

# Animal Health Laboratory



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10<sup>th</sup> annual CAHLN-RCTLSA meeting in Guelph a

### success!

Over 140 delegates attended the annual scientific meeting of the Canadian Animal Health Laboratorians Network - Réseau Canadien des Travailleurs des Laboratories de Santé Animale (CAHLN- RCTLSA), June 5-8, 2011, hosted by the University of Guelph, in Guelph, Ontario. The theme was *"New Technologies – New Horizons"*, and attendees were updated through 46 oral presentations, 21 posters, and 10 exhibitors.

- Also held concurrently were meetings of the Canadian Association of Veterinary Pathologists – L'Association Canadienne des Pathologistes Vétérinaries (CAVP-ACPV), the Canadian Association of Poultry Veterinarians – L'Association Canadienne des Vétérinaires Aviaires (CAPV-ACVA), the Canadian Animal Health Surveillance Network (CAHSN), and the National TSE Veterinary Laboratory Network.
- Selected for the 2011 Laboratorian of the Year Award for her meritorious service to veterinary laboratory medicine was **Dr. Josepha DeLay**, of the Animal Health Laboratory (photo). Dr. Delay is an accomplished veterinary pathologist and founder of the immunohistochemistry service at the AHL.
- The 2011 Graduate Student Presentation Award went to **Dr. Olivier Côté** for his presentation "*Role of Clara cell secretory protein in lung inflammation*". Dr. Coté, from the Department of Pathobiology, Ontario Veterinary College, was one of an excellent roster of 16 graduate student presenters at the meeting.
- The Constitution of the CAHLN-RCTLSA was adopted at the annual business meeting, and the Executive Committee members elected for 2011-2012 are **Dr. Grant Maxie**, President; **Dr. John Copps**, President-Elect; and **Ms. Marilyn Jonas**, Secretary-Treasurer.

The 2012 meeting will be held in Winnipeg Manitoba, June 2-6, at the National Center for Foreign Animal Disease (NCFAD). <u>http://www.cahln-rctlsa.com/index.html</u> AHL

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The Newsletter is available on-line at <u>http://ahl.uoguelph.ca</u> under "Publications".



Presentation of the 2011 CAHLN-RCTLSA Laboratorian of the Year Award to **Dr. Josepha DeLay** (left) by Dr. Murray Hazlett.



**Dr. Olivier Côté** (right) receives his CAHLN-RCTLSA Graduate Student Presentation Award from Dr. Grant Maxie.

### New 2011 AHL Bacteriology combined tests Durda Slavic

In addition to our regular bacteriology tests, this year AHL Bacteriology introduced a few additional combined tests. Our new tests are:

- **Combined aerobic and anaerobic culture**, code: **ancultn**, price \$65.00. No set-up fee applies.
  - Used for a maximum of 2 samples (1 aerobic and 1 anaerobic swab) when aerobic and anaerobic culture is needed.
  - Alternatively, this test can be used for 1 sample when aerobic and anaerobic culture is needed.
  - Susceptibility testing is included for fast-growing aerobic bacteria.
- Fecal culture with detection of *Clostridium perfringens* toxin, code: fecultn, price \$55.00. No set-up fee applies.
  - Used when a fecal sample is submitted for culture and detection of *C. perfringens* enterotoxin.
  - Susceptibility testing is included for fast-growing aerobic bacteria.
- Follow up test, code: cultn2, price \$25.00. No set-up fee applies.
  - Used for the same patient and same sample type if submitted within 2 months of the initial submission.
  - Susceptibility testing is included for fast-growing aerobic bacteria.

Additional bacteriology tests can be viewed and searched at http://www.ahl.uoguelph.ca AHL

### Tests ISO 17025 accredited in AHL Mammalian Virology by Standards Council of Canada:

- MOL-196 PRRSV Tetracore NA-EU real-time RT-PCR
- MOL-206 Swine influenza virus matrix real-time RT-PCR
- MMV-214 Bovine viral diarrhea virus antigen ELISA
- MMV-310 TGEV-Ab and PRCV-Ab EIA differentiating test
- MMV-334 Indirect fluorescent antibody assay (IFA) for the detection of IgM or IgG antibodies against *Porcine reproductive and respiratory syndrome virus* (PRRSV)

**Contributors to this issue:** 

- MMV-342 General procedure for hemagglutination inhibition (nested method appendix 16.3, 16.4, 16.5, 16.8)
- MMV-342 A16.8 Swine influenza virus A/H3N2/swine/Texas/4199-2/98 HI
- MMV-342 A16.10 Swine influenza virus A/H1N1/swine/Ontario/81 HI and A/H3N2/human/Colorado/77 HI
- MMV-345 PRRSV X3 & SIV (H1N1 and H3N2) antibody ELISA AHL

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| Our continued thanks to all of the non-author AHL clerical, tech-   |  |
| nical, and professional staff who contribute to the generation of results reported in the AHL Newsletter. |  |
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# A temporal study of Salmonella serovars in breeder flocks and hatcheries in Ontario between 1998 and 2008

Theva Sivaramalingam, Michele Guerin, Scott McEwen, David Pearl, Davor Ojkic

Under the Ontario Hatchery and Supply Flock Policy (OHSFP), routine *Salmonella* surveillance is carried out using fluff samples from hatcheries and environmental samples from poultry breeder flocks. In this study, OHSFP data from 1998 to 2008 were analyzed to:

(i) identify major *Salmonella* serovars and determine their prevalence and temporal patterns; and

(ii) identify temporal trends and clusters of the most frequent *Salmonella* serovars.

In breeder flocks, the prevalence of Salmonella was highest in broiler breeder flocks (47.4%), followed by layer breeder flocks (25.7%) and turkey breeder flocks (19.6%). However, this pattern did not continue at the hatchery level, in which the prevalence of Salmonella was highest in turkey-breeders (13.2%), followed by other-breeder birds (ducks, geese, quail, partridges, and pheasants; 11.9%), broiler-breeders (8.7%) and layer-breeders (3.1%). In breeder flocks, decreasing trends in the prevalence of Salmonella were observed in all poultry types. In contrast, in poultry hatcheries, trends in the prevalence of Salmonella were less consistent. Decreasing trends in the prevalence of Salmonella in broiler-, layer-, and turkey-breeder flocks were primarily due to decreasing trends in the prevalence of Salmonella Heidelberg. Similarly, in the hatcheries, the increasing trend in broiler-breeders was mainly driven by increasing trends in the prevalence of Salmonella Kentucky and Enteritidis, while the increasing trend in other-breeder birds was mainly due to increasing trends in Salmonella Enteritidis, Typhimurium, and Heidelberg. There was seasonal variation in *Salmonella* prevalence in breeder flocks, with an increased risk of Salmonella in the fall (dependent on the year). Among hatcheries, seasonal variation was also identified; however, the increased risk of Salmonella was associated with the summer season (dependent on the year).

One of the key findings of this study was that *Salmonella* Enteritidis was not isolated from layer- and turkey-breeder hatchery fluff samples. However, *Salmonella* Enteritidis was isolated from broiler-breeder and other-breeder bird hatcheries. The PT 13 and 8, and PT 9b were the most common phage types of *Salmonella* Enteritidis in

broiler-breeders and other-breeder bird hatchery fluff samples, respectively. A large cluster of 166 isolates of *Salmonella* Enteritidis from broiler fluff was detected from July 2005 to December 2008. During the entire study period, the prevalence of *Salmonella* Enteritidis from environmental samples from broiler breeder flocks in Ontario was low (<1% of the total broiler-breeder isolates) suggesting that domestic-origin broiler-breeders might not be an important source of this serovar to Ontario broiler hatcheries. The importation of hatching broiler eggs contaminated with *Salmonella* Enteritidis from the US might be one possible source of this serovar, and might explain the increasing prevalence of *Salmonella* Enteritidis, as imports from the U.S. increased between 2004 and 2007.

Salmonella Heidelberg was another prominent serovar. It was the most common serovar in broiler-, layerand turkey-breeder flocks, and among the 4 most common serovars in hatchery fluff samples from all poultry types. Of note, clusters of Salmonella Heidelberg in broiler-, layerand turkey-breeder flocks preceded or overlapped clusters in the respective hatcheries. Similarly, Salmonella Typhimurium and Hadar in broiler breeder flocks were temporally linked to clusters in the broiler-breeder hatcheries. These findings suggest that, for these 3 serovars, breeder flocks were the most probable source of the bacteria to the hatcheries. Interventions at the breeder flock level could reduce the transmission of these serovars to hatcheries, and possibly to lower levels of the production chain and poultry products.

A significant amount of variation in the prevalence of *Salmonella* could be explained by differences among breeder flocks, among hatcheries, and also among sampling visits. Future studies should investigate reasons for these differences, and implement control measures at each level in order to reduce the prevalence of *Salmonella* throughout the production chain. *AHL* 

#### Acknowledgment

This project was supported by the OMAFRA-UG Agreement through the Animal Health Strategic Investment fund (AHSI) managed by the Animal Health Laboratory of the University of Guelph.

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# AHL Lab Reports

# RUMINANTS

## Clostridial myositis (blackleg) in cattle in eastern Ontario

Andrew Brooks, Jan Shapiro

In June 2011, 5 cases of clostridial myositis, "blackleg", were diagnosed at the Animal Health Laboratory in Kemptville. Four cases, all from 1 farm, involved mixed breed beef cattle 14-20 months in age, and a fifth case involved a 12-month-old Holstein dairy heifer. The histories were sudden death with no premonitory signs except 1 animal that was recumbent and stargazing.

The gross lesions at necropsy were similar in all cases, with multiple regions of necrotic muscle in the neck, brisket, vertebral column, limbs, diaphragm or tongue. The affected muscle was dark red or black with variable amounts of emphysema and crepitus. Similar lesions were also present in the heart, and some animals also had fibrin deposits on the endocardium and pleura. There were no discernible skin wounds overlying the necrotic muscle. Fluorescent antibody testing was pursued in 2 cases, which confirmed the presence of *Clostridium chauvoei* in the lesions.

Blackleg is an acute fatal disease of ruminants caused by activation of latent spores of *C. chauvoei*. Black-

leg occurs most often in the summer in pastured beef animals. *C. chauvoei* inhabits the intestine, and fecal contamination of the soil is the likely source of infection for other animals. The ingested clostridial spores reside in muscle tissue and the disease is precipitated by local muscle injury or hypoxia which leads to spore activation, gangrenous muscle necrosis, and toxemia.

Blackleg is enzootic in certain regions and often occurs as local outbreaks. Increased prevalence has been associated with soil excavation and high rainfall. Affected cattle are typically between 6 months and 2 years of age, in good condition, rapidly growing, and on a high plane of nutrition. Most animals are found dead, but severe lameness, depression, anorexia, fever, tissue swelling, and crepitus may be detected.

Treatment is not effective, but the disease can be controlled by vaccination. None of the animals in this report were known to be vaccinated for blackleg. *AHL* 

### Hemonchosis on the rise in Ontario Paula Menzies, Andrew Peregrine

We seem to be experiencing an increase in small ruminant morbidity/mortality caused by *Haemonchus contortus* infections, perhaps as a result of wet spring and warm summer conditions. As well, a number of "type II hemonchosis" cases were seen in periparturient ewes this spring. These cases may be associated with anthelmintic resistance, and the veterinarian needs to investigate the producer's gastrointestinal parasite control program and, if AR is suspected, conduct a **fecal egg count reduction test** or a **drench response test**. A PDF of a handbook on the control of internal parasites of sheep can be obtained by emailing Dr. Menzies at <u>pmenzies@uoguelph.ca</u> *AHL* 

### Johne's disease in an alpaca Durda Slavic, Tony van Dreumel

A 5-year-old, male alpaca was off feed for almost 3 weeks with intermittent diarrhea that was unresponsive to antibiotics. The animal was euthanized, and during the field necropsy multiple 3 cm diameter lumps were found surrounding the mesenteric tree. A swab of a lump center and fixed pieces of lump and gut were submitted for bacteriology and histopathology work up. Histopathology revealed that the mass consisted of sheets of histiocytic type cells. The same cell type was also present in the lamina propria and submucosa of the gut section. An acid-fast stain of the gut section revealed abundant intracytoplasmic acid-fast bacilli. Based on histopathology findings, **Johne's disease was suspected and subsequently confirmed by PCR**.

A disease clinically resembling Johne's disease caused by *Mycobacterium avium* subsp *paratuberculosis* 

(MAP) was described in camelids as early as 1954. Clinically, disease in camelids is characterized by a history of weight loss and diarrhea, which is similar to clinical signs of this disease in other susceptible animal species. Unlike in other animal species, however, Johne's disease in alpacas can also affect animals under 2 years of age. On necropsy, the main finding in affected animals is enlarged lymph nodes. Further confirmation of the disease can be carried out by the presence of histopathological changes and acid-fast bacilli in affected lymph nodes, positive PCR, and/or culture for MAP. At present, the value of serological tests for disease detection in alpacas has not been well established.

There is no information about epidemiology and prevalence of Johne's disease in alpacas in Ontario. As a result, **this disease should be added to the list of differen**-

# SWINE

### New real-time PCR test for swine cytomegalovirus

Susy Carman, Li Ge

Swine cytomegalovirus (SCMV) is a betaherpesvirus, also known as *Suid herpesvirus 2* (SuHV-2). SCMV infection in adults is usually subclinical, but infection of piglets born to naïve non-immune gilts can result in stillbirths, mummified fetuses, and severe generalized disease when piglets are infected in utero or at parturition. Clinical signs in piglets may include lethargy, anorexia and respiratory signs, such as sneezing, nasal discharge, coughing, rhinitis, runting, and neurological disease. SCMV infection in piglets has been called **inclusion body rhinitis**, as a result of the basophilic intranuclear inclusions seen in cells of the nasal turbinates. Recently, **SCMV infection has been suggested as a cause of the post-weaning failure-to-thrive syndrome.** 

The AHL now offers a real-time PCR for SCMV. The fee is \$30.00 per test.

- For **aborted fetuses and neonates**, send cerebellum, olfactory lobe, liver, and bone marrow, since fetuses and neonates have generalized infection of reticuloendothial tissues.
- From live piglets, please send nasal swabs in 0.5 mL saline, or 2 mL of EDTA blood.
- For **dead piglets**, send lung, nasal turbinate mucosa, and kidney, as older piglets have infection of epithelial tissues. *AHL*

# Yellow fat and steatitis in a group of pigs

Murray Hazlett, George Branov, Debbie Brick

Seven barbecue pigs were slaughtered and found at inspection to have generalized severe yellow fat discoloration (Fig. 1). Both the sow and the piglets were fed a mixture of  $\frac{1}{2}$  ground flax seed and  $\frac{1}{2}$  grain mix (barley, oats and corn). The sow was not slaughtered. Dressed weights of the pigs ranged from 10-30 kg.

Samples of skin and subcutaneous fat were submitted to the AHL in Guelph for histopathology. The submitted tissue was composed primarily of adipocytes which contained variably sized fat vacuoles. Several large areas of fat necrosis could be seen, with secondary inflammation composed of lymphocytes and macrophages, and in some areas neutrophils were seen (Fig. 2).

The changes seen most likely represent a toxic or dietary issue in these pigs. In some animals (cats and mink), steatitis (or yellow fat disease) is associated with high-fat diets, in particular with some types of fish oils (cats) and low levels of vitamin E. This has also been seen in fattening pigs associated with a high fishmeal and oil diet, and was reported to be vitamin E responsive. Fat discoloration in hogs associated with feeding flaxseed high in linolenic acid has also been recorded, although microscopic examination of the tissues was not mentioned and we are unaware of any recent reports. The mechanism of action is not known, but we suspect it may have to do with oxidant activity or destruction of antioxidants, in particular vitamin E. *AHL* 

#### References

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Figure 1. Generalized yellow discoloration of body fat in a barbecue pig.



Figure 2. Fat necrosis with secondary inflammation in the yellow fat of a barbecue pig.

# AVIAN/FUR/EXOTIC SPECIES

# Inclusion body disease (IBD) of boid snakes

Emily Martin

A 1-year-old female boa with a short clinical history of 'head flipping', no previous signs of disease and sudden death was presented to the AHL for post mortem examination. At necropsy, the lungs and liver were dark red and there was accumulation of white material in the kidneys and white granular material in the ureters. On histopathology, there was pneumonia, pericarditis, urate accumulation in the kidneys and ureters, and numerous eosinophilic intracytoplasmic inclusion bodies in multiple organs. These inclusion bodies are consistent with a diagnosis of inclusion body disease (IBD) of boids.

IBD is seen in the families Boidae and Pythonidae and has a worldwide distribution. IBD is considered one of the most important diseases of captive boids and there is concern that this disease will become established in native wild populations by snakes bred for release into the wild. This disease can be subclinical and can be latent for long periods of time. Affected snakes show various clinical signs depending on species. Pythons have more rapid and severe disease progression than boas, and young snakes can have acute disease onset and high mortality whereas affected adults can experience long-term debilitating disease. There are no pathognomonic signs of IBD, however pythons and boas often have neurologic signs and boas can also display regurgitation. Other clinical signs and secondary infections include chronic wasting, stomatitis, pneumonia, lymphoproliferative disorders and round cell tumors. It is not known if there are different pathogenic strains to explain the clinical difference between species.

Diagnosis is by light microscopic demonstration of eosinophilic intracytoplasmic inclusions. **The etiologic agent is unknown.** The inclusions are not considered viral but consist of a protein called **inclusion body disease protein (IBDP)**. There is ongoing investigation into the possible role of viruses. In pythons, inclusions are mostly found within neurons in the CNS, and encephalitis, if present, is more severe in this species. In boas, inclusions are found in the CNS (neurons and glial cells), mucosal epithelium overlying and lymphoid cells within the esophageal tonsils, gastrointestinal epithelium (Fig. 1), respiratory epithelium, hepatocytes, pancreatic acinar cells (Fig. 2), and renal tubular epithelium.

The route of transmission is undetermined, but possibilities include direct contact, transfer by mites (*Ophionyssus natricis*), or vertical transmission from mother to offspring. Antemortem diagnosis is difficult as there are no serologic tests available. Antemortem tests described in the literature include screening blood samples for inclusions in lymphocytes or looking for inclusions in tissue biopsies of the liver, stomach or esophageal tonsils. **Currently, the**  main diagnostic test is post mortem identification of inclusion bodies within tissue sections. There are no vaccines available and no known treatment exists. **Preventing** introduction of this disease into collections is critical. *AHL* 

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Figure 1. Section of intestine with numerous eosinophilic intracytoplasmic inclusion bodies.



Figure 2. Section of pancreas with numerous eosinophilic intracytoplasmic inclusion bodies.

# HORSES

# Optimizing diagnostic value of equine endometrial cytology and biopsy submissions

Maria Spinato, Josepha DeLay, Beverly McEwen, Kristiina Ruotsalo, Tracey Chenier

Endometrial cytology and biopsy are valuable diagnostic procedures for the assessment of reproductive health in mares. A single small sample of tissue, usually measuring less than 2 cm in length and 0.5 cm in diameter, can be examined microscopically for features such as stage of reproductive cycle, acute versus chronic inflammation, infectious agents, fibrosis, and endometrial maldifferentiation. As another breeding season draws to a close, we have reviewed this year's submissions, and have compiled a few recommendations that will optimize the information that practitioners can obtain from their diagnostic submissions.

Similar to any other type of laboratory submission, a detailed history is critical to guiding diagnostic interpretation of endometrial biopsies. At a minimum, this should include: age of mare, # of years barren, breeding history, stage of estrus cycle, and clinical findings during the reproductive examination.

Endometrial cytology: Cytologic specimens should always be examined along with consideration of culture results. These are best prepared from a swab cap or tip, or low-volume uterine lavage. Some mares with negative cultures will have evidence of bacterial infection in cytologic smears. Conversely, a negative cytology result will assist in ruling out endometritis in cases where positive bacterial cultures are due to inadvertent contamination of the endometrial swab. The best cytology specimen is a smear that is prepared by the practitioner immediately after sampling. The swab cap or tip should be gently rolled onto a glass slide, followed by vigorous air drying. Low-volume uterine lavage is a recently described technique that increases the diagnostic sensitivity of cytology in mares with endometritis; a reference is included below. The worst cytology specimens are those prepared at the AHL from culture swabs, as the cells are invariably damaged beyond recognition. Therefore, please refrain from requesting cytology on culture swabs.

**Endometrial biopsy:** Histologic assessment of endometrial biopsies is based upon the internationally-recognized modified Kenney-Doig grading system. The

distribution and severity of inflammation and fibrosis in the biopsy are evaluated, and the endometrium is classified as normal (category I), or as showing mild (IIA), moderate (IIB), or severe (III) histologic lesions. As the histologic grade increases, the expected foaling rate decreases. The best diagnostic sample includes 2 endometrial biopsies, 1 collected from each uterine horn at the junction to the uterine body.

Careful handling and fixation of biopsies are critical to preventing artifacts that obscure important histologic features.

- Avoid excessive manipulation of the biopsy to prevent loss of luminal epithelium, distortion of glands and crushing artifact.
- Immerse the biopsy immediately in 10% neutral buffered formalin to avoid vacuolation and exfoliation of luminal epithelium.

For many years, Bouin's fixative was advocated as the fixative of choice for endometrial biopsies, due to better tissue hardening and preservation of cellular detail. However, over-hardening is a frequent undesirable event if the biopsy is not removed from Bouin's within 24 h and transferred to either 70% alcohol or 10% formalin. Since Bouin's fixative contains picric acid, a hazardous chemical, there are health and safety issues as well as complex disposal protocols for laboratory staff to follow.

Most pathologists and theriogenologists find that formalin fixation produces good preservation of cellular features, and actually prefer it to Bouin's. **Therefore, the AHL will no longer ship Bouin's fixative to veterinary clinics, and we strongly encourage the use of 10% buffered formalin as the fixative of choice for endometrial biopsies.** *AHL* 

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# **COMPANION ANIMALS**

### Giardia genotyping and Bartonella PCR are now available at the AHL Hugh Cai

**Giardia genotyping assay:** From 2008 to 2010, the AHL evaluated the effectiveness of various *Giardia* assemblage typing methods with 118 canine *Giardia*-positive fecal samples. We found that the combination of *ssu-rRNA* gene and *beta-giardin* gene sequencing methods was most effective, being able to type 64% (75/118) of the *Giardia*-positive canine fecal samples . The assay is now offered as a routine service at the AHL, consisting of *ssu-rRNA* gene and *beta-giardin* gene sequence analysis. To date, most of the positive canine fecal samples tested at the AHL contained *G. duodenalis* assemblages D and C, i.e., non-zoonotic assemblage types. **The fee for the** *Giardia* **genotyping assay is \$100 per sample.** 

**Bartonella PCR test**: This PCR method detects Bartonella species in canine and feline blood samples. The assay was previously optimized and evaluated from published assays by Dr. Prescott's group at the Department of Pathobiology, who found that the overall prevalence of *Bartonella* spp in Ontario was 4.3%. The analytical specificity was confirmed at the AHL by testing pure *Bartonella* cultures and a panel of non-*Bartonella* bacteria. **The fee for** *Bartonella* **PCR is \$32 per sample.** 

**Contact: Dr. Hugh Cai** or the **AHL Molecular Biology Lab** (519-824-4120, ext. 54086). *AHL* 

#### References

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### Testing of pet birds available for Coxiella and Chlamydophila

Hugh Cai, Marina Brash, Margaret Stalker, Emily Martin

The AHL has obtained funding from the Animal Health Strategic Investment (AHSI) program to carry out a project to determine the prevalence of *Chlamydophila*, *C. burnetii* and the avian *Coxiella*-like bacteria in pet birds submitted for necropsy to the AHL. The utility of different diagnostic tools is being evaluated using different sample types, including fecal samples.

Practitioners are encouraged to send in pet birds for necropsy and *Chlamydophila* and *Coxiella* testing. These tests will be free of charge on PM cases until August 31, 2012.

*Chlamydophila psittaci* is a well-known zoonotic pathogen which causes psittacosis in humans, and chlamydiosis in poultry and wild birds. *Chlamydophila abortus*, an ovine pathogen causing late-term abortions, may also play a role in avian *Chlamydophila* infection as it has been isolated from birds in a location with extensive ovine enzootic chlamydiosis, suggesting cross transmission of the pathogen between livestock and birds.

*Coxiella burnetii* is a second important zoonotic pathogen affecting primarily small ruminants, although infection in birds has also been reported sporadically. The prevalence of *C. burnetii* infection in a poultry farming region in Japan was 7%. In Spain, DNA of *C. burnetii* was detected in 11% of vultures and 14% of black kites.

In 2008, a **new avian** *Coxiella*-like bacterium was described from 7 psittacines and a toucan. A similar finding was reported in Ontario where a 7-month-old male blue-headed pionus parrot was found to contain a DNA sequence with 99% identity to this novel *Coxiella* sp. Birds with this *Coxiella*-like organism have anorexia, lethargy, weight loss, and respiratory distress prior to death. The public health significance of this organism is not yet clear. *AHL* 

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