Most of the pet bird owners prefer to visit their veterinarians during crisis period when possibility of recovery becomes low. Instead, visit to veterinarians in a regular interval, recently termed as ‘wellness examination’ will provide a knowledge package consisting of infection if present, balanced diet, proper exercise, behaviour and husbandry practices to be followed. Inexperienced bird owners and owners of aged birds with chronic ailment should have regular wellness examination of their pets. To provide this knowledge package the veterinarian should also follow appropriate diagnostic approaches with proper medicinal, nutritional, and husbandry recommendations.

5.1 Anamnesis

Taking history from the owner regarding health, diet and husbandry practices of their pet birds is the most primitive but still considered as most valuable diagnostic technique. This is a two-way technique which depends on co-operation of the owners, questioning ability and patience of the veterinarians and moreover, proper record keeping. The veterinarian can know whether the ailment is new or progressed from earlier stage. Co-operation from owners with truthful replies is required for proper diagnosis.

The veterinarians should primarily focus on age, species, sex, physical activities, urination and droppings (colour, consistency and amount) of the birds. Exposure to environment such as soil, garden, toxic fume or smoke, bird shows/fairs, shelters, aviaries, other infected birds and wild birds should be enquired from the owners. Evaluation of earlier recommendations regarding diet, bedding materials is necessary. What exactly the bird is taking as feed, not what is provided to them is
important to know. Excess or deficiency of nutrients is associated with different diseases. Excess fat in diet (present in seeds) causes obesity and hepatic lipidosis. Deficiency of vitamin and calcium causes osteoporosis and impairment of vision. The veterinarian can judge the possibility and severity of a specific ailment depending on the species, age and exposure of the birds. Sub-clinical carriers of zoonotic infections should also be identified specially when the birds are reared by aged or immunosuppressed owners. To perform all these functions properly the veterinarians should have an updated knowledge other than experience.

5.2 Physical Examination

Physical examination of the bird should be performed in a clean perch or small enclosure. Gentle restraining of the birds is necessary not only to avoid injury of the birds but also to gain the trust of the owners. Bad handling always creates doubt about the ability of a veterinarian. Mild sedation with midazolam (0.2–0.5 mg/kg body weight, intramuscular) or butorphanol (1–2 mg/kg body weight, intramuscular) will help to restrain hyperactive birds.

Alertness, weight, posture (straight and upright in healthy birds), feather condition (ruffled or normal), walking (leg twisting), breathing sound, colour, consistency and odour of droppings and urine, vision, behaviour (nervousness, relationship with owner, biting tendency) should be judged primarily (Fig. 5.1).

Fig. 5.1 Ruffled feather in a sick parrot (Courtesy Lefeber vet)
5.3 Collection of Clinical Samples

5.3.1 Blood

The volume of collected blood sample should be 0.5–1.0% of the patient’s body weight. EDTA is the most suitable anticoagulant except in crowned cranes, hornbills, eagle owl, laughing kookaburra, curassows and corvidae. In these species of birds, EDTA causes haemolysis and heparin is used as anticoagulant. Lithium heparin is suitable for blood biochemical parameters (except glucose and calcium), not for cellular study as it causes clumping of all avian blood cells. Blood is collected with a 23–25 s.w.g. needle bent at a single angle. The puncture site should be blocked with finger pressure immediately. Avian veins are fragile and possibility of haematoma formation is more. Blood can be collected from right jugular vein, brachial vein, medial metatarsal (caudal tibial) vein, external thoracic vein, and by cutting claws or direct heart puncture (Table 5.1).

Collection of avian serum in sufficient volume to conduct all the required tests is difficult due to presence of large fibrin clots which often decreases the volume. Heparinized plasma or whole blood is most common sample available from avian practitioners.

| Blood collection sites | Species | Remarks |
|------------------------|---------|---------|
| Right jugular vein     | Flamingos, budgerigars, raptors, penguins, ostrich | Unlike mammals, avian jugular vein is not located in any furrow. It is present subcutaneously at right neck. Due to fat deposition in neck, jugular vein is unsuitable for blood collection from pigeons |
| Brachial vein          | Most of the birds (for e.g. snow goose, gull, bald ibis, pigeon, bald eagle, Eurasian kestrel, hawk, thrush, crane, African grey parrot, rhea etc.) | Possibility of haematoma formation is more in small birds and racing pigeons |
| Medial metatarsal vein | Pigeons, raptors, ducks | Possibility of haematoma formation is low |
| Claw cutting           | Small finches | After cleaning the claws with antiseptic it is cut and the blood is collected in capillary tubes. The bleeding can be stopped with silver nitrate or ferrous subsulphate |
5.3.2 Droppings/Cloacal Swabs

Fresh droppings or faeces should be collected in sterile, screw capped short jars or plastic vials. Rubber caps should be avoided because the gas generated in the collected faeces may blow the cap. The faeces and urine should not be mixed together.

Cloacal swabs are collected by gently mopping the cloaca with sterile cotton swabs. The swabs should be moistened with transport medium before collection. The collected swabs are put into Stuart transport medium, 50% buffered glycerol, Sabouraud broth or BHI broth for transport (Figs. 5.2 and 5.3).

5.3.3 Crop/Proventriculus Washing

Normal saline (warm, 20 ml/kg body weight) is instilled into the crop or proventriculus with a blunt ended catheter or gavage tube. A part of the fluid is aspirated out with gentle negative pressure. For proventriculus washing, endotracheal tube can be used with general anaesthesia.

Fig. 5.2 Collection of cloacal swabs from birds (Courtesy Pratik Ghosh, Department of Veterinary Microbiology, WBUAFS, Kolkata, India)
5.3.4 Air Sac Washing

A sterile catheter attached with a syringe is inserted into the last intercostal space of a bird and sterile normal saline (3 ml for large birds) is injected and aspirated out immediately.

5.3.5 Tracheal Washing

Nylon tube (1 mm in diameter) or a canine catheter is inserted through the glottis and sterile normal saline (0.5–2 ml/kg body weight) is flushed and the washing is collected immediately. It is safe to collect tracheal washing from highly sedated birds.

5.3.6 Autopsy Samples from Vital Organs

The incised portion of the vital organ (liver) is wiped gently with a paper towel to remove excess blood. Indurated tissue is scraped with a sterile scalpel and the tissue is kept over the slide for further examination.
5.3.7 Bone Marrow

Bone marrow is collected from proximal tibiotarsal bone or keel of the sternum. Paediatric bone biopsy needle (23 s.w.g.) can be used for bone marrow collection. Bone marrow samples are useful for confirmation of leukaemia and non-regenerative anaemia.

5.4 Processing of Clinical Samples

Blood, exudates or aspirates from the lesions are required to mix with EDTA (or heparin) to prevent clotting. Physical parameters of the fluid samples such as colour (haemolysis), specific gravity, turbidity (presence of fat particles) are judged. Cellular part of the fluid is concentrated by low speed centrifugation (1500 rpm for 10 min).

5.5 Diagnostic Techniques Used in Laboratory

(a) Direct Examination

A smear can be prepared with the fluid sample for detection of microbes and parasites, morphology and differential counts of cellular components (heterophils, macrophages, leukocytes) and cells with malignancy.

Blood smear is primarily stained with Leishman’s, Wright’s or Giemsa stain. More time is required to stain avian blood cells than the mammalian. The pH of buffered water used for washing the slides should be more acidic (pH 5.0). Total and differential counting of avian blood samples requires expertise, as it is difficult to identify avian leukocytes, scattered throughout the microscopic field. Laboratory with expert technicians is required for estimation of blood biochemical parameters, enzyme and electrolyte concentration. Standard values of avian biochemical parameters, enzyme and electrolyte concentration are described in Table 5.2. These parameters although roughly indicate about occurrence of an infection or infestation or intoxication. It cannot confirm the diagnosis since wide variation exists in standard values between species, physiological conditions (moulting, egg laying, migration, age, sex etc.), and husbandry practices (nutrition, weather, wild or captive etc.). In some rare species no standard value is available to compare.

The smears prepared from tissue samples can be stained with Romanowsky, Wright’s, Giemsa stain or fungal stains (Grocott) (Fig. 5.4). Sudan III or IV along with new methylene blue stain is used if the fat content is more in the collected tissues. Use of bacterial stains such as Gram’s stain and acid fast stain is preferred in suspected clinical cases (Fig. 5.5).
### Table 5.2  Standard values of blood cells, biochemical parameters, enzymes, electrolytes in birds

| Parameter                                | Standard value | Remarks                                                                 |
|------------------------------------------|----------------|-------------------------------------------------------------------------|
| Packed cell volume (PCV)                 | 40–55%         | Young birds (not fully fledged) have lower PCV                          |
| Haemoglobin                              | 12.2–20 g/dl   | Decreased haemoglobin and PCV (haemolytic anaemia): blood parasite infection (*Haemoproteus, Plasmodium, Leucocytozoon*); gastrointestinal parasitism (*Capillaria, ascarids, coccidiosis, giardiasis); mite infestation; bacterial and yeast infection (salmonellosis, colibacillosis, yersiniosis, *Macrorhabdus*, proventricular ulceration, campylobacteriosis); viral infection (Pacheco’s virus, Herpes virus) |
| Mean corpuscular volume (MCV)            | 121–200 fl     | Increased value indicates regenerative or macrocytic anaemia            |
| Mean corpuscular haemoglobin concentration (MCHC) | 28–38 g/dl | Reduced MCHC index (Chronic non-regenerative anaemia): chronic infection (chlamydophilosisis, toxoplasmosis, aspergillosis, salmonellosis, yersiniosis, colibacillosis, campylobacteriosis); toxicosis [lead, copper, zinc, chloramphenicol, pesticides (DDT, carbamates), aflatoxins]; starvation and malnutrition |
| Polychromatic index                      | Raptors: above 3.5% | –                                                                 |
| Total erythrocyte count (TEC)            | $2.1–5.5 \times 10^{12}$/l | Young birds (not fully fledged) have lower TEC; Higher in migratory birds during flying |
| Leukocyte count                          | $1–32 \times 10^9$/l | Leucopenias-toxicosis Leucocytosis-infection with bacteria, fungi, parasite, neoplasia, trauma Heteropenia, lymphopenia-Viral infection Lymphocytosis-neoplasia |
| Thrombocyte count (platelet)             | $20–30 \times 10^9$/l | Thrombocytosis-bacterial infection Thrombocytopenia-severe septicaemia |
**Table 5.2 (continued)**

| Parameter               | Standard value | Remarks                                                                                     |
|-------------------------|----------------|----------------------------------------------------------------------------------------------|
| Total serum protein     | 3–5 g/dl       | Hypoproteinaemia—Hepatopathy, gastrointestinal parasitism, nephritis, trauma, lead toxicosis, Pacheco’s disease, anaemia, malnutrition etc. Hyperproteinaemia—prior to egg laying stage in physiological condition, dehydration, acute infection and shock |
| Total plasma protein    | 0.15 g/dl above the value of serum protein | –                                                                                           |
| Serum albumin           | 1.0–2.2 g/dl   | –                                                                                           |
| Albumin/globulin ratio (A/G) | 1.4–4.9 | Decreased A/G ratio—acute and chronic infections (e.g. *Chlamydomphila*, aspergillosis, mycobacteriosis) |
| Serum glucose           | 200–500 mg/dl (Emu: 158 mg/dl) | Normal value varies with age, diet, breeding season of the birds. The serum glucose level is decreased during day time and increases during night (reverse for nocturnal birds) *Hyperglycaemia*—stress, lead toxicosis, pancreatitis, Diabetes mellitus in granivorous birds, budgerigars, cockatoos, Amazon parrots, macaws, cockatiels, toucans *Hypoglycaemia*—starvation, hypovitaminosis, septicaemia |
| Uric acid               | 2–15 mg/dl     | Normal value is more in carnivorous birds than the granivorous birds. High level of uric acid—starvation, gout, trauma, toxicity (due to excess gentamicin, sulphonamides,azole group antifungals), hypervitaminosis D₃, bacterial or viral infection |
| Urea                    | 2.4–4.2 mg/dl  | High level of plasma urea—dehydration, cardiopathy, cloacal impaction, neoplasm, blockage of renal tubules with urate crystals during salt poisoning |

(continued)
Table 5.2 (continued)

| Parameter                      | Standard value | Remarks                                                                                           |
|--------------------------------|----------------|--------------------------------------------------------------------------------------------------|
| Cholesterol                    | 108–330 mg/dl  | High level—fatty degeneration of liver, xanthomatosis (yellowish cholesterol deposition in any tissue) |
|                                |                |                                                                                                   |
| Bile acids                     | 18–144 µmol/l (psittacines) | High bile acid level in plasma—hepatopathy                                                      |
|                                |                |                                                                                                   |
| Aspartate amino transferase (AST) | 52–270 IU/l | High level indicates hepatopathy, Pacheco’s disease, chlamydomillosis, toxicosis due to pesticides, adverse reaction of drugs (doxycycline injection, azole group of antifungals) |
|                                |                |                                                                                                   |
| Alanine amino transferase (ALT) | 6.5–263 IU/l | High level—hepatopathy                                                                             |
|                                |                |                                                                                                   |
| Lactate dehydrogenase (LDH)    | 46–442 IU/l    | High level—hepatopathy                                                                             |
|                                |                |                                                                                                   |
| Alkaline phosphatase (ALP)     | 42–479 IU/l    | High ALP level—osteomyelitis, bone neoplasms, fractures, aflatoxin poisoning, rickets, hyperparathyroidism, physiologically high during egg laying period |
|                                |                |                                                                                                   |
| Creatine phosphokinase (CPK)   | 110–480 IU/l   | High CPK level—convulsions, lead toxicity, chlamydophilositis, bacterial septicaemia, vitamin E deficiency |
|                                |                |                                                                                                   |
| Calcium                        | 8–12 mg/dl (Budgerigars: 6.4–11.2 mg/dl; chicken: 13.2–23.7 mg/dl) | Hypercalcaemia: ovulation (physiological), dehydration, bone tumour Hypocalcaemia: muscular spasm, seizure, steroid therapy |
|                                |                |                                                                                                   |
| Sodium                         | 127–170 mEq/l  | Hypernatraemia: salt poisoning in wild birds, feeding of excess salt with feed (peanuts, potato crisps) in pet birds |
|                                |                |                                                                                                   |
**Fig. 5.4** *Penicillium* hyphae present in lung tissue of African grey parrot (Grocott stain)  
(*Courtesy* Dr. Giovanni Lanteri, University of Messina, Italy)

**Fig. 5.5** *Klebsiella* spp. in gram stained smear isolated from birds (100×, *Courtesy* Achintya Mahanti, Department of Veterinary Microbiology, WBUAFS, Kolkata, India)
Fresh faeces/droppings can be visualized unstained for detection of parasite eggs, yeast and few bacteria (Fig. 5.6).

(b) Different serological, immunological and molecular biology based tests can be performed for detection of avian infections, infestations or intoxications (Table 5.3). Lack of species specific reagents (polyclonal and monoclonal antibodies), standardized and reproducible techniques, considerable variations in results between laboratories are the major constraints in diagnostics of avian medicine. All the laboratory test reports should be correlated with signs and symptoms of the birds by the veterinarian.
| Table 5.3  Diagnostic approaches for avian infections, infestations and intoxications |
|---------------------------------|---------------------------------|
| **Avian infection**             | **Diagnostic approaches**       |
| Avian aspergillosis             | Antigen detection ELISA, PCR, isolation |
| Avian influenza                 | Hemagglutination-inhibition (HI), agar gel immunodiffusion (AGID), virus neutralization, enzyme-linked immunosorbent assay (ELISA), PCR, isolation of virus |
| Beak and feather disease        | Haemagglutination inhibition (HI), blocking ELISA, histopathology, PCR |
| Avian Bornavirus infection      | ELISA, indirect immunofluorescence assay, isolation of virus, histopathology, reverse transcriptase-PCR, radiography |
| Campylobacteriosis              | Direct examination, isolation of bacteria, ELISA |
| Avian chlamydophilosus          | Direct examination, micro immunofluorescence (MIF) test, ELISA, CFT, elementary body agglutination test, isolation, PCR |
| Avian cryptococcosis            | Direct examination, Detection of Cryptococcal antigen by ELISA, isolation, PCR |
| Avian cryptosporidiosis         | Direct examination, Capture enzyme-linked immunoassays, PCR |
| Avian giardiasis                | Direct examination, ELISA (antigen capture), immunofluorescence, PCR |
| Lyme disease                    | Direct Examination, isolation, ELISA, PCR |
| Avian mycobacteriosis           | Direct examination for acid-fast organisms, histopathology, PCR, isolation |
| Newcastle disease               | Virus neutralization test, plaque neutralization, hemagglutination-inhibition, agar gel immunodiffusion, enzyme-linked immunosorbent assay, isolation of virus, real-time reverse-transcriptase polymerase chain reaction |
| Salmonellosis                   | Isolation of bacteria, rapid whole blood/serum agglutination test, ELISA, PCR |
| Avian yersiniosis               | Direct examination, isolation of bacteria |
| Mycoplasmosis                   | Direct examination with contrast phase microscopy, dark phase illumination techniques, serum plate agglutination test, PCR, isolation of Mycoplasma |
| Pasteurellosis                  | Direct examination, isolation of bacteria |
| Escherichia coli infection      | Isolation of bacteria, PCR |
| Avian candidiasis               | Wet mount with 10% KOH, histopathology, isolation, PCR |
| Mycotic proventriculitis        | Direct examination by Gram’s staining, histopathology |
| (Macrorhabdus ornithogaster)    |                                |
| Avian sarcocysticosis           | Visualization of sarcocystis in the muscles after post-mortem, muscle biopsy, indirect immunofluorescent assay, PCR |
| Avian trichomoniasis           | Wet mount |
| Coccidiosis                     | Examination of faeces for oocysts or macrogametes |
|                                 | (continued) |

(continued)
5.6 Diagnostic Techniques in Avian Clinics or Hospitals

5.6.1 Radiography

Digital radiography is a useful diagnostic technique used currently in avian medicine for rapid detection of underlying hidden etiology. It is easy to perform in birds due to smaller size and one exposure is sufficient to take the image of the whole body. Avian air sac system acts as a negative contrast to the organs which make the interpretation easier for the radiologists. Other than high power X-ray apparatus, film-screen combination and developing system, expert technician is required for
interpretation of avian radiographic images. Short exposure times (0.015–0.05 s) are recommended for taking avian images because image quality is detoriated due to high respiration rate of the birds even under anaesthesia. For taking images of internal organs and skeleton of birds, rare earth screens and mammography screens are recommended, respectively. In general, fine film screen combinations are preferred for avian images.

5.6.2 Ultrasonography (USG)

In ultrasonography, the image is produced with transmission and reflection of sound waves. In birds, use of USG is limited because the ultrasound cannot invade a gas filled air sac. Other hindering factors include circulatory and respiratory distress of birds during examination, application of coupling gels to make contact between transducer and avian skin, and lack of experienced veterinarians. Fasting of the birds for 2–4 h is required before USG examination. Confirmation of hepatomegaly, cardiac disease, disorders of kidney and reproductive tract, and ascites is possible through USG examination.

5.6.3 Computed Tomography (CT)

This technique generates a cross-sectional image for accurate visualization through the use of X-rays. General anaesthesia is required and the whole procedure takes 10–15 min time to be completed. Examination of avian skull, sinuses and lower respiratory tract is possible through CT.

5.6.4 Magnetic Resonance Imaging (MRI)

This technique also generates cross sectional images through strong external magnetic force. MRI can detect the presence of caseous plug, granuloma, mucocele, polyp in brain, spinal cord, coelomic organs and upper respiratory tract. Detection of accurate location of these lesions helps in surgery. MRI takes longer time than CT for examination.

5.6.5 Myelography

In larger birds (1 kg or more), images are taken after injection of non-ionic iodinated contrast medium (0.8–1.2 ml/kg body weight) into subarachnoid space at thoraco-synsacral junction. The technique is indicated for detection of compressive and traumatic lesions in the spinal cord.
5.6.6 Echocardiography

Echocardiography provides useful information of cardiac function and structure of the heart. Earlier it was difficult in birds due to presence of air sacs which block the passage of ultrasound waves. Currently echocardiograph machines with advanced technology (7.5 MHz or higher frequency, doppler function, more than 100 frames/s) is used successfully in avian medicine. Fasting for 2–12 h (psittacine, pigeons) or longer period (raptors) is recommended before echocardiography. Food filled enteric tract may create an obstruction between the machine and heart. Ventromedian (psittacines, raptors) and parasternal (pigeons) approaches are followed for avian echocardiography.

5.6.7 Electrocardiography (ECG)

ECG is used to observe cardiac function during anaesthesia and for recording cardiac stages (systole, diastole). Use of ECG in avian medicine is not frequent due to difficulties in making connection of leads with skin of the birds, lack of reference values and alterations of ECG values under stress or anaesthesia.

5.6.8 Endoscopy

Avian endoscopy helps the clinicians to examine internal organs (lungs, air sacs, heart, intestinal tract, liver, kidneys, adrenal glands, spleen, pancreas, gonads, oviduct, and shell gland) through a small and single incision. Endoscopy of oral cavity also allows examination of esophagus, crop, proventriculus, glottis and trachea. In addition to physical examination, clinicians can collect tissue biopsies from vital organs, coelomic musculature and abnormal soft tissue structures.