melopsittacus undulatus</i> )	Switzerland	Enlarged spleen, necrotizing myocarditis, hepatitis, intestinal pneumonia are observed in naturally infected budgerigars. Hyperemia and hemorrhagic to fibrino-necrotic enteritis, multifocal areas of necrosis in intestine and brain (with tachyzoites) are detected in experimentally inoculated budgerigars
	Regent parrot ( <i>Polytelis anthopeplus</i> ), superb parrot ( <i>P. swansonii</i> ), crimson rosella ( <i>Platycercus elegans</i> )	Australia	–
	Vinaceous Amazon parrot ( <i>Amazona vinacea</i> )	Brazil	Edema and congestion of lungs, cloudy air sacs, mild hepatomegaly, necrosis in myocardium
	Red lory ( <i>Eos bornea</i> ), Black-winged lory ( <i>Eos cyanogenia</i> )	United States	Enlarged spleen, necrotizing myocarditis, hepatitis, intestinal pneumonia
	Swainson's lorikeet ( <i>Trichoglossus moluccanus</i> )	Netherland	–

(continued)

**Table 2.13** (continued)

Order	Birds	Country/continent	Gross lesions
	North Island kaka ( <i>Nestor meridionalis</i> )	New Zealand	Hepatosplenomegaly and swollen lungs
<i>Columbiformes</i>	Rock dove ( <i>Columba livia</i> )	Brazil, United States	Mild encephalitis and neuritis
	Crown pigeons ( <i>Goura cristata</i> , <i>G. Victoria</i> , <i>G. scheepmaker</i> )	Belgium, Netherlands, United states	–
	Strait pigeons ( <i>Ducula spilorrhoa</i> ), Wonga pigeon ( <i>Leucosarcia melanoleuca</i> )	Australia	–
	Bleeding heart dove ( <i>Gallicolumba luzonica</i> )	Netherland	–
	Nicobar pigeons ( <i>Caloenas nicobarica</i> ), luzon bleeding-heart pigeons ( <i>Gallicolumba luzonica</i> ), orange-breasted green pigeon ( <i>Treron bicincta</i> )	United States	–
	Kereru ( <i>Hemiphaga novaeseelandiae</i> )	New Zealand	–
<i>Strigiformes</i>	Barred owl ( <i>Strix varia</i> )	Canada	Multifocal necrotic areas (1 mm diameter) containing numerous tachyzoites and surrounded by inflammatory cells
<i>Galliformes</i>	Domestic turkey	Germany	–
	Partridges ( <i>Perdix perdix</i> )	Czech Republic	–
	Erckel's francolin ( <i>Francolinus erckelii</i> )	Hawaii, United States	Focal discoloration of liver and heart, edematous lungs
<i>Anseriformes</i>	Hawaiian goose nene goslings ( <i>Nesochen sandicensis</i> )	Hawaii, United States	Edematous, consolidated lungs and necrosis in liver, brain, heart and muscles
<i>Apterygiformes</i>	North Island brown kiwi ( <i>Apteryx mantelli</i> )	New Zealand	Hepatosplenomegaly and swollen lungs

### 2.3.1.4 Transmission

*Toxoplasma gondii* has three stages in life cycle—oocyst (with sporozoites), tissue cyst with bradyzoites and rapidly multiplying form or tachyzoites. Oocysts are infective stage of the protozoa present in cat faeces (definitive host) . The infected cats can shed millions of oocysts within 3–10 days of infection, regardless of the presence of clinical signs. The oocysts are activated within 1–5 days after faecal excretion and can survive in soil and water for prolonged period (up to 1 year). The feral cats bury the faeces into soil but earthworms and other soil associated

insects bring them into the top layer of soil. The oocysts are ingested by intermediate hosts (birds, rodents, sheep, marine mammals and human) from the soil or water. The parasite invades tissues of intermediate hosts and produce bradyzoites within tissue cyst.

### 2.3.1.5 Pathogenesis

After ingestion of oocysts by the intermediate hosts, sporozoites prefer to invade most of the vital organs, with predilection for the reticuloendothelial and central nervous systems. The parasite enters the host cell by an active process. After intracellular multiplication, the progeny parasites go into the blood circulation by lysis of the infected cells and finally reach vital organs through the blood circulation. Onset of clinical symptoms depends on type of organs invaded by the parasite.

The parasitemia (presence of parasite in blood) declines after development of host immunity. The parasites localize and persist in the form of 'tissue cysts'. Most of these tissue cysts are benign in nature and do not produce any clinical symptom during persistence. In immunosuppression due to stress, concurrent viral infection or immunosuppressive therapy, the tissue cysts are ruptured. Granulomatous lesions develop with invading inflammatory cells into the surrounding tissues of the site where the cyst persisted.

### 2.3.1.6 Clinical Symptoms

In canaries infected with toxoplasmosis, weight loss, diarrhoea, dyspnoea, crusty exudates around eye lids, collapsed eyeballs, inflammation of choroid with or without retinal involvement, cataracts and blindness, head twitch, walking in circles (due to encephalitis) are commonly observed. Dull, visionless, closed eyes and encephalitis are consistent features in passerine birds (Fig. 2.26). In psittacine birds, non-specific clinical symptoms are observed. In lories, respiratory distress is distinct. In pigeons infected with *T. gondii*, anorexia, emaciation, high fever, weakness, conjunctivitis and convulsions are detected.



**Fig. 2.26** Finches with closed eye (Courtesy LafeberVet)

### 2.3.1.7 Lesion

In passerines (canaries), gross lesions include osseous replacement of eye globe, non-suppurative inflammation of optic nerve, anterior and posterior uveitis, swelling of the lenticular fibers in the lens, choroiditis, focal necrosis and detachment of retina and atrophy of periocular tissue (sunken appearance of eye). *T. gondii* tachyzoites are detected in choroid, retina (nerve fiber layer), vitreous and lens of the affected birds. Histological evidence of pneumonia and non-suppurative encephalitis associated with tissue cysts is also observed. Gross lesions observed in affected psittacine and other birds are enlisted in Table 2.13.

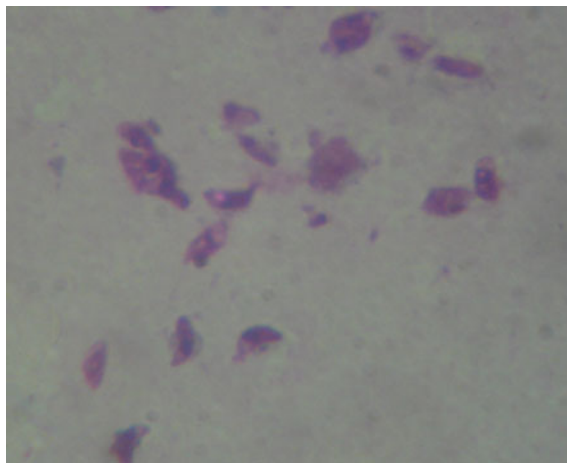
### 2.3.1.8 Diagnosis

#### Clinical Specimens

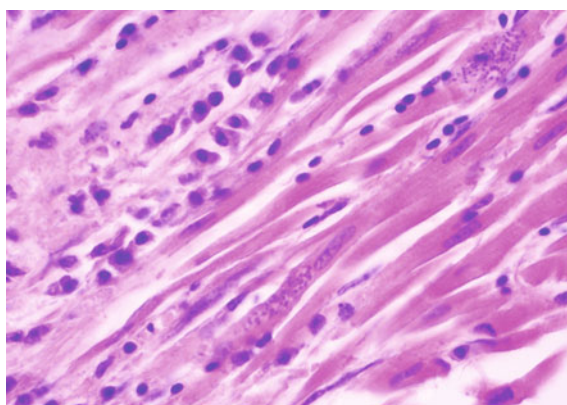
Blood, serum, eye suspensions from live birds and organs (spleen, liver, intestine, eye, brain, gizzard, proventriculus) collected after post mortem in buffered neutral 10% formalin can be used as clinical specimens. For bioassay (isolation), collected tissues are homogenized in 0.85% normal saline solution and brain tissue homogenates are digested with acidic pepsin before bioassay.

#### Diagnostic Techniques

- (a) *Direct examination*: Preliminary diagnosis is made by demonstration of *T. gondii* tachyzoites in Giemsa stained impression smears prepared from collected organs. In smears, tachyzoites are crescentic to globular in shape (Fig. 2.27).
- (b) *Histological examination*: In formalin fixed tissues, globular to oval shaped *T. gondii* tachyzoites are detected which are smaller in size than their appearance in impression smear (Fig. 2.28). *T. gondii* 'tissue cysts' appear as a globular structure with a thin cyst wall and small, slender bradyzoites are present within it (50–500). The bradyzoites can be visualized by periodic acid Schiff (PAS) staining. Immunohistochemical staining with polyclonal antibody raised against whole parasite can confirm the presence of *T. gondii* in formalin fixed tissues.
- (c) *Serological tests*: Modified agglutination test (MAT) is a sensitive, specific and easy to do serological test for detection of *T. gondii* antibodies in different species of birds. ELISA, indirect FAT in tissues can also be performed.
- (d) *Bioassay*: *T. gondii* can be isolated in laboratory mice by subcutaneous inoculation of eye suspensions or tissue homogenates (liver, brain) collected from suspected birds. In positive cases, inoculated mice will die and *T. gondii* tachyzoites or tissue cysts are detected from dead mice.
- (e) *Molecular biology*: The parasite can be confirmed by *T. gondii*-specific PCR. Nested PCR analysis based on *T. gondii* *pppk-dhps* gene is recently developed for confirmation. Genotyping of *T. gondii* depends on restriction fragment length polymorphism of surface antigen 2 gene (SAG2).



**Fig. 2.27** *Toxoplasma gondii* tachyzoites from infected mice and stained with Giemsa ( $\times 100$ , Courtesy Surajit Baidya, Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, India)



**Fig. 2.28** Necrosed myocardium of a Vinaceous Amazon parrot associated with intracellular tachyzoites and lymphocytes, macrophages and plasma cell infiltration ( $\times 40$ , H & E stain, Courtesy Prof. Roselene Ecco, Veterinary School, Universidade Federal de Minas Gerais, Brazil)

### 2.3.1.9 Zoonosis

Human get the infection by ingestion of vegetables, fruits and other foods or water contaminated with *T. gondii* oocysts. Direct transmission of *T. gondii* from pet birds to human is not documented. Feral or domestic cats may ingest the *T. gondii* infected carcasses of pet birds disposed into the surroundings without proper measures. The cats may become infected with toxoplasmosis and start to shed oocysts into the environment.

Person to person transmission is rarely possible during organ transplantation or blood transfusion. Most of the human infection is asymptomatic but sometimes cervical or occipital lymphadenopathy is observed. In immunosuppressed persons, encephalitis, chorioretinitis and pneumonitis are detected. In pregnant women, abortion and fetal infection causing congenital hydrocephalus, intracranial calcifications and mental retardation is documented.

### 2.3.1.10 Treatment and Control Strategy

Pyrimethamine (0.5 mg/kg body weight, orally, 12 h interval) is recommended for treatment of clinical toxoplasmosis in birds. Use of Tiamulin fumarate (300–400 mg/kg feed for 7 days in different species of birds; 225–250 mg/l drinking water for 3–7 days in pigeons and poultry) and clindamycin (25 mg/kg body weight, oral, 48 h interval) is also observed. In canaries, treatment with trimethoprim (80 mg/ml) and sulfadiazine (400 mg/ml) in drinking water for 14 days was apparently successful.

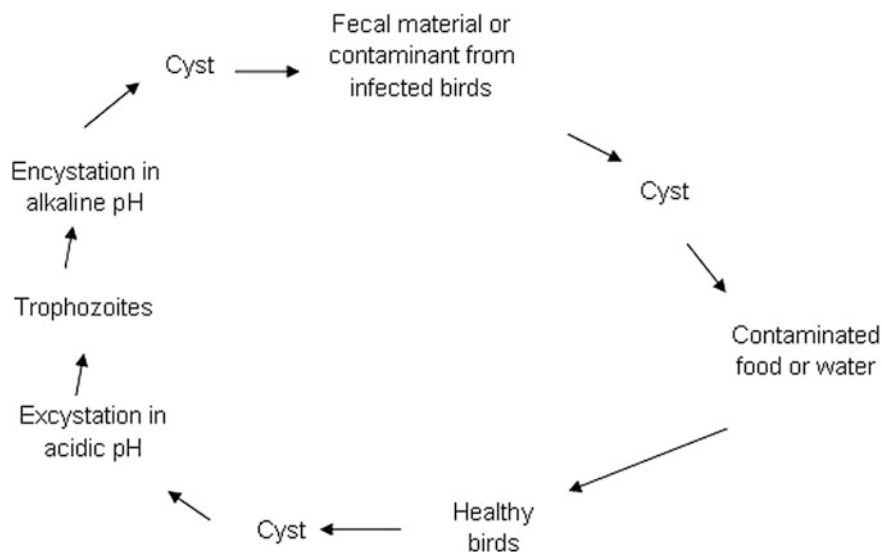
Prophylactic use of coccidiostats (monensin, decoquinate) is recommended in calves and lambs to prevent toxoplasmosis. Low acute toxicity of decoquinate is observed in avian species and no regulation or evidence is available regarding its prophylactic use. No vaccine is also available for birds to prevent toxoplasmosis.

## 2.3.2 Giardiasis

### 2.3.2.1 History

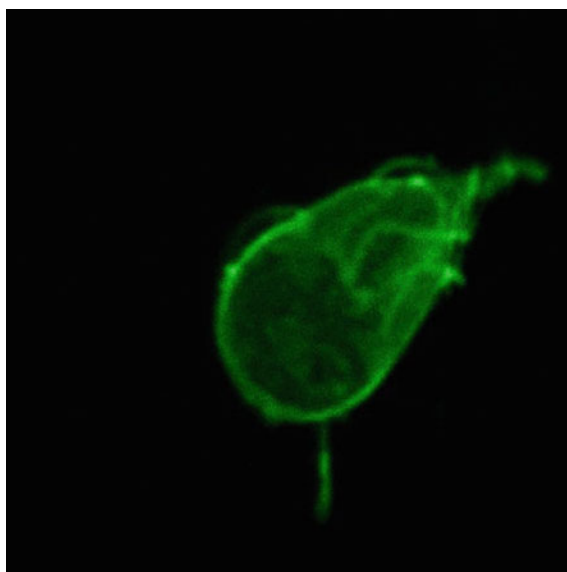
Antony van Leeuwenhoek (Delft, Netherlands, 1681) first observed *Giardia* under his self made simple microscope during investigation of his own diarrhoeic stool. He described it as ‘animalcule’ with ‘flattish belly’ and ‘sundry little paws’ (flagella). Under microscope, the organisms showed a slow and helical motion with occasional rapid movement by ‘paws’. Later (Dobell 1932) it was concluded as trophozoite stage of *Giardia* spp. In 1859, Vilem Dusan Lambl (Prague, Czech Republic) described the organism in more details and named it as *Cercomonas intestinalis*. In 1915, Charles Wardell Stiles coined the name *Giardia lamblia* in honour of Professor Alfred Mathieu Giard (Zoologist, France) and Dr. Vilem Dusan Lambl (Czech) for their contribution in progress of *Giardia* associated knowledge.

Leibovitz (1962) first described *Giardia* infection in a budgerigar in United States with a history of chronic diarrhoea and debility. In 1977, Jones and Carroll also observed presence of *Giardia* in the intestine of budgerigars in United Kingdom. In 1978, *Giardia* infection causing high mortality in parakeets was reported from United States which was successfully treated by dimetridazole (Panigrahy et al. 1978).



**Fig. 2.29** Life cycle of *Giardia* (schematic)

**Fig. 2.30** Trophozoites of *Giardia* under confocal microscopy using fluorescent tagged antibody (Courtesy Sandipan Ganguly, National Institute of Cholera and Enteric Diseases, Kolkata, India)



### 2.3.2.2 Etiology

Avian Giardiasis is caused by *Giardia* spp., an eukaryotic, multicellular, binucleate, flagellated protozoan belonged to Sarcomastigophora phylum. Six valid species of *Giardia* are recognized based on morphological observations using light and

electron microscopy. The valid species are *Giardia psittaci* and *G. ardeae* in birds; *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*) in human, livestock and wildlife; *G. microti* and *G. muris* in rodents; and *G. agilis* in amphibians. *G. duodenalis* has eight identified genotypes (assemblages, A–H). Assemblage A and B are common in both human and animals and assemblage C–H are restricted within animals only.

*Giardia* spp. has two stages in life cycle-motile ‘trophozoite’ and ‘cyst’ (Fig. 2.29). The cysts are smaller than trophozoites ( $10\ \mu\text{m} \times 8\ \mu\text{m}$ ), dormant, resistant to adverse environmental condition (like bacterial spore) and infectious form of the protozoa. They are common in streams, lakes and ponds. They can survive for months in cold water also ( $8\ ^\circ\text{C}$ ). After transmission of the cysts into the host, the cysts pass through acidic pH, increased  $\text{CO}_2$  level and slight alkaline pH (proximal small intestine) consecutively and excystation takes place. One trophozoite from each cyst emerges which undergoes cytoplasmic division to produce two trophozoites. Trophozoites are pear shaped (pyriform) structure which measures  $12\text{--}18\ \mu\text{m}$  in length,  $10\ \mu\text{m}$  in breadth and  $2\text{--}4\ \mu\text{m}$  in thickness (Fig. 2.30). The trophozoites have a concave disc with a raised ridge at the ventral surface of anterior site (broad portion) and eight flagella arranged bilaterally. The trophozoites attach with enterocytes at duodenum and jejunum with the ventral disc (‘sucker’) for feeding on mucosal secretions. The colonization is followed by binary fission.

Some trophozoites detach from the enterocytes and move forward (tumbling and skipping) with their flagella to re-attach with a new enterocyte. A few of the trophozoites instead of re-attachment prefer to be excreted through the faeces as cyst. During the process of encystment, the trophozoites stop their active motility, become rounded in shape and covered with a cyst wall. Nuclear division takes place and a quadrinucleate, matured cyst is excreted into the environment.

### 2.3.2.3 Host Susceptibility

*Giardia* is identified in faecal samples of more than 20 species of birds specially in psittacines. *Giardia psittaci* associated clinical infection is fatal in young budgerigars. Avian giardiasis is also reported in cockatiels, lovebirds, finches, great blue herons, raptors and gray-cheeked parakeets throughout the world. Some psittacines such as blue-fronted Amazon (*Amazona aestiva*), blue and yellow macaw (*Ara ararauna*), scarlet macaw (*Ara macao*) act as carrier (transport host) of *Giardia duodenalis* (assemblage A) without showing clinical symptoms.

### 2.3.2.4 Transmission

The infection is transmitted by faeco-oral route through ingestion of the food or water contaminated with infective cysts. Feeding or watering trough, cage materials, toys or other inanimate objects contaminated or soiled with faeces of the infected birds (captive or wild) may serve as a source of infection. Asymptomatic birds generally shed the cyst intermittently and thus serve as a potential source of infection.



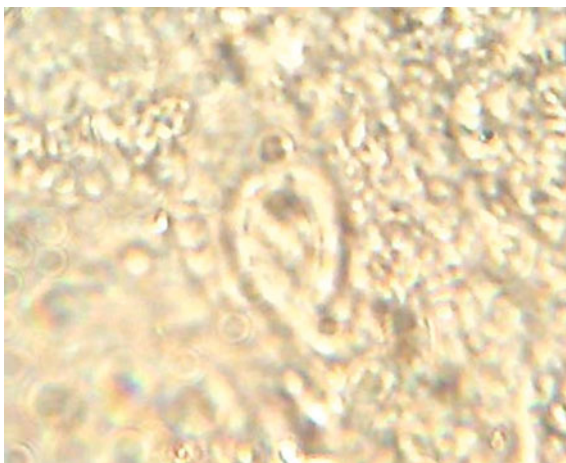
### 2.3.2.5 Pathogenesis

*Giardia psittaci* mostly reside in the duodenum with manifestation of diarrhoea and malabsorption syndrome in birds. The trophozoites adhere to the intestinal villi with ventral suckers. The adherence results inflammatory cell infiltration, villous atrophy, reduced villous to crypt ratio and reduction in disaccharidase enzymes (e.g. lactase). Food absorption is hampered and the food particles are accumulated in the lumen which increases the osmotic pressure and causes diarrhoea. With inhibition of food absorption, chronic weight loss may be noticed in affected birds. There may be deficiency of vitamins and minerals due to absorption failure. Unlike mammals where immunity has a direct relation with occurrence of giardiasis, avian immunity rarely shows such association. Some risk factors are also noticed in birds for giardiasis such as overcrowding, unhygienic cage condition and inadequate nutrition. The studies revealed that giardiasis may be more common among heavily inbred population of birds.

### 2.3.2.6 Clinical Symptoms

Mucoid and persistent diarrhoea with loose, brown or pale coloured and foul smelling faeces, anorexia, depression, hypoproteinemia, weight loss, ruffled feathers are common clinical symptoms in birds infected with *Giardia* spp. Stunted growth and high mortality are observed in young budgerigars and cockatiels. In cockatiels, feather picking and pruritus are also detected. Feather picking from wings, flanks and legs along with screaming is common. Feather damage is sometimes observed in non-cockatiel birds also. Secondary bacterial, viral or yeast infection are identified in birds with avian giardiasis. Concurrent infection of *G. psittaci* and *Polyomavirus*, *Cryptosporidium*, *Chlamydia* and *Macrorhabdus* spp. (Megabacterium) are observed in budgerigars.

**Fig. 2.31** Trophozoites of *Giardia* present in stool smear under wet mount preparation (Courtesy Sandipan Ganguly, National Institute of Cholera and Enteric Diseases, Kolkata, India)



### 2.3.2.7 Lesion

No gross lesion or sometimes distended small intestine with mucous, yellowish or creamy fluids is detected in most species of the birds. Atrophy of villous, infiltration of inflammatory cells, hyperemia of intestinal mucosal layer and presence of numerous trophozoites throughout the entire length of villi are observed.

In cockatiels, thickened skin, haemorrhage and areas of feather loss are observed in patagium (membranous structure that helps in flight) and axillary area. It may progress to squamous cell carcinoma.

### 2.3.2.8 Diagnosis

#### Clinical Specimens

Fresh faeces collected in saline from the suspected birds, blood or serum, intestine (duodenum) in 10% formalin can be used as clinical specimen.

#### Diagnostic Techniques

- (a) *Direct examination*: Wet mount examination of collected faecal sample (mixed with warm saline, not tap water) or the content of duodenum can be performed for direct visualization of motile trophozoites (pear shaped) or cysts (Fig. 2.31). The faecal sample can be concentrated with formalin-ethyl acetate or SAF (sodium acetate, acetic acid, formaldehyde) and zinc sulphate. Multiple fresh faecal samples (three consecutive samples) should be tested from a single suspected bird due to *intermittent shedding* of trophozoites and cysts. If the faecal sample is more than 10 min old, possibility of trophozoite visualization is low. If immediate processing is not possible, faecal samples may be preserved in polyvinyl alcohol for trichrome staining. The trophozoites are also destroyed in salt or sugar flotation solutions for faecal sample observation. Faecal sample can be stained with Lugol's iodine for visualization of *Giardia* cysts.
- (b) *Molecular biology*: For extraction of DNA from *Giardia*, oocysts present in faecal samples are concentrated. Faecal suspension prepared with sterile distilled water is kept over sucrose solution (1 M) and is centrifuged at 400 g for 15 min at room temperature. The water-sucrose interface is removed with a Pasteur pipette, washed in normal saline and centrifuged at 600 g for 10 min. DNA is extracted from the sediment by a standard nucleic acid extraction kit. PCR targeting  $\beta$ -*giardin* gene, *Giardia* elongation factor 1 alpha gene (*ef1 $\alpha$* ) or *Giardia* glutamate dehydrogenase gene (*gdh*) can be performed for confirmation.
- (c) *Antigen detection tests*: ELISA (antigen capture) and immunofluorescence tests are available for detection of *Giardia* antigen in clinical samples.

### 2.3.2.9 Zoonosis

Human giardiasis is caused by *G. duodenalis* and it has two phases i.e. acute and chronic. Flatulence, belching, abdominal distension with cramps, frequent watery diarrhoea with offensive smell occurs in acute phase. In chronic phase, malabsorption syndrome takes place with chronic weight loss. The stools are usually pale or yellow, frequent and of small volume. The prevalence of giardiasis in developing countries is approximately 20% compared to about 5% in the developed world. Transmission of *Giardia* to humans can occur through ingestion of food and water contaminated with infectious cysts. Waterborne transmission is associated with contaminated community water systems of municipality or corporation in urban area, and ponds, rivers and streams in rural area. Contaminated swimming pool also plays a role in transmission of giardiasis. Giardiasis is not specific for any human race and sex but it is more prevalent in children below 4 years of age.

The cysts are excreted in the environment through the faeces of infected human, animals and birds. The studies revealed that psittacine birds (blue fronted Amazon, blue and yellow macaw, scarlet macaw) can carry *Giardia duodenalis* (Assemblage A) cysts which may be disseminated into human.

### 2.3.2.10 Treatment and Control Strategy

Metronidazole (10–20 mg/kg body weight, oral, 12–24 h interval for two days for psittacines and pigeons) is the drug of choice in confirmed cases of avian giardiasis. Necrosis at site of injection in all species of birds and toxicity in finches are adverse drug reactions of metronidazole. Fenbendazole (50 mg/kg body weight, oral, 24 h interval for 3 days) is an alternative choice for effective treatment. Other drugs such as tinidazole (20 mg/25 ml of drinking water for 7–14 days) or paromomycin (100 mg/kg twice daily for 7 days) are also used successfully against avian giardiasis in budgerigars and barred parakeets. In budgerigars, amphotericin B and metronidazole combination is indicated for synergistic infection of *Giardia*, *Candida*, *Megabacterium*, *Trichomonas* and other bacterial infections.

With the discontinuation of therapy the infestation may relapse. It is necessary to properly rinse and dry the cage, feeding, watering and other in contact inanimate objects. Cleaning and drying reduce both the number of cysts and their viability and helps to prevent reinfestation. Feeding of boiled water in clean water bottles also helps to reduce the risk of infestation.

## 2.3.3 Cryptosporidiosis

### 2.3.3.1 History

Dr. Ernest Edward Tyzzer (1929, United States) first described cryptosporidiosis in bird (chicken) although *Cryptosporidium* species was not identified. It resembled *Cryptosporidium muris* as described in mice earlier by same worker (Tyzzer 1907, 1910). *C. meleagridis* was first identified in turkeys by Slavin (1955). In 1986, Current, Upton and Haynes isolated another species from chickens and coined the name *C. baileyi*.

### 2.3.3.2 Etiology

*Cryptosporidium* is a protozoon under phylum Apicomplexa, class Sporozoasida, order Eucoccidiorida, and family Cryptosporidiidae. Three *Cryptosporidium* species such as *C. meleagridis*, *C. baileyi*, and *C. galli* are commonly associated with avian infection. Recent molecular epidemiological studies have also identified several genotypes of *Cryptosporidium* causing infection in birds. The genotypes are: avian genotypes (I–V), goose genotypes (I–IV), black duck genotype, and Eurasian woodcock genotype. In addition, *C. hominis*, *C. parvum*, *C. serpentis*, *C. muris* and *C. andersoni* are detected in a number of birds due to accidental ingestion of Cryptosporidial oocysts. Among all these species and genotypes, *C. baileyi* is considered as the most common cause of avian infection.

*Cryptosporidium* has a direct life cycle which requires a single host. ‘Oocyst’ with four sporozoites is the infectious form and it is shed in faeces and cough of infected birds, animals and human. The oocyst can enter a new host and after excystation the sporozoites penetrate to epithelial cells of the gastrointestinal or respiratory tract. The sporozoites undergo six developmental stages such as merogony (asexual multiplication), gametogony (gamete formation), fertilization, oocyst formation, and sporogony (sporozoite formation). The sporozoites of *C. baileyi* produce 3 types of meronts (type I, II, III) during merogony to generate merozoites. Two types of oocysts (thin and thick walled) are observed in *C. baileyi* infection. Thin-walled oocysts are not excreted and they excyst in situ within the host to begin auto-infection. Whereas, thick walled (multilayered) oocysts are more resistant to environment and are excreted out to enter new hosts. Sometimes, auto-infection takes place after excretion and the excreted oocysts may infect the same host.

### 2.3.3.3 Host Susceptibility

*Cryptosporidium* is detected in more than 30 bird species (Anseriformes, Charadriiformes, Columbiformes, Galliformes, Passeriformes, Psittaciformes and Struthiniiformes) in different countries such as Australia, Argentina, Canada, China, Czech Republic, Denmark, Egypt, Germany, Greece, Hungary, Japan, Korea, The Netherlands, Romania, Scotland, Spain, South Africa, Taiwan, Turkey, and United States (Table 2.14).

### 2.3.3.4 Transmission

Avian cryptosporidiosis is transmitted by ingestion of contaminated food and water (faeco-oral) or inhalation of sporulated oocysts. Feeding or watering trough, cage materials, toys or other inanimate objects contaminated with faeces of the infected birds may serve as a source of infection. In the same aviary or premises, transmission is possible from one avian species to another through contaminated equipments or personnel. Once the oocyst enters the premises possibility of large scale outbreak increases in the aviaries or zoos.

**Table 2.14** Susceptible avian hosts of *Cryptosporidium* spp. in different geographical locations

Parasite	Susceptible host	Organs affected	Geographical location
<i>Cryptosporidium baileyi</i>	Black-headed gulls ( <i>Chroicocephalus ridibundus</i> )	Bursa of Fabricius, conjunctiva, kidney, respiratory tract, cloaca, rectum	Africa, Asia, Europe, North America, South America
	Great cormorant ( <i>Phalacrocorax carbo</i> )		
	Cranes ( <i>Gruidae</i> )		
	Channel-billed toucan ( <i>Ramphastos vitellinus</i> )		
	Eastern golden-backed weaver ( <i>Ploceus jacksoni</i> )		
	Cockatiels ( <i>Nymphicus hollandicus</i> )		
	Grey-bellied bulbul ( <i>Pycnonotus cyaniventris</i> )		
	Red-rumped cacique ( <i>Cacicus haemorrhous</i> )		
	Crested oropendola ( <i>Psarocolius decumanus</i> )		
	Red crowned amazon ( <i>Amazona viridigendilis</i> )		
	Rose-ringed parakeet ( <i>Psittacula krameri</i> )		
	Grey partridge ( <i>Perdix perdix</i> )		
	Mixed-bred falcons		
	Black vulture ( <i>Coragyps atratus</i> )		
	Saffron finch ( <i>Sicalis flaveola</i> )		
	Ruddy Shelducks ( <i>Tadorna ferruginea</i> )		
	Chickens ( <i>Gallus gallus domesticus</i> )		
	Turkey ( <i>Meleagris gallopavo</i> )		
	Brown quail ( <i>Coturnix ypsilophora</i> )		
	Duck ( <i>Anas</i> spp.)		
	Goose ( <i>Branta canadensis</i> )		
	Black-billed magpie ( <i>Pica pica</i> )		
	Bohemian waxwing ( <i>Bombycilla garrulus</i> )		
	Common myna ( <i>Acridotheres tristis</i> )		
	Crested Lark ( <i>Galerida cristata</i> )		
	Gouldian finch ( <i>Chloebia gouldiae</i> )		
	Red-billed leiothrix ( <i>Leiothrix lutea</i> )		
	White Java sparrow ( <i>Padra oryzivora</i> )		
	Zebra finch ( <i>Taeniopygia guttata</i> )		
	Ostriches ( <i>Struthio camelus</i> )		

(continued)

Table 2.14 (continued)

Parasite	Susceptible host	Organs affected	Geographical location
<i>Cryptosporidium meleagridis</i>	Parrots	Small intestine, large intestine	Africa, Asia, Europe, Oceania, North America, South America
	Cockatiel ( <i>Nymphicus hollandicus</i> )		
	Red-legged partridge ( <i>Alectoris rufa</i> )		
	Rose-ringed parakeet ( <i>Psittacula krameri</i> )		
	Chickens ( <i>Gallus gallus domesticus</i> )		
	Turkey ( <i>Meleagris gallopavo</i> )		
	Bohemian waxwing ( <i>Bombycilla anarctus</i> )		
	Fan-tailed pigeon ( <i>Columba livia</i> )		
	Rufous turtle dove ( <i>Streptopelia orientalis</i> )		
	Indian ring-necked parrot ( <i>Psittacula krameri</i> )		
	Vinaceous-breasted amazon parrot ( <i>Amazona vinacea</i> )		
	Plain parakeet ( <i>Brotogeris tirica</i> )		
	Chopt blackbird ( <i>Gnorimopsar chopi</i> )		
<i>Cryptosporidium galli</i>	Cockatiel ( <i>Nymphicus hollandicus</i> )	Proventriculus	Asia, Europe, Oceania, South America
	Green-winged saltator ( <i>Salpator similis</i> )		
	Double-collared seedeater ( <i>Sporophila caerulea</i> )		
	Atlantic Canary ( <i>Serinus canarius</i> )		
	Saffron finch ( <i>Sicalis flaveola</i> )		
	Rufous-collared sparrow ( <i>Zonotrichia capensis</i> )		
	Silver-eared Mesia ( <i>Leiothrix argentauris</i> )		
	Turquoise parrots ( <i>Neophema pulchella</i> )		
	Flamingo ( <i>Phoenicopterus ruber</i> )		
	Hornbill ( <i>Buceros rhinoceros</i> )		
	Canaries ( <i>Serinus canaria</i> )		
	Indian peafowl ( <i>Pavo cristatus</i> )		
Avian genotype I		-	Oceania, South America
(continued)			

Table 2.14 (continued)

Parasite	Susceptible host	Organs affected	Geographical location
Avian Genotype II	Cockatiel ( <i>Nymphicus hollandicus</i> )	Cloaca, rectum, bursa of Fabricius	Asia, Oceania, South America
	Major Mitchell cockatoo ( <i>Cacatua leadbeateri</i> )		
	Eclectus ( <i>Eclectus roratus</i> )		
	Galah ( <i>Eolophus roseicapilla</i> )		
	Sun conure ( <i>Aratinga solstitialis</i> )		
	Princess parrot ( <i>Polytelis alexandrae</i> )		
	Alexandrine parrot ( <i>Psittacula eupatria</i> )		
Avian Genotype III	White-eyed parakeet ( <i>Aratinga leucophthalma</i> )	Proventriculus	Asia, Oceania, North America, South America
	Cockatiel ( <i>Nymphicus hollandicus</i> )		
	Red-billed blue magpie ( <i>Urocissa erythrorhyncha</i> )		
	Peach-faced lovebird ( <i>Agapornis roseicollis</i> )		
	Galah ( <i>Eolophus roseicapilla</i> )		
	Sun conure ( <i>Aratinga solstitialis</i> )		
	Japanese white-eye ( <i>Zosterops japonicus</i> )		
Avian genotype IV	Blue-fronted amazon ( <i>Amazona aestiva</i> )	–	Europe
Avian Genotype V	Cockatiel ( <i>Nymphicus hollandicus</i> )	–	Asia, South America
	Mitchell's cockatoo ( <i>Lophochroa leadbeateri</i> )	–	–
	Anseriformes	–	North America
	Anseriformes	–	Oceania
	Charadriiformes	Proventriculus	Europe
	Galliformes	–	Europe
	Ostriches ( <i>Struthio camelus</i> )	–	Asia, Europe
<i>Cryptosporidium muris</i>	Cockatiel ( <i>Nymphicus hollandicus</i> )	Small intestine, caecum	Asia, Europe, North America, South America
<i>Cryptosporidium parvum</i>			(continued)

Table 2.14 (continued)

Parasite	Susceptible host	Organs affected	Geographical location
<i>Cryptosporidium</i> <i>blagburni</i>	Gouldian Finch ( <i>Erythrura gouldiae</i> )	—	
	Red-faced Auroora Finch ( <i>Pytilia hypogrammica</i> )		
	Plum-headed Finch ( <i>Aidemosyne modesta</i> )		
<i>Cryptosporidium</i> spp.	Budgerigar ( <i>Melopsittacus undulatus</i> ) Lovebirds ( <i>Agapornis</i> sp.)	—	



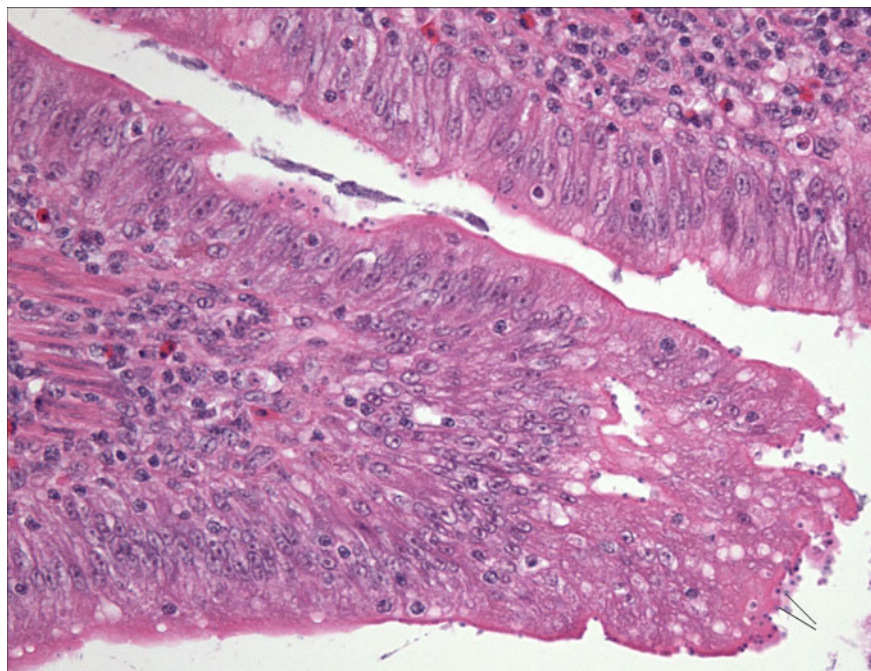
### 2.3.3.5 Pathogenesis

Following ingestion of oocysts by susceptible birds, it can enter salivary and esophageal glands, proventriculus, small intestine, caecum, colon, cloaca, and bursa of Fabricius and developmental stages of *Cryptosporidium* begins. The intracellular stages of the organism occur within a parasitophorous vacuole. Detachment of enterocytes and villous atrophy in small intestine are common lesions which hamper the absorption of nutrients and causes osmotic diarrhoea and malabsorption syndrome.

Following inhalation of oocysts, primary colonization in upper respiratory tract (sinus) is followed by colonization in lower respiratory tract of the birds (trachea, bronchi, air sacs, lungs). Mucoïd exudates are detected in sinus, nasal passage, and trachea. The air sacs and lungs become cloudy and mottled grey-red, respectively.

The site of predilection also varies with species of *Cryptosporidium* infecting the birds. *C. baileyi* prefers to invade mucosal epithelium of a number of organs (small and large intestines, caeca, cloaca, trachea, air sacs, urinary system, bursa and conjunctiva), whereas *C. meleagridis* and *C. galli* are restricted within the intestine and proventriculus, respectively (Table 2.14).

Concurrent viral, bacterial or parasitic infections are observed in birds suffering with both clinical forms of cryptosporidiosis due to bursitis and impairment of



**Fig. 2.32** Adherence of cryptosporidial oocysts with the intestinal epithelium, 40 $\times$ , H & E stain (Courtesy Prof. Oscar Fletcher, North Carolina State University, United States)

immunity. *Escherichia coli* and *Isospora* sp. infection are common with *C. galli* infestation.

### 2.3.3.6 Clinical Symptoms

Two clinical forms such as respiratory and gastrointestinal cryptosporidiosis are observed in birds. In respiratory form, increased mortality, depression, lethargy, anorexia, unthriftiness, coughing, sneezing, gurgling, dyspnoea, conjunctivitis and sinusitis are the common clinical symptoms. In gastrointestinal form, lethargy, decreased bodyweight gain, lower pigmentation, and diarrhoea with lime-green stool, chronic or intermittent regurgitation, lockjaw, aspiration pneumonia, seizure, egg binding are the common clinical observations.

*C. galli* infection in passerine birds is characterized by chronic apathy and weight loss. In Brazil in a cockatiel infected with *C. galli* lethargy for approximately 1 year before death was observed. Lethargy and slow crop emptying was also detected in an Indian ring-necked parrot infected with *C. meleagridis*. In peach-faced lovebirds infected with avian genotype III, chronic vomiting and weight loss was detected.

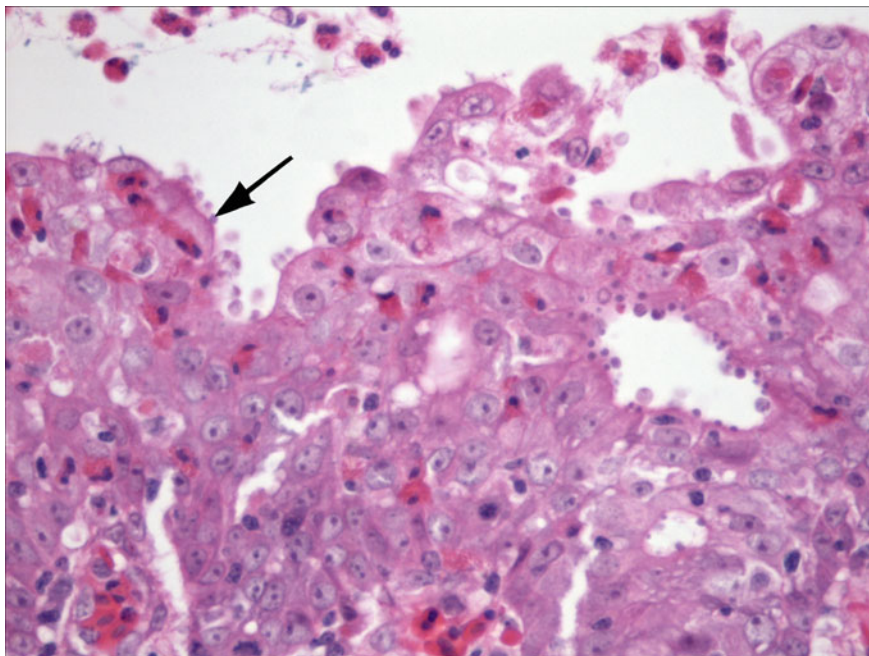
### 2.3.3.7 Lesion

Presence of too much mucoid exudates in conjunctival sacs, nasal passage, sinuses and trachea, chemosis, hyperemia, mucus gland distension or cystic hypertrophy/hyperplasia, mottled grey-red lungs, cloudy air sacs, bursal atrophy, hepatosplenomegaly are common gross lesions in respiratory form of cryptosporidiosis in birds.

In gastrointestinal form, distended intestine filled with mucoid contents and gas, detachment of enterocytes, villous atrophy, bursal epithelial cell hypertrophy and hyperplasia and necrosis in the bursa are common gross and microscopic lesions in birds. Histopathology demonstrates the presence of cryptosporidial oocysts adhered to the surface of intestinal brush border (Fig. 2.32). In finches, purulent nephritis with enlarged and pale kidneys is detected. Recently, visceral gout with renal and cloacal lesions is detected in Mitchell's cockatoo infected with *Cryptosporidium* sp.

Dilated proventriculus with thickened proventricular wall is common in lovebirds, cockatiels, red-faced Aurora finch, Australian diamond firetail finch and bronze mannikin finches. Histopathology demonstrates the presence of cryptosporidial oocysts adhered to the surface of proventricular glandular epithelial cells along with hyperplasia and necrosis of epithelial cells.

The organs affected by different *Cryptosporidium* spp. are enlisted in Table 2.14.



**Fig. 2.33** Histological section of trachea of a turkey showing cryptosporidial oocysts, 40 $\times$ , H & E stain (Courtesy Prof. Oscar Fletcher, North Carolina State University, United States)

### 2.3.3.8 Diagnosis

#### Clinical Specimens

Fresh faeces or cloacal swabs collected in potassium dichromate solution (2.5–5%) from the suspected birds, blood (collected from wing vein) or serum, and formalin fixed intestine, proventriculus can be used as clinical specimen. The cloacal swabs are filtered through wire mesh (0.3 mm) and the filtrate is centrifuged at room temperature at 1000 g for 10 min. After discarding the supernatant, the concentrated faecal sample is used for further analysis. The faecal samples can be preserved at 4 °C in phosphate buffered saline solution (pH 7.2) with antibiotics and antifungals (streptomycin @ 100 mg/ml, penicillin G @ 100 IU/ml and amphotericin B @ 0.25 mg/ml).

#### Diagnostic Techniques

- (a) *Direct examination:* Detection of cryptosporidial oocysts in clinical samples is the most common diagnostic technique used in laboratories. It is a low sensitive method which requires minimum 5000–50000 oocysts/g of faeces for detection. The oocysts are non-refractile, spherical (5–6  $\mu$ m in diameter) in shape and are often confused with yeast cells. Gram's stain, Kinyoun acid-fast

stains are used in light microscopy. In Kinyoun stained smear, oocysts appear as red spherical bodies against blue background whereas yeast cells take blue stain. Phase contrast microscopy provides better resolution and the oocysts appear as bright bodies with 1–4 dark granules (granules are absent in yeast cells).

Faecal samples can be concentrated by centrifugal flotation in high specific-gravity salt or sugar solutions (Sheather's sugar flotation technique, discontinuous density sucrose gradient) for detection of oocysts. The sample after concentration should be used rapidly because in presence of sugar solutions oocysts are distorted or collapsed.

In histologic sections stained with hematoxylin and eosin (H & E), cryptosporidia appear as basophilic bodies (Fig. 2.33).

- (b) *Molecular biology*: *Cryptosporidium* is detected by means of PCR, followed by either restriction fragment length polymorphism (RFLP) or sequencing of the amplified fragments. The gene commonly used for determining the species or genotype is 18S rRNA. Other target genes are actin, heat shock protein (HSP-70) and *Cryptosporidium* oocyst wall protein (COWP). Subtyping of *C. meleagridis* is based on 60-kDa glycoprotein (GP60) gene. Duplex-real-time PCR is recently developed for confirmation of *C. galli* and avian genotype III.
- (c) *Serological tests*: ELISA can be used for detection of anti-*Cryptosporidium* antibodies in serum samples.
- (d) *Antigen detection assays*: Capture enzyme-linked immunoassays (ELISA), direct fluorescent antibody (DFA) assay using commercially available antibodies are used for detection of *Cryptosporidium* antigen which is more sensitive and specific than staining. The detection of cryptosporidial species is not possible due to cross reactivity.

### 2.3.3.9 Zoonosis

Cryptosporidial oocysts are excreted in the faeces of infected birds and animals which can be transmitted to human by faeco-oral route. Human cryptosporidiosis is mostly caused by *C. hominis*, *C. parvum* and *C. meleagridis*. The pet birds and domestic chicken may act as source of *C. parvum* and *C. meleagridis* oocysts in the environment. Watery diarrhoea, abdominal cramping, and increased gas production are common clinical signs of *Cryptosporidium* infection in human. Severe complications such as pancreatitis, cholangitis, respiratory distress and death are observed in immunosuppressed individuals. Person to person transmission is possible.

### 2.3.3.10 Treatment and Control Strategy

Macrolide antibiotic (erythromycin) is used successfully against cryptosporidiosis in cliff swallow and owls. Azithromycin (40–45 mg/kg body weight, 24 h interval,

oral) is recommended for treatment of avian cryptosporidiosis in most of the bird species. United States Food and Drug Administration (FDA) have approved nitazoxanide for use in humans suffering with cryptosporidiosis.

The cryptosporidial oocysts are highly resistant to common disinfectants used in aviaries and environmental stress such as temperature variation, desiccation, humidity and change of pH. Maintenance of strict hygiene and biosecurity in the aviaries to reduce the possibility of oocyst contamination is the control strategy for avian cryptosporidiosis.

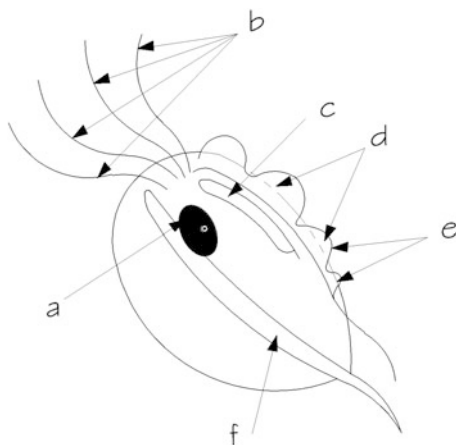
### 2.3.4 Other Parasitic Infections

Other parasites affecting pet birds include protozoa, helminths (nematodes, cestodes, trematodes), and arthropods. Captive birds kept in small and dirty cages are more susceptible to parasitic infestations. Capability of a parasite to survive and reproduce in pet birds ('parasite fitness') depends on host immunity and nutrients for parasite available in the host body. The parasites may avoid the birds with good health condition due to strong immune response, but the parasites also avoid the birds with very poor health due to less possibility to obtain nutrients. The balance between immunity and nutrient availability is crucial for selection of host by the parasites.

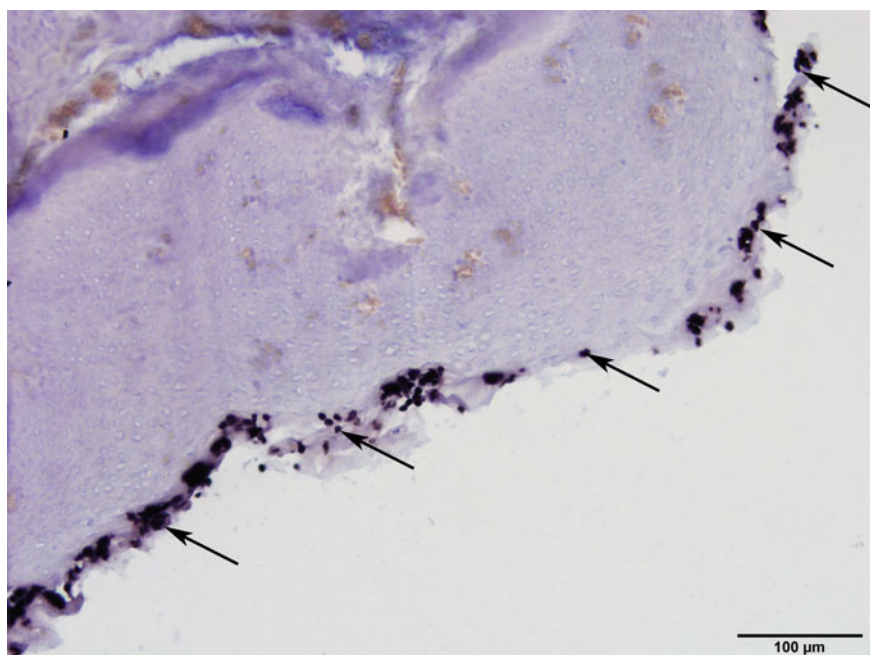
#### 2.3.4.1 *Sarcocystis falcatula* (Avian Sarcocysticosis, Sarcosporidiosis)

*Sarcocystis* belongs to the phylum Apicomplexa, suborder Eimeriorina and the family Sarcocystidae. It is a protozoon with two different hosts in their life cycle. Opossum (*Didelphis virginiana*) acts as definitive host in which infectious oocysts are generated and are excreted with faeces. Sporulated oocyst contains two sporocysts and each of them contains 4 sporozoites. Insects (flies, cockroaches), cowbirds (*Molothrus* sp.), and grackles (*Quiscalus* sp., *Hypopyrrhus* sp., *Lamprosar* sp., *Macroagelaius* sp.) act as natural intermediate hosts. Raptors (owls, eagles) may act as both definitive and intermediate hosts. The sporocysts after transmission into intermediate hosts (via ingestion) undergo schizogony or merogony and produce sarcocysts in the cardiac or striated muscles. When the definitive hosts further ingest the intermediate hosts with sarcocysts in the muscles, the life cycle is completed.

Cockatoos, cockatiels, grey parrots can get the protozoon by the ingestion of insects with sarcocysts. Three clinical forms such as acute pulmonary form, muscular form, and neurologic forms are observed in birds. In acute form, death without development of sarcocysts in the muscle occurs. In other two forms, clinical signs for instance dullness, weakness, respiratory signs, yellow urates are detected. Pulmonary hemorrhage and edema are the cause of death. Presence of sarcocysts in muscles of heart, breast, thigh, neck, and esophagus is the major lesion. Hepatosplenomegaly, lung consolidation, pulmonary edema are also observed.

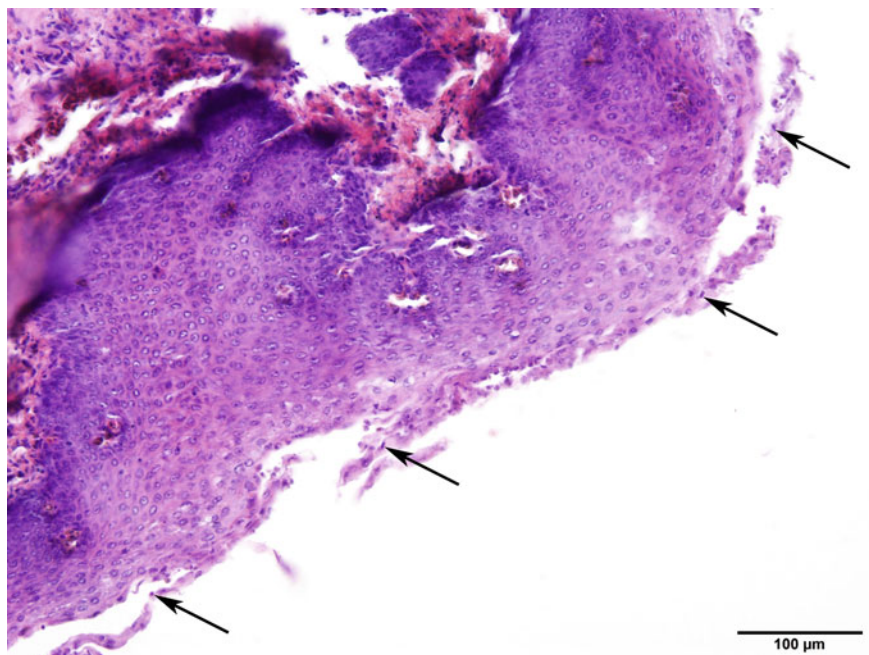


**Fig. 2.34** Morphology of *Trichomonas gallinae* (schematic). *a* Nucleus *b* anterior flagella *c* parabasal body *d* undulating membrane *e* posterior flagella *f* axostyle



**Fig. 2.35** Section of a pigeon crop infected with *Trichomonas gallinae* (Haematoxylin and eosin). Arrows indicate some of the parasites in the epithelium (Courtesy Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria)





**Fig. 2.36** In situ hybridization (ISH) confirmed the presence of *Trichomonas gallinae* in crop epithelium of a pigeon (Courtesy Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria)

Diagnosis is usually based on visualization of sarcocysts in the muscles after post-mortem. Ante-mortem diagnosis is possible by muscle biopsy, indirect immunofluorescent assay and PCR. Successful treatment of birds can be done with pyrimethamine (0.5 mg/kg body weight, 12 h interval for 14–28 days) and sulphadiazine-trimethoprim (30 mg/kg body weight, 8–12 h interval). Removal of insects from cages, maintenance of cleanliness, fencing of outdoor aviaries to prevent opossum access can prevent the occurrence of sarcocysticosis in birds. Avian sarcocystosis is usually not considered to be a public health hazard.

#### **2.3.4.2 *Trichomonas gallinae* (Avian Trichomoniasis)**

*Trichomonas* sp. is a motile pear shaped protozoon which contains a central, longitudinal rod up to the posterior end (axostyle) and four flagella attached to the anterior end. An undulating membrane extending from the anterior to posterior end is present (Fig. 2.34). The membrane encloses a flagellum which does not have any free end. *T. gallinae* has direct life cycle without any intermediate hosts or/and vectors. They multiply by longitudinal binary fission.

In budgerigars, finches, and cockatiels, transmission of *T. gallinae* occurs through ingestion of contaminated food and water. Young domestic pigeons

(squabs) get the infection through ingestion of contaminated 'pigeon milk'. Adult pigeons act as carrier of *T. gallinae*.

After entry through the oral route *T. gallinae* can invade the mucosal surface of the buccal cavity, sinuses, pharynx, esophagus, crop, proventriculus and conjunctiva (rare invasion) depending on the species of affected birds (Figs. 2.35 and 2.36). Clinical signs include weight loss, hypersalivation, vomiting, and diarrhoea. Death due to starvation occurs with progression of the infection. Gross lesion includes formation of diphtheritic membrane or white plaques on gastrointestinal mucosa (oropharynx to esophagus), blockage of esophagus lumen with cheesy material and ingluvitis (inflammation of crop). Solid, white to yellow circular masses appears in liver of affected pigeons (Fig. 2.37).

Buccal cavity and crop can be collected as clinical specimens after post-mortem of the bird. Wet mount prepared from fresh crop wash with saline can be observed under microscope for detection of typical pear shaped *Trichomonas* sp. In live birds, wet mount prepared from fresh faeces or crop swabs can be observed under microscope for detection of *Trichomonas* sp. However, diagnosis of avian trichomoniasis is difficult in ante-mortem samples because the parasite is unstable in the environment due to lack of cyst formation capacity.

Metronidazole (10–20 mg/kg body weight, 12–24 h interval, for 2 days), ronidazole (6–10 mg/kg body weight, 24 h interval, for 6–10 days), carnidazole (100–200 mg/kg body weight, oral, once), dimetridazole [1 tea spoonful/gallon



**Fig. 2.37** Oral fluke in barn owl (Courtesy Dr. M. Scott Echols, The Medical Center for Birds, California)



(4.5 L) drinking water] and ipronidazole (500 mg/gal drinking water for 7–30 days) are recommended for avian trichomoniasis.

#### 2.3.4.3 *Coccidia* (*Eimeria* spp., *Isospora* spp.)

*Eimeria* spp. and *Isospora* spp. belongs to phylum Apicomplexa and they have a direct life cycle without any intermediate host or vector. Sporulated oocysts are the infectious form and ingestion of food and water contaminated with oocysts is the major way of transmission. The oocyst wall is crushed in gizzard and the sporozoites are released. The sporozoites enter intestinal mucosa and undergo schizogony or merogony to produce progeny oocysts. The new oocysts leave intestinal mucosa and are excreted through the faeces. The direct life cycle requires 6–8 days time to complete.

Coccidiosis is common in mynahs, toucans, pigeons, lorries, finches, budgerigars and canaries. Clinical symptoms do not appear until the birds are immunosuppressed or infected with enormous numbers of coccidial oocysts. During schizogony in intestine, mucosa is damaged and enteritis is produced. Lethargy, weight loss, severe haemorrhagic diarrhoea is detected as clinical symptoms within 4–6 days after infection. Nonsporulated oocysts are shed with the faeces.

Diagnosis is made through examination of faeces and smears taken from the suspected lesion. The oocysts or macrogametes are detected in the smears in positive cases. The oocysts of *Eimeria* contain four sporocysts each with two sporozoites, whereas, two sporocysts each with four sporozoites are present in oocysts of *Isospora*.

Treatment with amprolium (50–100 mg/l drinking water for 5–7 days), trimethoprim and sulphamethoxazole (25 mg/kg body weight, 24 h interval, oral) are recommended for coccidiosis in pet birds. Amprolium produces toxicity in falcons (@22 mg/kg body weight, 24 h interval) and trimethoprim-sulphamethoxazole is recommended for toucans and mynahs.

#### 2.3.4.4 *Atoxoplasmosis*

Atoxoplasmosis is a dreadful disease caused by the protozoa *Atoxoplasma* sp. causing significant mortality among the fledgling birds. Although atoxoplasmosis is mostly asymptomatic among the adult birds, fatal infection with hepato-splenomegaly may be noticed among the young birds of canaries and Passeriformes.

The infected birds usually shed the oocysts via faeces and the healthy one picks up the infection by consuming the oocysts. Asymptomatic adults may also shed such oocysts and can be a potent but silent source of infection for the in-contact and prone young birds. Interestingly, the *Atoxoplasma* sp. from one host may not be infectious to other species of birds exhibiting certain degree of host specificity. Infected birds may periodically shed large number of oocysts. In general, the birds were detected to shed the oocysts up to eight months of infection. The oocysts are environmentally stable and can persist for along period.

Birds of less than 1 year of age, usually exhibit clinical symptom and adults are usually asymptomatic. Clinical findings are usually non-specific and include anorexia, depression, weight-loss, chronic diarrhoea, lethargy and depression. In few cases mortality may reach up to 80%. The enlarged liver, spleen and dilated

intestinal loops can be palpated in few cases. During the period of parasitaemia, the protozoa *A. serini* undergoes scizogony in the polymorphomononuclear cells (PMNs) and spread throughout the body circulation. Thereafter, the parasites reach out to their site of predilection reticuloendothelial cells (RE cells) of vital internal organs like liver, spleen, pancreas and intestinal epithelial cells. During this process of multiplication and propagation, the parasite causes heavy damage to these organs resulting in hepatosplenomegaly and enteritis in young birds.

When any adult bird is suspected as an asymptomatic carrier, microscopic examination of the fecal samples may confirm the presence of the oocysts ( $20.1 \times 19.2 \mu\text{m}$ ). In the height of severe infection, zoite form of the parasite may be detected in the lymphocytes following processing the buffy coat smear with Romanowsky stain. Reddish intracytoplasmic inclusion bodies are detected in the PMN cells when stained with Giemsa. Impression smear prepared from the enlarged liver, spleen or other affected organ may be helpful. PCR or nucleic acid detection method may give more confirmatory diagnosis.

There is no effective therapy available for the disease. The birds may remain infected and continues to show periodic clinical symptoms even after therapy up to 4 months. Primaquine has been recommended to suppress the tissue form of the parasite where as sulfachlorpyridazine can be used to decrease the faecal shedding of the oocysts. Generally, toltrazuril is employed @ 12.5 mg/kg/day, orally for 14 days and sulfachlorpyridazine @ of 150–300 mg in one liter of drinking water. This sulfachlorpyridazine should be given for 5 days in a week for at least 2–3 weeks.

#### 2.3.4.5 Ascaridiasis (Roundworm Infestation)

*Ascaridia hermaphrodita*, *A. columbae*, *A. nymphi* and *A. galli* have detected in hyacinth macaw, pigeon, Australian parrots, budgerigar, cockatiel, and princess parrot. In some ground feeding species of birds (e.g. dove), *Baylisascaris procyonis* (raccoon roundworm) and *Baylisascaris columnaris* (skunk roundworm) infestation is observed due to occasional consumption of food, water and faeces contaminated with *Baylisascaris* eggs. Ingestion is the major route of transmission in pet birds for other Ascarids also.

Clinical signs include lethargy, diarrhoea and death. Engorgement of duodenal loop with roundworms is major necropsy finding. In *Baylisascaris procyonis* infestation, considerable damage of central nervous system and subsequent ataxia, depression, head tilt, stumbling, recumbancy, torticollis, wing paralysis and death is common (Fig. 2.38).

Detection of thick shelled Ascarid eggs in faecal sample is the major diagnostic approach. During necropsy, presence of roundworm in the intestine and larval stage in tissue section can aid in diagnosis. Confirmation can be done by PCR targeting ribosomal DNA, internal spacers (ITS-1, ITS-2), and mitochondrial gene (mtDNA-cox2) of Ascarids.

Treatment with fenbendazole (20–100 mg/kg body weight, once) is recommended for ascaridiasis in most of the birds except finches, marabou storks and vultures due to acute toxicity. The drug is contraindicated during growth of feathers



**Fig. 2.38** Sternal recumbency of a lovebird (Courtesy LafeberVet)

also. Other common anthelmintics such as piperazine dihydrochloride and mebendazole are also contraindicated in psittacines, pigeons, cormorants, pelicans, raptors, finches and ostriches due to toxicity and reported death. Regular deworming of birds kept in crowded and unclean cages is needed to avoid ascaridiasis.

Other parasitic infections of pet birds are described in Table 2.15 (Figs. 2.39, 2.40, 2.41, 2.42, 2.43 and 2.44).

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## 2.4 Fungal Diseases

### 2.4.1 Cryptococcosis

#### 2.4.1.1 History

*Cryptococcus* was first isolated from peach juice in Italy and it was named as *Saccharomyces neoformans* (Sanfelice 1894) and subsequently, it was also isolated from a German patient (Busse 1894). Two years later, an encapsulated bacilliform yeast, which was named *Saccharomyces subcutaneous tumefaciens*, was detected from an apparently healthy man in France, later identified as *Cryptococcus gattii* (Curtis 1896). Vuillemin (1901) examined several of these cultures and due to lack of *Saccharomyces* specific characteristics he placed these species in the genus *Cryptococcus*. In 1905, von Hanseemann reported the first case of meningoencephalic cryptococcosis. In 1951, Emmons isolated *C. neoformans* from pigeon nests and their droppings. Disseminated cryptococcosis in a macaw was described later (Clipsham and Britt 1983).

**Table 2.15** Other important parasitic infection of pet birds

Parasite	Susceptible hosts	Clinical signs/gross lesions	Treatment
<i>Thelazia</i> spp.	Parrots, cockatoos, other companion birds	Eye spasm, hyperaemia, eye itching and irritation, conjunctivitis, chemosis	The worms can be manually removed after they are incapacitated with 0.125% demecarium bromide
<i>Ceratospira</i> spp.			Ivermectin (0.2 mg/kg, PO, SC, or IM, repeated in 10–14 days) may be used to kill the worms followed by manual removal or flushing
<i>Capillaria</i>	Budgerigars, canaries, macaws, pigeons etc.	Anorexia, chronic haemorrhagic diarrhoea, weight loss, regurgitation, anemia	–
<i>Encephalitozoon hellum</i> (protozoa)	Peach-faced, masked and Fischer's lovebirds ( <i>Agapornis roseicollis</i> , <i>A. personata</i> , <i>A. fischeri</i> ), European goldfinch ( <i>Carduelis carduelis</i> ), canary ( <i>Serinus canaria</i> ), budgerigar ( <i>Melopsittacus undulatus</i> ), eclectus parrots ( <i>Eclectus roratus</i> ), double yellow-headed Amazon parrots ( <i>Amazona ochrocephala</i> ), yellow-streaked lory	Enteritis, hepatitis, nephritis, keratoconjunctivitis, sinusitis, and lower respiratory tract infections. Infection is associated concurrent viral or bacterial infection	Fenbendazole (20–100 mg/kg body weight, once). It is toxic for finches, marabou storks and some vultures. The anthelmintic should not be used when birds are actively growing feathers
<i>Cochlosoma</i> spp. (protozoa)	Cockatiel ( <i>Nymphicus hollandicus</i> ), finch (subclinical infection in adults)	Diarrhoea, weight loss, pruritis and feather picking, respiratory signs	Metronidazole, ronidazole
<i>Spironucleus meleagridis</i> (protozoa)	Australian king parrots ( <i>Alisterus scapularis</i> ), cockatiel ( <i>Nymphicus hollandicus</i> ), splendid grass parakeet ( <i>Neophema splendida</i> )	Chronic diarrhoea, weight loss and death.	Nitroimidazoles (ronidazole, carnidazole)
<i>Haemoproteus</i> spp. (haemoprotozoa)	Cockatoos ( <i>Cacatuidae</i> ), parrot (carrier). It is transmitted by flies ( <i>Hippoboscidae</i> ) and midges ( <i>Culicoides</i> )	Pathogenicity uncertain. Splenomegaly, hepatomegaly, pulmonary edema are detected in heavily infested birds	Chloroquine and primaquine (not used in asymptomatic birds)

(continued)

**Table 2.15** (continued)

Parasite	Susceptible hosts	Clinical signs/gross lesions	Treatment
<i>Plasmodium</i> spp. (Avian malaria)	Passerine birds are the definitive host and act as reservoir. It is transmitted by <i>Culex</i> and <i>Aedes</i> mosquitoes to other birds. Documented in canaries, waterfowl, raptors, pigeons and parrots	Depression, anorexia, vomiting, dyspnoea, hemoglobinuria, bright green faeces due to increased biliverdin, pale mucus membranes, conjunctival edema and death	Chloroquine and primaquine (Therapeutic use: 25 mg/kg body weight, followed by 15 mg/kg body weight at 12, 24, and 48 h in conjunction with 0.75–1.0 mg/kg body weight primaquine at 0 h)
			Quinacrin HCl (@7.5 mg/kg body weight, 24 h interval for 7–10 days)
			(Prophylactic use: weekly dosing during the season when mosquito population increases)
			Pyrimethamine (0.5 mg/kg body weight, 12 h interval for 14–28 days)
			Azithromycin (45 mg/kg body weight, 24 h interval)
<i>Leucocytozoon</i> (protozoa)	Waterfowl, turkeys, young raptors and some passerines. Relatively uncommon in parrots. Black flies (family Simuliidae) act as vector	Anorexia, depression, dehydration, hemolytic anemia and associated hemoglobinuria	Chloroquine and primaquine
Trematoda (Fluke)	Cockatoos, owls, falcons, Gouldian finches.	Anorexia, Depression, anaemia, diarrhoea, hepatomegaly, increased firmness of liver, striation and mottling of liver. Flukes are present in any location such as blood vessel, mouth etc.	Fenbendazole (20–50 mg/kg body weight, 24 h interval for 3 days)
			Praziquantel (10–20 mg/kg body weight, oral, repeat after 10–14 days). For intramuscular injection: 9 mg/kg body weight, 24 h interval for 3 days, then oral administration for 11 days. It is toxic for finches in higher dosage.
	Birds get the infection by ingestion of insects or mollusc (second intermediate host)		Chlorsulon (20 mg/kg body weight, oral, three times, 2 weeks apart) Mebendazole (25 mg/kg body weight, 24 h interval for 5 days)

(continued)

**Table 2.15** (continued)

Parasite	Susceptible hosts	Clinical signs/gross lesions	Treatment
<i>Capillaria</i> (nematode, hairworm or threadworm)	Parrots, macaws, budgerigars, canaries, pigeons, and raptors. Direct or indirect transmission takes place. Insects and earthworms act as intermediate hosts	Anorexia, dysphagia, diarrhoea, weight loss, hyperemic streaks and diphtheritic lesions in organs	Anthelmintics are recommended
<i>Raillietiaenia</i> , <i>Choanataenia</i> , <i>Gastronemia</i> , <i>Idiogenes</i> , <i>Amoebataenia</i> (tapeworm)	Finches, African grays, cockatoos, and eclectus parrots. Tapeworms have indirect life cycles and require an intermediate host (grasshoppers, beetles, ants, horse flies)	Anorexia, weight loss, diarrhoea. Tapeworm segments are observed in droppings or hanging from the vent	Praziquantel
<i>Knemidocoptes pilae</i> (scaly face and leg mite)	Budgerigars, New Zealand parakeets, grass parrots, Polytelis parrots	Crusty lesions on beak, cere, face, legs, margins of vent and wing tips	Ivermectin (0.2 mg/kg body weight, repeat after 14 day. Frequent use can cause ataxia and depression. In budgerigars, finches, kingfishers and woodpeckers, very small dose (0.02 ml) may be toxic. In small birds one drop of the drug is directly applied on the lesion) Moxidectin
<i>Dermanyssus gallinae</i> (red or roost mite)	Reported in canaries. Common in birds having direct contact with wild birds	These mites feed on the birds during night and leave them at morning. Skin irritation, anaemia in young birds is observed.	Ivermectin, Moxidectin, carbaryl dusting powder (toxicity in higher dose)
<i>Cytodites nudus</i> , <i>Sternostoma tracheacolum</i> (air sac mite)	Canaries, Gouldian finches, parrots, budgerigars, cockatiels	Lethargy, change of voice, production of sucking or clicking noise due to irritation caused by the mite. Visualization of mites in the trachea with powerful light source, or detection of eggs in faeces or tracheal wash can diagnose it	Ivermectin with antibiotic (in Gouldian finches, a drop of ivermectin is applied onto the skin between the scapulae to avoid toxicity)

(continued)

**Table 2.15** (continued)

Parasite	Susceptible hosts	Clinical signs/gross lesions	Treatment
<i>Neopsittaconirmus</i> spp., <i>Psittaconirmus</i> spp., <i>Eomenopon</i> spp., <i>Pacifimenopon</i> spp., <i>Ciconiphilus</i> spp., <i>Menacanthus</i> spp. (Chewing lice)	Budgerigars, cockatiels, lovebirds, raptors, falcon, cattle egret, snowy egret, rusty-margined guan, Indian peafowl,	The entire life cycle of chewing lice (egg, three nymph, adult) is developed on a single host. Severe bite-induced pruritus, anorexia, weight loss are observed. Self-mutilation activities of the bird increase the risk of secondary bacterial infection	Carbaryl dusting powder, pyrethrin sprays
Hippoboscid fly (Louse fly/parrot fly)	Raptors, pigeon, wild parrots, red crowned parakeets, ostriches, swift, swallows	Blood sucking parasite which lays eggs on the host body (abdomen). Non-pathogenic but it can transmit blood parasite ( <i>Haemoproteus</i> spp.). Clinical myiasis and anaemia is detected in young birds. It can be transmitted into human handlers also	Carbaryl dusting powder, pyrethrin sprays
<i>Echidnophaga gallinacea</i> (Stickfast flea)	Psittacines, raptors, pigeons	Stickfast flea attaches firmly around the head and ear of the bird. In severe cases hyperkeratinisation, irritation and anaemia may occur	Pyrethrin sprays

### 2.4.1.2 Etiology

*Cryptococcus* belongs to the Filobasidiella clade of the Tremellales, under the order Tremellomycetes, phylum Basidiomycota. The genus *Cryptococcus* includes over 37 species majority of which do not cause any infection in mammals. Important pathogenic species are *C. neoformans*-*C. gattii* species complex, which includes *C. neoformans* var. *neoformans*, *C. neoformans* var. *grubii*, and *C. gattii* (*C. bacillisporus*). Among them, *C. neoformans* var. *neoformans* and *C. gattii* are considered as primary pathogens in immunocompetent avian hosts. Other cryptococcal species such as *C. uniguttulatus*, *C. albidus* and *C. laurentii* are occasionally detected in droppings and crops of pigeons and psittacine birds although clinical significance is uncertain.

*Cryptococcus* life cycle is predominantly divided into two phases i.e. vegetative and sexual growth phase. Two major morphological forms (yeast and pseudohyphae) exist in the vegetative growth phase. The predominant form found in the environment and avian and animal hosts is unicellular budding yeast. The yeast





**Fig. 2.39** Bird feather infected with lice (Courtesy Dr. Sychra Oldřich, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic)

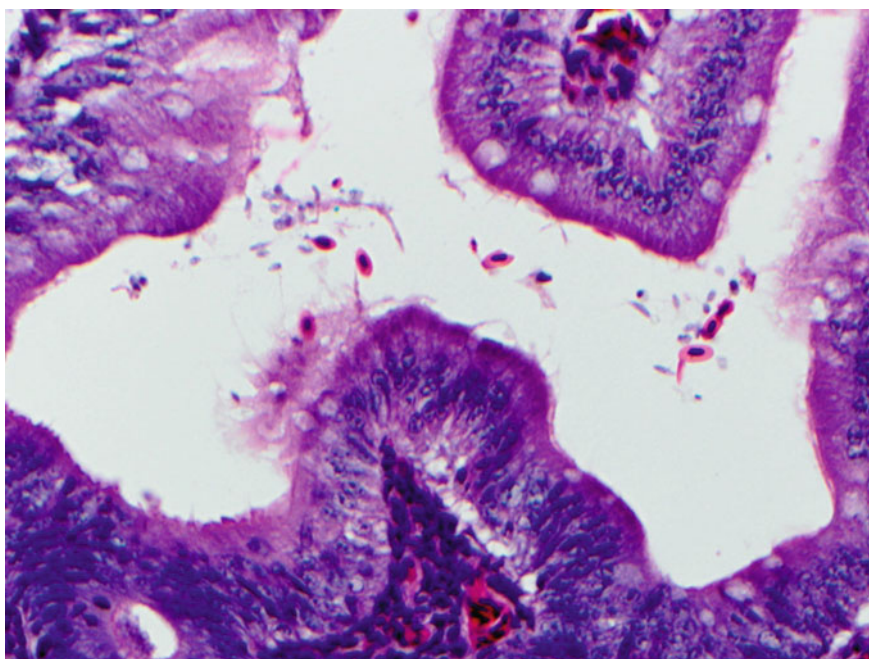


**Fig. 2.40** Layer birds infested with *Dermanyssus gallinae* (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)





**Fig. 2.41** Canaries infested with *Dermanyssus gallinae* (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)



**Fig. 2.42** Section of a female lutino cockatiel (*Nymphicus hollandicus*) infested with *Spirunculus meleagridis* (Courtesy Dr. Lauren V Powers, Carolina Veterinary Specialists, United States; Prof. John Barnes, NC State University, North Carolina, United States)



**Fig. 2.43** Budgerigar infected with mite (*Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy*)



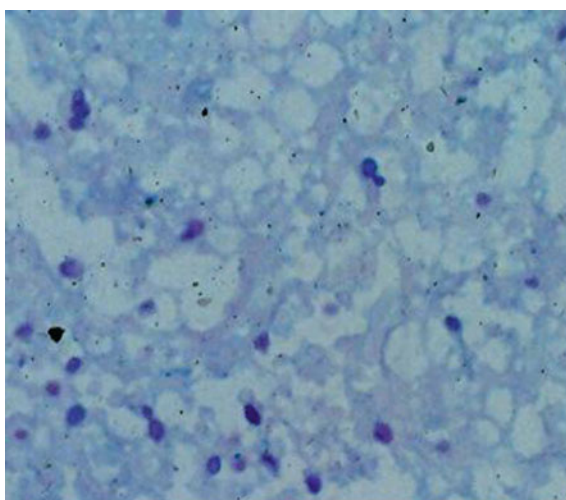
**Fig. 2.44** Bird infected with scaly leg mite (*Knemidokoptes*) infestation of a bird (*Courtesy Petra Maria Burgmann, Canada*)

cells are thin walled, spherical to oval with varying diameter (2–20  $\mu\text{m}$ ) and they reproduce by mitotic division. The buds are present at the narrow base (Fig. 2.45).

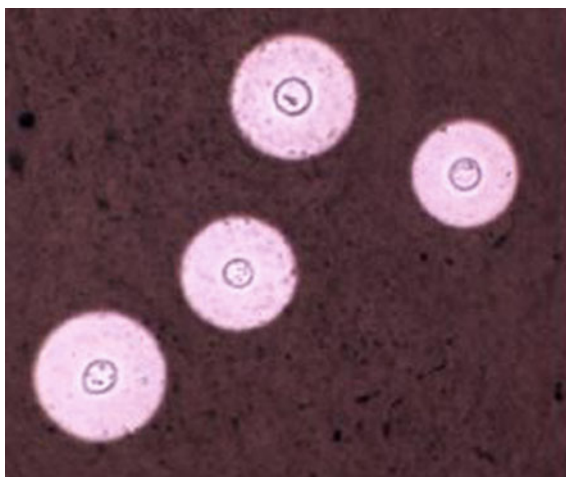
The morphological transition from the yeast phase to hyphal phase (pseudohyphae) is noticed during sexual mating. They are not considered as true dimorphic fungi probably due to their predominant existence as yeast form in the environment and hosts and the lack of involvement of this transition in the infection process. Further, both at 25 and 37  $^{\circ}\text{C}$  they can produce yeast like colonies in the isolation media. Recently unusually large yeast like morphological form (30–100  $\mu\text{m}$ ) is also detected in clinical samples, known as ‘giant’ or ‘titan’ cells.

Another unique morphological feature of *Cryptococcus* is the presence of capsule. The capsule can be best observed in fresh preparations by staining with diluted India ink or phase contrast microscopy (Fig. 2.46). Giemsa can also partially stain

**Fig. 2.45** Budding  
*Cryptococcus* spp. (Courtesy  
Prof. P.P. Gupta, Ex-Director  
of Veterinary Research,  
Punjab Agricultural  
University, Punjab, India)



**Fig. 2.46** Encapsulated  
*Cryptococcus* spp. in India  
ink preparation, 400 $\times$   
(Courtesy Prof. P.P. Gupta,  
Ex-Director of Veterinary  
Research, Punjab Agricultural  
University, Punjab, India)



the capsule. Like other eukaryotic organism *Cryptococcus* also possesses mitochondria which serves as source of energy, and is involved in processes of aging, calcium homeostasis, apoptosis, and regulation of virulence.

### 2.4.1.3 Host Susceptibility

Presence of *C. neoformans* in the faeces of different avian species except raptors (Table 2.16.) is a saprobiotic phenomenon. Avian faeces rich in creatine, urea, uric acid and protected from sunlight and ultraviolet light, high flock density and poor sanitary conditions create a microenvironment for *C. neoformans*. Pigeons as mechanical carrier of *C. neoformans* in their feathers, feet and crop are mostly studied avian species.

Description of clinical cryptococcosis in birds is relatively rare although reported in pigeons, kiwis, major Mitchell's cockatoo, moluccan cockatoos, thick-billed parrot, African grey parrot, green-winged macaw, Papua lorries, blackcapped lorries, Goldie's lorikeet, and ring necked parrot.

### 2.4.1.4 Transmission

*Cryptococcus* is transmitted primarily through inhalation route in birds, animals and human. The basidiospores are major infectious particles, small in size (2–3  $\mu\text{m}$ ) which can easily invade the lung alveoli than the encapsulated yeast (10–60  $\mu\text{m}$ ). Rarely ingestion of large number of organisms may cause the infection as observed during *C. gattii* infection of psittacine birds in Brazil. Psittacine birds have the habit of chewing wooden objects, such as the perches made of *Eucalyptus* spp. In tropical countries, *C. gattii* is commonly associated with *Eucalyptus* trees.

### 2.4.1.5 Pathogenesis

The basidiospores lodge in the lung alveoli of the birds after inhalation. The capsule of the fungi helps in evasion of immune system and survival within the host. The capsule is constituted with mannan (polysaccharide) which is highly hydrophilic. It makes a gelatinous zone surrounding the yeasts that conceals the pattern recognition receptors (PRR) of the yeast from the immune system. The capsule also prevents antibody binding and phagocytosis of the yeasts. *C. gattii* can produce extracellular fibrils which can prevent the phagocytosis by neutrophils and help to establish the primary pulmonary infection.

After primary colonization in upper respiratory tract, haematogenous spread of yeast into different organs such as heart, liver, spleen, intestine, kidneys, and central nervous system is observed in different psittacine birds. Occasionally, coelomic dissemination occurs between organs within close proximity. Superficial colonization of yeast is detected in choanas, sinus, upper beak, and infraorbital sinus of African grey parrot, Goldie's lorikeet and Beccaris's crowned pigeon. Minimal inflammatory response with epithelioid macrophages, multinucleated giant cells and heterophils or absence of inflammatory response is detected in different organs of psittacine birds.



**Table 2.16** *Cryptococcus* spp. detected in avian hosts in different countries

<i>Cryptococcus</i> spp.	Avian hosts	Country
<i>C. neoformans</i> var. <i>grubii</i> molecular type VNI	Budgerigars ( <i>Melopsittacus undulatus</i> )	Brazil, Germany
<i>C. neoformans</i> , <i>C. gattii</i>	White eyed parakeet ( <i>Aratinga leucophthalmus</i> ), Peach-fronted parakeet ( <i>Aratinga aurea</i> ), Jandaya parakeet ( <i>Aratinga jandaya</i> ), Scaly-headed parrot ( <i>Pionus maximiliani</i> ), Cockatiel ( <i>Nymphicus hollandicus</i> ), Alexandrine parakeet ( <i>Psittacula eupatria</i> ), Nanday parakeet ( <i>Nandayus nenday</i> ), Festive amazon ( <i>Amazona festiva</i> ), Red-browed amazon ( <i>Amazona rhodocorytha</i> ), Mealy amazon ( <i>Amazona farinose</i> )	Brazil
<i>C. neoformans</i>	Budgerigars ( <i>Melopsittacus undulatus</i> ), monk parakeet ( <i>Myiopsitta monachus</i> )	Brazil
<i>C. neoformans</i>	Sun parakeet ( <i>Aratinga solstitialis</i> ), blue fronted amazon ( <i>Amazona aestiva</i> )	Brazil
<i>C. neoformans</i>	Red-cowled cardinal ( <i>Paroaria dominicana</i> ), yellow canary ( <i>Serinus canarius</i> , <i>Serinus flaviventris</i> ), saffron finch ( <i>Sicalis flaveola</i> ), double-collared seedeater ( <i>Sporophila caerulea</i> )	Brazil
<i>C. neoformans</i>	Pigeons ( <i>Columba livia</i> )	United States
<i>C. neoformans</i>	Kiwis ( <i>Apteryx australis mantelli</i> )	New Zealand, Australia
<i>C. neoformans</i> var. <i>gattii</i>	Major Mitchell's Cockatoo ( <i>Cacatua leadbeateri</i> )	Australia
<i>C. neoformans</i> (serotype AD)	Exotic birds	Chile
<i>C. neoformans</i> var. <i>neoformans</i>	White face duck ( <i>Dendrocygna viduata</i> ), eagle owl ( <i>Bubo africanus cinerascens</i> ), peacock ( <i>Pavo cristatus</i> ), spotted eagle owl ( <i>Bubo africanus</i> )	Nigeria
<i>C. uniguttulatus</i> , <i>C. laurentii</i>	Pigeons ( <i>Columba livia</i> )	Sweden
<i>C. uniguttulatus</i>	Slender billed parakeet ( <i>Enicognathus leptorhynchus</i> ), bluecheeked rosella ( <i>Platycercus adscitus</i> )	Chile
<i>C. albidus</i>	Bluefronted Amazon parrot ( <i>Amazona aestiva</i> )	Chile
<i>C. neoformans</i> var. <i>gattii</i> (serovar B)	Papua lori ( <i>Charmosyna papou</i> ), blackcapped lorries ( <i>Lorius lory</i> ), Goldie's lorikeet ( <i>Trichoglossus goldiei</i> ), ring necked parrot ( <i>Psittacula krameri</i> ), African grey parrot ( <i>Psittacus erithacus</i> )	Brazil

### 2.4.1.6 Clinical Symptoms

Clinical cryptococcosis causes respiratory distress, lethargy, emaciation, diarrhoea, anaemia, incoordination, progressive paralysis and development of gelatinous mass in choana, sinus, upper beak, and infraorbital sinus in birds (Figs. 2.47 and 2.48). In psittacines, ocular infections causing conjunctival, corneal and intraocular lesions



**Fig. 2.47** A soft palpable swelling at the head of a lovebird suffering with cryptococcosis  
(*Courtesy* Prof. Adjunta Karin Werther, Universidade Estadual Paulista, Brazil)



**Fig. 2.48** Presence of a gelatinous mass at subcutaneous space of the infected lovebird's head  
(*Courtesy* Prof. Adjunta Karin Werther, Universidade Estadual Paulista, Brazil)

and blindness are also detected. In domestic pigeons (*Columba livia*), rare report of clinical cases revealed the central nervous system signs, weight loss, dyspnoea, and infraorbital sinus mass. Development of thick crust over the right naris is observed in Major Mitchell's cockatoo suffering with clinical cryptococcosis.

#### 2.4.1.7 Lesion

Gelatinous myxomatous mass (granulomata) in respiratory tract, abdominal cavity, sinuses and brain is the most common lesion observed in birds. In psittacines, hepatomegaly, multifocal hepatitis, yellow areas in the capsule and parenchyma of liver, congestion of lung, replacement of pulmonary parenchyma with yellow gelatinous material, thickened air sacs, sinuses filled with yellow coloured substance, nephromegaly, tubular and glomerular degeneration, splenomegaly, congested intestine with black coloured material in lumen are observed.

#### 2.4.1.8 Diagnosis

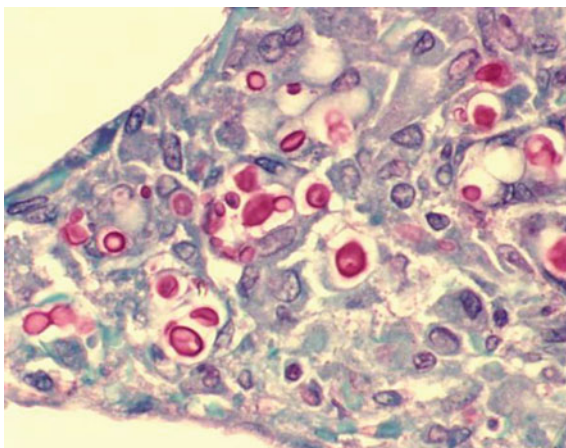
##### Clinical Specimens

Cloacal/choanal swabs, dry faeces, blood/serum, aspiration biopsy of the gelatinous mass present in upper beak, infraorbital sinus and choana, viscera collected in 10% formalin after post mortem are considered as clinical specimens of avian cryptococcosis. Dry faecal samples (2.5 gm) are suspended in 15 ml sodium chloride solution in tubes containing chloramphenicol (0.1 mg/ml).

##### Diagnostic Techniques

- (a) *Direct examination:* The smear can be prepared from the aspiration biopsy samples and is stained with India ink, Nigrosin, or Romanowski for demonstration of capsule. The Romanowski stain produces clearer capsule against the lightly stained background. The tissue samples can be stained with Periodic Acid Schiff base (PAS)—Haematoxylin stain which will outline the yeast cell and the capsule will appear as clear zone surrounding the cell (Fig. 2.49).

**Fig. 2.49** *Cryptococcus* spp. in PAS stained smear  
(Courtesy Prof. P.P. Gupta, Ex-Director of Veterinary Research, Punjab Agricultural University, Punjab, India)



In Mayer's mucicarmine stain, the capsule and cell wall appears as red. Another characteristic feature of *Cryptococcus* is narrow base budding (Fig. 2.45) in comparison to other yeasts (*Blastomyces*) having broad based budding. Sometimes false positive results are produced due to confusion with globules of myelin, lysed cells, lymphocytes and dead yeasts after successful treatment.

- (b) *Isolation of Cryptococcus spp. from clinical samples*: Faecal suspension (dry faeces mixed with sodium chloride and chloramphenicol) of the suspected birds can be inoculated into corn meal agar, Sabouraud's Dextrose agar, blood agar, honey agar, brain heart infusion agar and malt agar. The specific medium is birdseed agar/Staib medium (Niger seed agar)/sunflower seed extract agar with antibiotics. The plates are incubated at 28–37 °C for 2 days to 2 weeks. The colonies are initially small, convex, mucoid, creamy in colour, increases in diameter up to several centimeters after prolonged incubation. In birdseed agar the colonies appear as brown coloured in the center of the plate due to the production of melanin by the action of phenyl oxidase. The optimum capsule production is detected in chocolate agar after incubation at 37 °C with 5% CO<sub>2</sub> tension. The isolates are confirmed by API system or different biochemical tests such as urea hydrolysis, assimilation of inositol and creatinine, non-fermentation of carbohydrates, and melanin production. The L-Canavanine glycine bromothymol blue media can differentiate *C. neoformans* and *C. gattii* by the formation of distinctive blue coloration with the growth of *C. gattii*.
- (c) *Detection of Cryptococcal antigen*: Detection of Cryptococcal antigen in clinical specimens can be performed by latex particles coated with polyclonal serum and antigen-capture ELISA. The detection of antigen is possible from both live and dead organisms. During initial phase of therapy, disintegration of the yeast cells releases the capsule which produces high titer. So, the tests should not be done within 6–8 weeks after initiation of the therapy. The titer can be observed even after successful treatment as the dead organisms also have the intact capsule.
- (d) *Molecular biology*: Multiplex PCR and mating type-PCR are used for determination of molecular and mating types of *C. neoformans* var. *neoformans* and *C. gattii* isolates.

#### 2.4.1.9 Zoonosis

In human the patients with suppressed immunity (suffering from AIDS, lymphoma, haematologic malignancy and using corticosteroids for prolonged period) are mostly susceptible to the cryptococcal infection. It causes meningitis, meningoencephalitis and death in human. Inhalation of cryptococcal basidiospores is the major way of transmission in human. Cryptococcal infection in patients after exposure to the birds is observed. It was first reported from United States in a renal transplanted patient who developed cryptococcal meningitis transmitted from her pet cockatoo (Nosanchuk et al. 2000). Report of cryptococcal meningitis is also documented in



immunocompetent patient who acquired the infection from her pet magpie, although, her contact with the bird was limited to passing by the cage when entering home (Lagrou et al. 2005).

#### 2.4.1.10 Treatment and Control Strategy

Fluconazole (5–15 mg/kg body weight, oral, 12 h interval for 15–60 days) and itraconazole (10 mg/kg body weight, oral, 24 h interval for 15–90 days with food) are recommended for treatment of avian cryptococcosis in pet birds. Use of itraconazole is contraindicated in African grey parrots.

Proper ventilation, regular cleaning of droppings and organic debris from the cages can prevent the occurrence of avian cryptococcosis. In tropical countries, wooden perches made of *Eucalyptus* tree should be avoided because psittacine birds like to chew the perches.

### 2.4.2 Aspergillosis

#### 2.4.2.1 History

In 1729, Micheli first described *Aspergillus* who found the similarity between the spore chain of the fungi with the brush ('Aspergillum') used for sprinkling holy water in the churches. Later in 1842, pathogenic *Aspergillus* (*A. candidus*) was detected in air sac lesion of a bullfinch by Rayer and Montagne. Whereas, *A. fumigatus* was first detected in lung of a great bustard (*Otis tarda*) in 1863 by Fresenius, who was the first to use the term 'aspergillosis' for this respiratory infection. The major members of aflatoxins were first detected and its association with *Aspergillus flavus* was established in 1961 during investigation of mysterious 'turkey-X disease' causing high mortality in turkey poults (Sargeant et al. 1961). In 1962, the name 'aflatoxin', using first letter from 'Aspergillus' and the first 3 letters from 'flavus' was proposed.

#### 2.4.2.2 Etiology

*Aspergillus* is the fungus which belongs to Trichocomaceae family under the order Eurotiales and phylum Ascomycota. The genus *Aspergillus* contains more than 250 species grouped into sub genera and species complex. There are eight such sub genera i.e. *Aspergillus*, *Fumigati*, *Circumdati*, *Candidi*, *Terrei*, *Nidulantes*, *Warcupi*, and *Ornati*. The important species complexes are *A. fumigatus*, *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans*, and *A. ustus*. Among them, *A. fumigatus* is the most common cause of avian aspergillosis. Other species complexes such as *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans* are occasionally associated with avian aspergillosis. *A. fumigatus* is the major cause probably due to smaller size of the spores than other species which helps in easy transmission.

#### 2.4.2.3 Host Susceptibility

All species of domestic, pet and wild birds including chicken, duck, quail, and geese are susceptible to avian aspergillosis due to anatomic and physiologic characteristics

**Table 2.17** *Aspergillus* spp. detected in avian hosts in different countries

<i>Aspergillus</i> spp.	Avian hosts	Country
<i>Aspergillus</i> spp.	Goliath Heron ( <i>Ardea goliath</i> ), Great horned owl ( <i>Bubo virginianus</i> ), Rhea ( <i>Rhea Americana</i> ), palm cockatoo ( <i>Probosciger aterrimus</i> ), African grey parrot ( <i>Psittacus erithacus</i> ), Moluccan cockatoo ( <i>Cacatua moluccensis</i> ), cape parrot ( <i>Poicephalus robustus</i> ), yellow naped Amazon parrot ( <i>Amazona ochrocephala</i> ), quaker parrot ( <i>Myiopsitta monachus</i> ), blue and gold macaw ( <i>Ara ararauna</i> ), eclectus parrot ( <i>Eclectus roratus</i> ), harlequin macaw ( <i>Ara ararauna</i> , <i>Ara chloroptera</i> ), blue-fronted parrot ( <i>Amazona aestiva</i> )	United States, Brazil
<i>A. fumigatus</i>	Pigeons ( <i>Columba livia</i> ), common peafowl ( <i>Pavo cristatus</i> ), bearded vulture ( <i>Gypaetos barbatus</i> ), Eurasian black vultures ( <i>Aegypius monachus Linnaeus</i> ), Himalayan Griffon Vulture ( <i>Gyps himalayensis</i> ), herring gulls ( <i>Larus a. argentatus L.</i> ), seagulls ( <i>Larus cachinnans micaellis</i> ), love bird ( <i>Agapornis roseicollis</i> ), red-faced love bird ( <i>Agapornis pullaria</i> ), stitchbird or hihi ( <i>Notiomystis cincta</i> ), wild geese ( <i>Chloëphaga poliocephala</i> ), pink-footed Geese ( <i>Anser brachyrhynchus Baillon</i> )	Sudan, Chile, Belgium, United Kingdom, Bulgaria, Italy, Spain, New Zealand
<i>A. flavus</i>	Canada geese ( <i>Branta Canadensis</i> ), king shag ( <i>Phalacrocorax albivenier</i> ), juvenile red-crowned crane ( <i>Grus japonensis</i> ), Magellanic penguin ( <i>Spheniscus magellanicus</i> ), gentoo penguin ( <i>Pygoscelis papua</i> ), chinstrap penguin ( <i>Pygoscelis antarctica</i> ), king penguin ( <i>Aptenodytes patagonica</i> ), little blue penguin ( <i>Eudyptula minor</i> ), yellow-eyed penguin ( <i>Megadyptes antipodes</i> ), rockhopper penguin ( <i>Eudyptes chrysocome</i> ), adelic penguin ( <i>Pygoscelis adeliae</i> ), peruvian penguin ( <i>Spheniscus humboldti</i> ), black footed/jackass penguin ( <i>Spheniscus demersus</i> ), budgerigar ( <i>Melopsittacus undulatus</i> )	Canada, Spain, Scotland, Australia, New Zealand, Antarctica, South Africa
<i>A. terreus</i>	pigeon ( <i>Columba livia</i> )	India
<i>A. niger</i>	great horned owl ( <i>Bubo virginianus</i> )	Canada

of avian respiratory system (Table 2.17). Among the wild birds, some species such as goshawks (*Accipiter gentilis*), gyr falcons (*Falco rusticolus*), penguins, and auk (*Alca torda*) are more susceptible to respiratory fungal infections. Diving birds (auk, penguin) are more susceptible due to air re-circulation during diving.

#### 2.4.2.4 Transmission

Inhalation of fungal spores (conidia) is the major way of *Aspergillus* transmission in birds. In addition to inhalation route, ingestion of contaminated feed (seed mixture) with the fungal spores is another way of transmission. Due to small non-expanding lung and presence of air sacs, the birds are more susceptible to *Aspergillus* infection. Primary colonization of the spores takes place in the air sacs because the inhaled air passes through the sacs to reach the lungs. Other predisposing factors for *Aspergillus* infection include higher body temperature which helps in fungal growth, lung injury, stress due to malnutrition and vitamin deficiency, use of immunosuppressive drugs, very young or old age and use of hay or straw contaminated with fungal spores in preparation of aviary litter.

#### 2.4.2.5 Pathogenesis

The conidia of *Aspergillus* spp. reach the lung parenchyma through inhaled air. The conidia are trapped between atrium and infundibulum of parabronchus region of the lungs. Conidial sialic acids act as ligand for adherence with the alveolar epithelial cells specially with fibrinogen and fibronectin, commonly found in the wounded epithelial surfaces. So, lung injury acts as major predisposing factor for causation of invasive aspergillosis. Gliotoxin, fumagillin, and helvolic acid produced by the fungi causes damage in the respiratory mucosa and slow ciliary movement facilitating the attachment of the conidia.

The conidial maturation begins which causes loss of hydrophobic layer and exposure of the inner cell wall. The cell wall is composed of galactomannan, chitin, and  $\beta$  glucan which act as ligand for the soluble and cell associated pattern recognition receptors (PRR). The soluble receptors act as opsonin and can bind with fungal cell wall carbohydrate which enhances phagocytosis by the alveolar macrophages. Most of the conidia are killed by reactive oxygen species (ROS) or acidified phago-lysosome produced within the alveolar macrophages. In immunosuppressed birds having defective alveolar macrophage function the conidia are able to escape the phago-lysosome mediated killing.

The conidia which survive the first line of defense can germinate. The germination involves conidial swelling (isotropic growth) followed by protrusion of germ tube (polar growth). They produce a necrotic focus (plaque) without a structured granuloma which can obstruct the trachea or bronchi and fill up the air sacs. Hyphae with fruiting bodies can penetrate the air sacs or lungs and produce serositis. Fungal growing hyphae also invade the endothelial cell lining of blood vessels (angioinvasion) from the albuminal side to the luminal side. Dissemination of infection into vital organs occurs through the haematogenous route.

#### 2.4.2.6 Clinical Symptoms

Avian aspergillosis is considered as an opportunistic infection in birds with immunosuppression. Primary infection in immunocompetent hosts sometimes takes place when numerous spores are inhaled. Acute and chronic types of aspergillosis may develop in affected birds. Mostly young birds suffer with acute infection in which sudden death of birds occurs without prior symptoms probably due to hepatic damage by aflatoxins



**Fig. 2.50** Rhinolith in a Timneh African Grey parrot (*Courtesy Dr. Kenneth R. Welle*)

released by the fungi. If the birds are alive for a few days, general symptoms such as laboured breathing, anorexia, diarrhoea, polydypsia, and cyanosis develop.

Chronic aspergillosis is more common in adult birds. It produces non-specific syndrome such as fever, diarrhoea, respiratory distress, change in voice ('sore throat' sound due to tracheal and syringeal inflammation), change in behaviour, emaciation, cheesy deposit in conjunctival sac, and nares, development of rhinolith ('nose-stone') and facial swelling, neurological disorders, dermatitis, wing droop due to humerus involvement (Fig. 2.50). Death occurs due to obstruction of airways and respiratory failure.

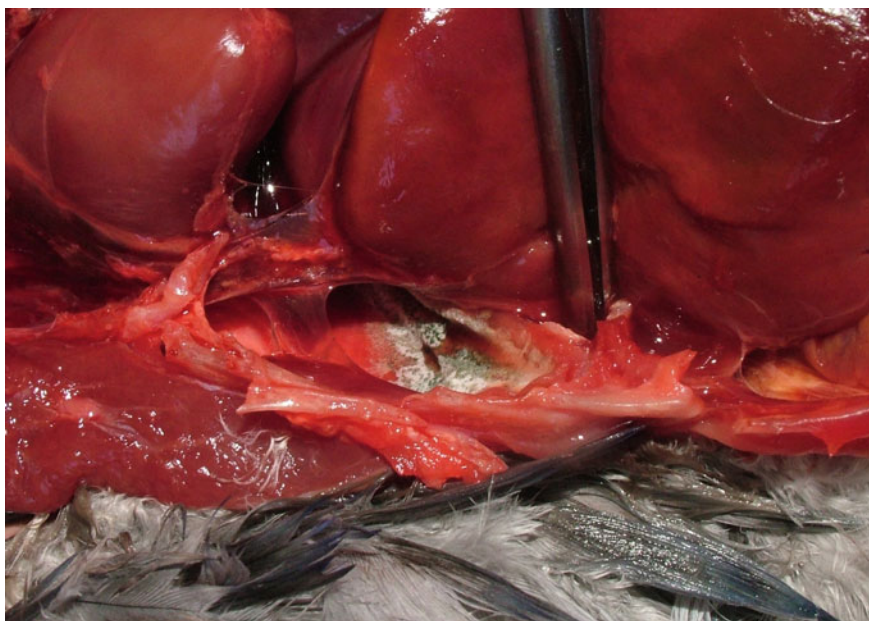
#### **2.4.2.7 Lesion**

Congestion and yellow nodules in lungs, thickened air-sacs with small whitish-yellow plaques are detected in acute aspergillosis (Figs. 2.51 and 2.52). In chronic form, granulomatous lesions (nodules and plaques) are observed in periphery of lungs and air sacs which may occlude the trachea and bronchi. Typical granulomas are also detected in kidneys, oviduct, and ovaries.

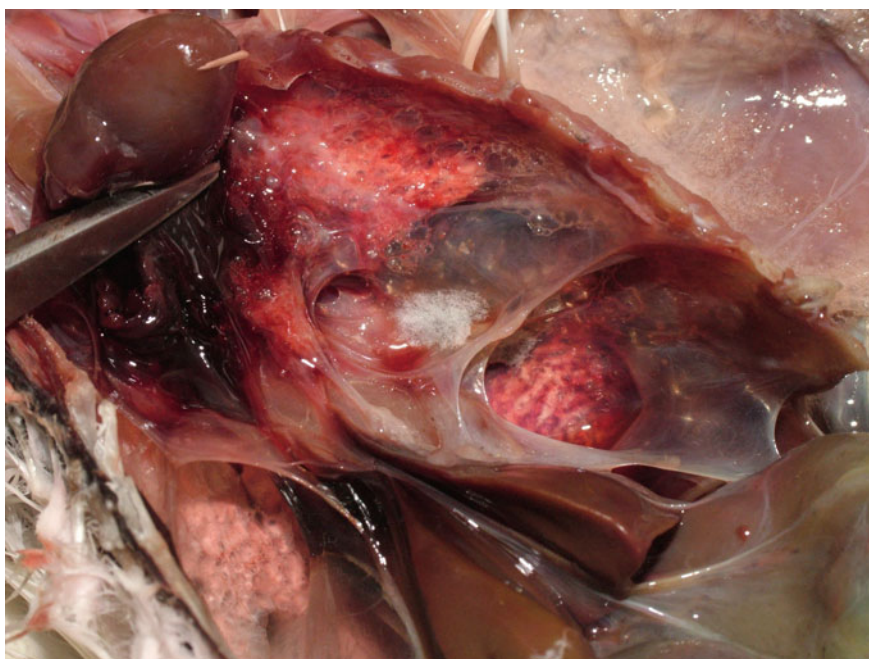
#### **2.4.2.8 Diagnosis**

##### **Clinical Specimens**

Whole blood, tracheal washings, air sac fluids, tissue biopsies from respiratory tract granuloma and vital organs such as lungs, air sacs, kidney, liver collected after post



**Fig. 2.51** Air sac lesion in African grey parrot infected with *Aspergillus* spp. (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)



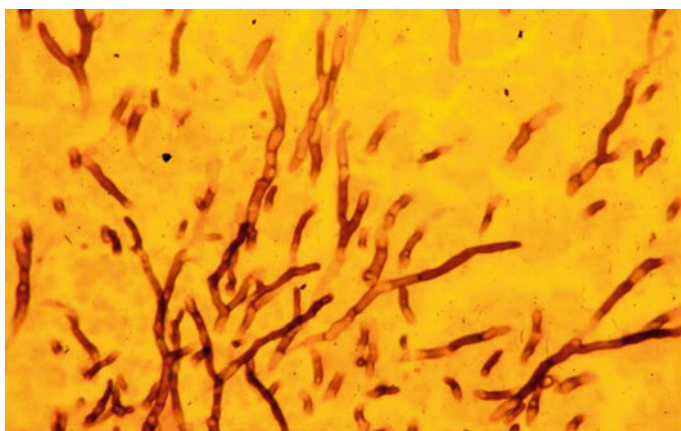
**Fig. 2.52** Air sac lesion in cockatoo infected with *Aspergillus* spp. (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)



mortem can be used as clinical specimens for laboratory confirmation of avian aspergillosis.

### Diagnostic Techniques

- (a) *History and clinical symptoms*: History of immunosuppressive treatment and clinical symptoms such as sudden change of voice along with other general symptoms primarily suggests about clinical aspergillosis.
- (b) *Direct examination*: The tissues collected as clinical specimens should be cleared with 10% KOH and are observed under microscope. Histopathological staining can be performed with periodic acid Schiff (PAS), Grocott's silver or methenamine silver stain for detection of tissue invasion. In the tissue section, *Aspergillus* hyphae are narrow and septate which are not easily distinguishable from other fungi (Fig. 2.53). Different species of *Aspergillus* has characteristic fruiting body structures which can be identified by an expert. Confirmation of aspergillosis is also possible by immunohistochemistry.
- (c) *Isolation of Aspergillus from clinical specimens*: *Aspergillus* can be isolated in Sabouraud dextrose agar (SDA) with or without chloramphenicol (0.05 gm/l) and other common bacteriological media such as blood agar. The cycloheximide can inhibit the growth. The plates are incubated at 37 °C for 4–5 days. *A. fumigatus* is thermophilic which is able to grow at 55 °C and can survive at more than 75 °C. However, repeated isolation from the clinical specimen is required, along with correlation with history, clinical signs, histopathological observations for proper diagnosis of clinical aspergillosis.
- (d) *Blood biochemical tests and radiography*: Haematological parameters such as leukocytosis (20,000–100,000), heterophilia with a left shift (degenerative shift), monocytosis, lymphopenia, and change in  $\beta$ -globulin concentration



**Fig. 2.53** Septate and dichotomously branching *Aspergillus* spp. hyphae in lungs (GMS) (Courtesy Prof. P.P. Gupta, Ex-Director of Veterinary Research, Punjab Agricultural University, Punjab, India)

indicate about occurrence of infection such as aspergillosis. Radiographic changes of pneumonia and airsacculitis (lateral and ventrodorsal views) also suggests respiratory tract infection such as aspergillosis. Both these biochemical tests and radiography cannot confirm the infection.

- (e) *Detection of Aspergillus antigen*: Detection of *Aspergillus* antigen is useful in acute infection. Galactomannan (GM) is the predominant antigen released by *A. fumigatus* in the circulation during angioinvasion which can be detected by latex agglutination test, sandwich ELISA in blood samples. The detection limit of both the tests is 15 ng/ml and 1 ng/ml, respectively. However, GM detection assay is not specific for *Aspergillus* as it is cross-reacting with other fungi such as *Penicillium*, *Fusarium*, *Alternaria*, and *Histoplasma*. Similarly,  $\beta$ -D-glucan (BDG) can be detected for identification of *Aspergillus*. It is also produced by a lot of other fungi such as *Candida*, *Fusarium*, *Pneumocystis* etc. So the test can predict the general fungal infection rather than specifically aspergillosis.
- (f) *Serological tests*: Serological tests such as counter-immunoelectrophoresis, agar gel immunodiffusion and enzyme-linked immunosorbent assays (ELISA) can be employed as supportive diagnostic assays to detect the antibodies in chronic infection. In immunosuppressed birds production of antibody is undetectable.
- (g) *Molecular biology*: Confirmation of *Aspergillus* spp. can be done by conventional PCR and real-time PCR from clinical samples. *A. fumigatus* isolates can be characterized by PCR-RFLP using *BccI*, *MspI* and *Sau3AI* restriction enzymes.

#### 2.4.2.9 Zoonosis

*A. fumigatus* causes invasive aspergillosis in immunocompromised human which involves lung parenchyma, pleura, trachea and bronchi. It is common in the patients with haematological malignancy, prolonged antibiotic users or stem cell transplant recipients. Transmission of clinical aspergillosis from pet birds is not documented so far.

#### 2.4.2.10 Treatment and Control Strategy

Removal of granulomatous lesion is essential for effective treatment. It is difficult due to remote location of granuloma within respiratory tract, risk of surgical trauma and anaesthesia. In most of the confirmed cases, treatment is restricted with antifungals. Nebulization, oral, intravenous, nasal or air sac flushing, surgical irrigation of the abdominal cavities are ways for drug application. Antifungals used in avian aspergillosis with suitable dosage are described in Table 2.18. Sometimes, immunostimulants, for example, levamisole and microbial products ( $\beta$ -glucans) can be used for boosting of the immune system.

No vaccine is currently available against avian aspergillosis. Maintenance of hygiene and nutrition, avoiding mouldy feeds, proper ventilation and spraying of fungistatic agents [nystatin, thiabendazole, copper sulphate (1 g per 2 L of water)] in large aviaries can prevent avian aspergillosis.

**Table 2.18** Antifungals used in birds against aspergillosis

Antifungals	Route and Dosage	Comments
Amphotericin-B	Nebulization: 1 mg/kg body weight for 20 min, 3–4 times a day for 10–14 days	It should be slowly injected via intravenous route, otherwise cardiac arrhythmia and mild convulsion occurs. The drug is potentially nephrotoxic for birds (except raptors). It is rapidly excreted by raptors
	Intravenous: 1.5 mg/kg body weight, 8 h interval, for 3–5 days in most species	
	Intravenous: 1 mg/kg body weight, 8–12 h interval, in raptors, psittacines (tracheal granuloma)	
Clotrimazole	Intratracheal: 2 mg/kg body weight, 24 h interval for 5 days in psittacines (tracheal granuloma)	–
	Nebulization: 1% in 2 or 3 ml saline 1.5 h/day for 4–6 weeks	
Fluconazole	Oral: 15 mg/kg body weight, twice a day for 15–60 days	It shows variable activity against <i>Aspergillus</i> spp. Use of nephrotoxic drug with fluconazole is contraindicated
Itraconazole	Oral: 10 mg/kg body weight once a day for 7–35 days	It is well absorbed when taken with food. In African Grey parrot, it should be avoided as it causes severe anorexia and depression
Ketoconazole	Oral: 10–30 mg/kg body weight, twice a day for 30–60 days	Some species of <i>Aspergillus</i> show resistance. Tablets may be mixed with food or fruit juice for feeding. Hepatotoxic drugs should be avoided along with ketoconazole

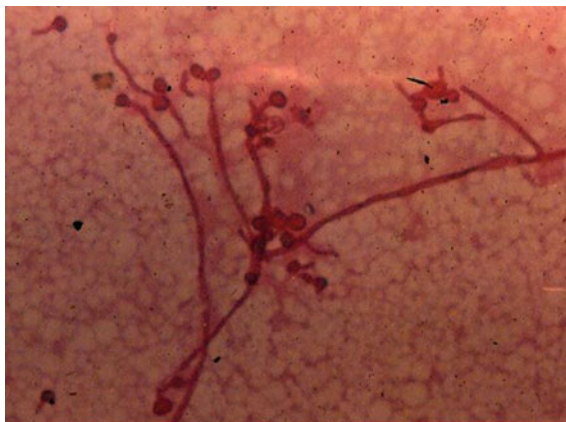
## 2.4.3 Other Fungal Infections of Pet Birds

### 2.4.3.1 Avian Candidiasis

*Candida* spp. is a common inhabitant of avian enteric tract, although, it can cause infection in young, immunosuppressed and stressed birds and during prolonged use of antibiotics. *Candida albicans*, *C. krusei*, and *C. tropicalis* are major cause of avian candidiasis. *C. parapsilosis* is rarely isolated from the crops of the birds. Among the pet birds, clinical candidiasis is reported from peach-faced lovebirds, Fisher's lovebirds, pigeons, cockatiels, Amazon parakeets, budgerigars and peacocks. In Eclectus parrot, concomitant infection of histoplasmosis and candidiasis is detected. Healthy cockatiels and some other psittacines are also detected to act as carrier of *Candida* spp. Infected birds show whitish, pseudomembranous lesions in oral cavity, crop and esophagus. Crop is the most suitable organ for fungal growth due to its pouch like structure and availability of nutrients. General symptoms such as anorexia, depression, delayed crop emptying, and regurgitation is sometimes observed. Presence of thickened mucosa (pseudomembranes) and ulcers in oral cavity, esophagus and crop is the major necropsy findings. The smear prepared



**Fig. 2.54** Budding yeast, hyphae and pseudohyphae of *Candida albicans* (PAS) (Courtesy Prof. P. P. Gupta, Ex-Director of Veterinary Research, Punjab Agricultural University, Punjab, India)



from the clinical specimens can be observed under microscope either by 10% KOH preparation or by staining with Gram's stain method. In the tissue section *Candida* can be observed by PAS-haematoxylin or methenamine silver stain. They appear as unicellular budding yeast or hyphae and pseudohyphae (Fig. 2.54). *Candida* can be isolated in common fungal or bacteriological media such as Sabouraud dextrose Agar (with penicillin, streptomycin, chloramphenicol to prevent the bacterial growth), potato dextrose agar, blood agar and brain heart infusion agar. The plates are incubated at 25–30 °C for 2–3 days. Immunohistochemistry is also useful in detection of *Candida* spp. in birds. Several types of PCR such as semi nested and nested PCR, real time PCR, multiplex PCR followed by DNA sequencing or pyrosequencing are developed for detection of Candidal DNA. The target gene includes rRNA (5.8S, 18S, 28S) gene, internal transcribed spacer (ITS) and intergenic spacer (IGS) region genes. Treatment with nystatin (3,00,000–600,000 IU/kg body weight, 8–12 h interval for 7–14 days in psittacines; 1,000,000 IU/kg body weight, 12 h interval in raptors and pigeons) is recommended for pet birds in confirmed cases of avian candidiasis. The drug is not well absorbed from the gastro intestinal tract and it should be mixed with food for better absorption. Except vomition in some species the drug is safe and cost-effective.

#### 2.4.3.2 Mycotic Proventriculitis (*Macrorhabdus ornithogaster* Infection)

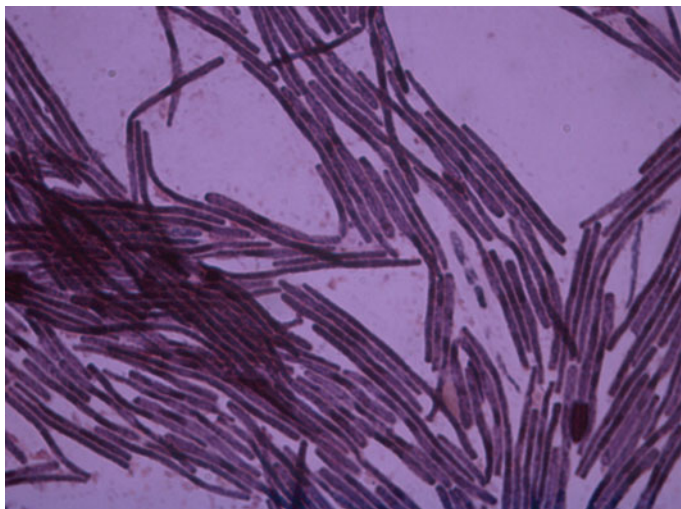
Proventriculitis was originally described as *Megabacterium* associated infection in aviaries. Due to presence of a eukaryotic nucleus, mycotic staining properties, presence of chitin in cell wall, and finally phylogenetic analysis based on 18S rRNA and 26S rRNA gene, *Megabacterium* is classified as yeast. It is renamed as *Macrorhabdus ornithogaster*. The infection is reported from captive-bred budgerigars (*Melopsitticus undulatus*), parrotlets (*Forpus* spp.), canaries (*Serinus canaria*), pheasants (*Phasianus colchicus*), red-legged partridges (*Alectoris rufa*), helmeted guinea fowl (*Numida meleagris*) and gray partridges (*Perdix perdix*) as primary pathogen or concomitant with other infections. Faecal-oral route is the



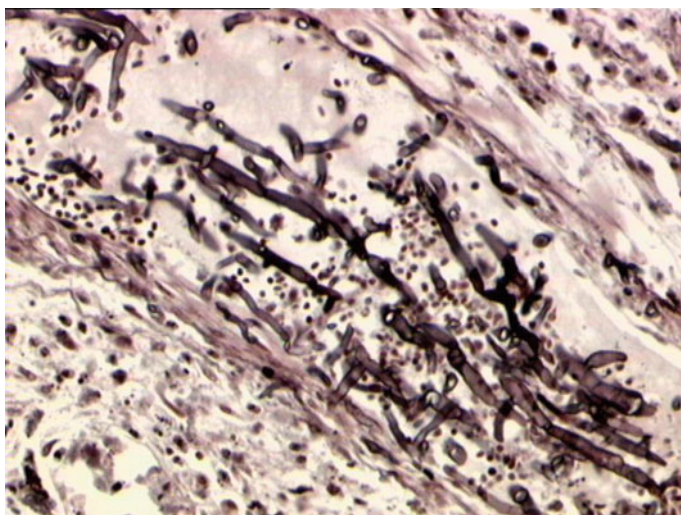
**Fig. 2.55** Regurgitation of a budgerigar infected with *Macrorhabdus ornithogaster* (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)

probable transmission route of *Macrorhabdus* infection in birds. The yeast prefers to colonize isthmus of proventriculus or ventriculus in birds. The infection is chronic and it produces certain non-specific symptoms such as anorexia, continuous loss of body weight, depression, plumage disorder, regurgitation, diarrhoea and dehydration (Fig. 2.55). Sudden death due to rupture of proventriculus is observed in budgerigars and parrots. Swollen and hyperemic proventriculus, covering of isthmus with thick, transparent-to-white mucus, and mucosal erosions in gizzard are specific necropsy findings. Fresh faecal samples from live birds and tissues from vital organs (esophagus, crop, proventriculus, gizzard, pancreas, liver etc.) can be collected in 10% buffered formalin after post-mortem. Smears prepared from faecal samples can be stained by Gram's method. The tissue sections are stained with periodic acid-Schiff (PAS), Grocott's methenamine silver nitrate (GMS) and Mayer's hematoxylin and eosin. Detection of typical gram-positive, large rod shaped organisms (20–60  $\mu\text{m}$  long and 2–3  $\mu\text{m}$  wide) suggests *Macrorhabdus* infection (Fig. 2.56). Isolation of *Macrorhabdus* is difficult because it does not grow in commonly used solid media. It can be isolated only in liquid medium (minimum essential media) with 20% foetal bovine serum (FBS) and 5% sucrose and more than two weeks incubation. Antifungals for instance nystatin and amphotericin-B can be used for the treatment (Figs. 2.57, 2.58 and 2.59).

Other fungal infections of pet birds are described in Table 2.19.

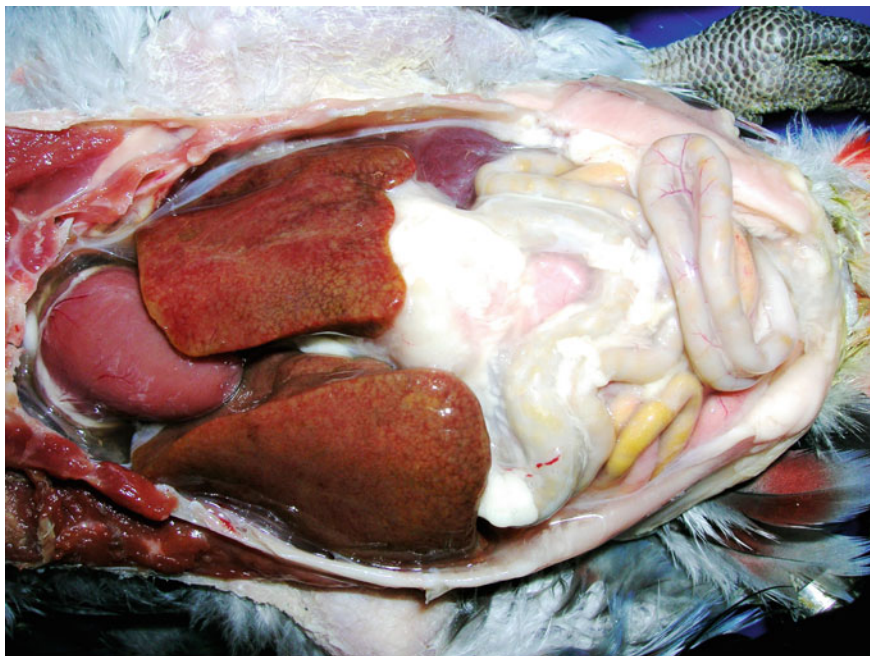


**Fig. 2.56** Microscopic appearance of *Macrorhabdus ornithogaster* (Gram's stain) (Courtesy Prof. Elena Circella, Avian Diseases Unit, Department of Veterinary Medicine, University of Bari, Italy)



**Fig. 2.57** *Penicillium* hyphae present in blood vessels of African grey parrot (Grocott stain) (Courtesy Giovanni Lanteri, University of Messina, Italy)





**Fig. 2.58** Numerous yellowish white nodules in the liver of African grey parrot infected with *Penicillium* spp. (Courtesy Giovanni Lanteri, University of Messina, Italy)



**Fig. 2.59** Dense yellowish fluid in the crop of African grey parrot infected with *Penicillium* spp. (Courtesy Giovanni Lanteri, University of Messina, Italy)

**Table 2.19** Other important fungal infection of pet birds

Fungi	Susceptible hosts	Clinical signs/Gross lesions	Treatment
<i>Penicillium chrysogenum</i>	African gray parrot ( <i>Psittacus erithacus</i> )	Cheesy yellowish deposit in oral cavity, yellowish fluid in crop, pyogranulomatous lesions and whitish nodules in lung, liver, kidney	Enilconazole (6 mg/kg body weight, 12 h interval, oral)
<i>Histoplasma capsulatum</i>	Eclectus parrot ( <i>Eclectus roratus</i> )	Oral and peri-ocular masses, chronic lameness	–
<i>Absidia corymbifera</i>	African gray parrot ( <i>Psittacus erithacus</i> )	Lesions in air sac and kidney	–
<i>Rhizomucor pusillus</i>	African gray parrot ( <i>Psittacus erithacus</i> )	Diarrhoea, unsteady gait, twisted neck	–
<i>Rhizopus microsporus</i> var. <i>chinensis</i>	Eclectus parrot ( <i>Eclectus roratus</i> )	Concomitant infection with <i>Candida krusei</i> and produces acute necrotising ventriculitis. It is characterized by vomition and bright green faeces	–

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