



AHL Newsletter

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Dr. Margaret Stalker rejoins the AHL

Dr. Marg Stalker was the successful candidate in our recent competition for a Veterinary Pathologist, Mammalian/Avian, and rejoined the AHL 3 days/wk as of Sept 5/06, and full-time as of May 1/07.

Dr. Stalker worked for VLSB from 1994-97, the AHL from 1997 to 2002, and has been an Assistant Professor in OVC Pathobiology for the last 3 years. She holds a BSc degree from Queen's ('82), a DVM from WCVM ('87), a PhD from Guelph ('94), and was board-certified by the American College of Veterinary Pathologists in anatomic pathology in 1996.

Welcome back Marg!



Helen Kocmarek, Clinical Pathology Technical Supervisor

Helen Kocmarek received an Honors B.Sc. from the University of Guelph (1978) and a diploma in Medical Laboratory Technology from Mohawk College (1980). She has worked in Clinical Chemistry and the Core labs at the Hamilton and Hender-son General Hospitals. She is a member in good standing with the Canadian Society of Medical Laboratory Scientists and the College of Medical Laboratory Technologists of Ontario.

In 2000, Helen joined the Clinical Pathology section of the Animal Health Laboratory. She has been involved in committee work for continuous improvement, process redesign and our new laboratory information system.

Welcome to the technical supervisor position Helen!



AHL Christmas hours, 2006

Between Christmas and New Year's, the AHL will be open with limited staffing and offering limited services 9 AM to 5 PM, December 27, 28, 29 (Weds, Thurs, Friday) in both Guelph and Kemptville. Emergency necropsy service is available and Specimen Reception is staffed in the Guelph lab on weekends and holidays except for Christmas Day, Dec. 25 (lab is closed) - our specimen drop box is available 24/7.

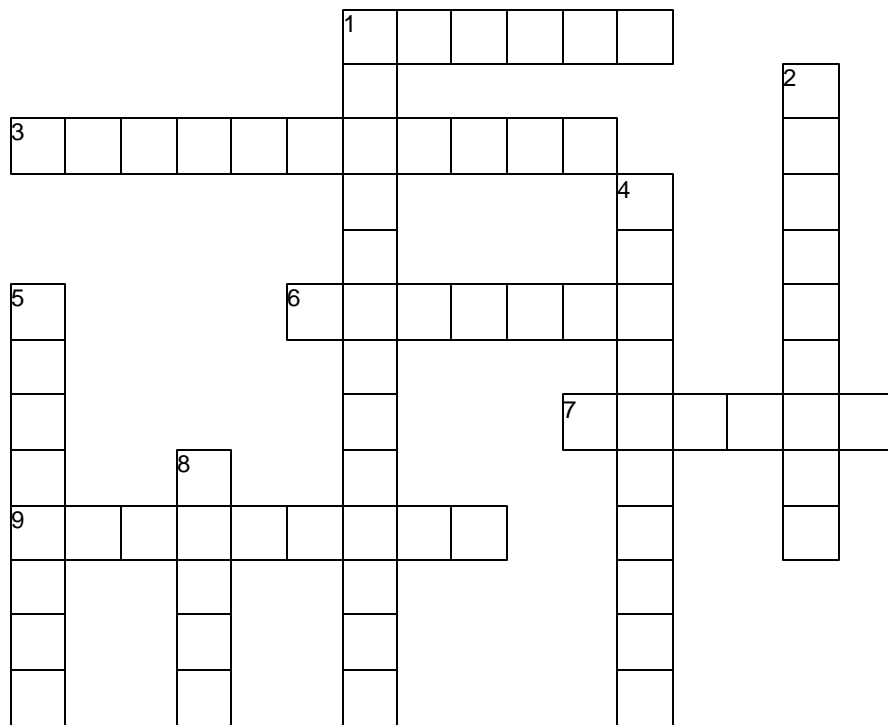
If you would like to schedule testing over this period, please call by Dec 11. If you have testing that must be done urgently over the holidays, please call the lab to determine the availability of the testing that you require. We will do our best to oblige you and your clients.

Please note that the last day for shipping samples to US laboratories will be Weds. Dec. 20/06. Shipments to the US will resume Tuesday Jan. 2/07.

Season's Greetings from
the staff of the Animal
Health Laboratory



Murray's Dec/06 AHL Newsletter crossword *Murray Hazlett*



www.CrosswordWeav

Across

- 1. Most common equine abortifacient agent
- 3. A skin infection in fish handlers
- 6. Returning hero with villainous name
- 7. Common leptospiral species from bovine abortions
- 9. *Eucoleus aerophilus* ova can easily be confused with ova of

Down

- 1. Latest hemotropic *Mycoplasma* from a dog in Ontario
- 2. New polyclonal antibody used to look for ...
- 4. Now more RFLP type 321 found
- 5. Gram stain reaction of *Erysipelothrix*
- 8. Centre looking for influenza in wildlife

Answers on p. 32.

Tulathromycin - new antimicrobial

A new antimicrobial agent of the macrolide class, **tulathromycin (Draxxin, Pfizer)**, has been added to the **porcine and bovine respiratory antimicrobial susceptibility panel**. Tulathromycin is being tested against *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, *Bordetella bronchiseptica* and *Actinobacillus pleuropneumoniae* using the disk diffusion method. *AHL*

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 Editorial Assistant: **Ms. Helen Oliver**

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Our continued thanks to all of the non-author AHL clerical, technical, and professional staff who contribute to the generation of results reported in the AHL Newsletter.

AHL Lab Reports

RUMINANTS

Bovine *Leptospira interrogans* serovar *pomona* abortion

Beverly McEwen, Josepha DeLay, Henk Ten Oever, Davor Ojkic

Tissues from a bovine fetus and placenta were submitted for examination following 4 late-term abortions (7-8 months gestation) in a group of 9 bred heifers. The heifers were pasture fed and drank water from a creek. They had been vaccinated against BoHV-1/BVDV/BRSV/BPIV-3 but not *Leptospira* sp.

Histological lesions included hepatocellular atrophy and dissociation, mild periportal lymphocytic infiltration, and moderate cholestasis. Mild fetal pneumonia was present and there was edema and mineralization of the chorioallantois. Large numbers of individualized organisms with morphology consistent with leptospire were identified in the liver by immunohistochemistry using an anti-*Leptospira* spp. polyclonal antibody cocktail. Other abortifacient pathogens were not identified or isolated.

Two of the heifers that aborted were seropositive to *Leptospira interrogans* serovar *pomona* (*L. pomona*) with titers of 1:2560 and 1:640. For both animals, titers to *L. hardjo* and *L. icterohaemorrhagiae* were in the suspicious range of 1:80-1:160. Although serological cross-reactions to

multiple serovars occur with *Leptospira* sp. infections, **the high titers to *L. pomona* strongly suggest that it was the significant serovar in this case.**

Previous serological studies in Ontario cattle have found that exposure occurs commonly to *Leptospira* serovars

pomona, *hardjo* and *icterohaemorrhagiae*. Infections tend to increase in the fall, especially during periods of wet weather, although they can occur at any time of year.

Depending on the serovar, infection with *Leptospira* spp. in cattle can cause abortion, infertility, systemic ill-

ness, neonatal death, and atypical mastitis. Abortions usually occur in the third trimester and there may be a delay between acute infection in the dam, fetal death and abortion. A wide range of vertebrates including swine, dogs, horses, rodents, small ruminants and humans are susceptible to infection and disease. Transmission occurs via contact with infected urine, soil, water, and fomites.

In addition to **serology**, the AHL offers **PCR** for *Leptospira* sp. on urine and tissues (from untreated animals) and **immunohistochemistry**. AHL

Infection with *Leptospira* spp. in cattle can cause abortion, infertility, systemic illness, neonatal death, and atypical mastitis

AVIAN SPECIES

Wildbird avian influenza virus survey update

Davor Ojkic

A Canada-wide survey for avian influenza viruses in wild birds is again taking place in 2006. The survey involves the Canadian Cooperative Wildlife Health Centre (CCWHC), the Government of Canada, provincial and territorial governments and university laboratories, including the Animal Health Laboratory.

The main goal of the survey is to provide an early warning system for potential detection of highly pathogenic strains of avian influenza virus in Canada. The survey will also help achieve a better understanding of the characteristics of influenza viruses circulating in wildbirds in Canada.

As its part of the survey in 2006, the AHL has tested 600 samples collected from live urban Canada geese in Southern Ontario. All 600 samples were negative for avian influenza viruses.

As a part of dead-bird surveillance in Ontario, we have tested 851 samples from various birds species to date; **17 samples contained low-pathogenicity influenza A viruses, but none was of H5 or H7 avian influenza subtype.** More information about results of the nationwide survey can be found at:

http://wildlife1.usask.ca/en/aiv/aiv_latest_results.php AHL

Erysipelas in turkeys

Lloyd Weber, Marina Brash

During the late summer and early fall, the AHL-Guelph lab diagnosed multiple cases of erysipelas in male turkeys approaching market age. The histories reported a sudden spike in mortality. At necropsy, birds were in good body condition with very little feed in crops and gizzards. Generalized tissue congestion with enlargement of the liver and spleen was observed. Differential diagnoses included gram-negative bacterial septicemia, i.e., *E. coli*, *Salmonella* spp., or *Pasteurella multocida*. Histologically, vascular congestion with microvascular thrombosis containing gram-positive slender rod-shaped bacterial colonies and foci of acute necrosis with similar appearing bacterial colonies were present in many organs (Fig. 1). On bacterial culture, *Erysipelothrix rhusiopathiae* was recovered in large numbers and pure cultures from blood-filtering tissues.

Erysipelas is an acute septicemic disease occurring most commonly in older male turkeys. Outbreaks occur most often in the late fall and winter following periods of cold, wet weather. It is thought that the bacterium can persist in the soil for an extended period of time increasing the risk of infection for turkeys raised on range. Due to biosecurity concerns, most turkeys are now raised in confinement, however many grow-out barns have dirt floors, so the risk of exposure still exists.

The actual portal of entry in birds has not been established. Entry through breaks in the skin or mucous membranes has been suggested. Infection by ingestion of contaminated material is also a possibility. Scientific articles have historically reported outbreaks of erysipelas in turkeys and chickens grown on farms where infected swine and sheep have been raised previously. Healthy swine can be carriers and shed the bacteria in manure thereby continually recontaminating the environment. The role of vectors such as caretakers' footwear, soil-contaminated bedding, rodents and wild birds cannot be ruled out.

Bacterial culture of liver, spleen or bone marrow is required in order to confirm the diagnosis. *Erysipelothrix rhusiopathiae* is a slender gram-positive rod. It is facultatively anaerobic. It grows well on blood culture media, and colonies are slow growing and appear small, gray, and may be alpha hemolytic.

In humans, the infection caused by *Erysipelothrix rhusiopathiae* is known as **erysipeloid**, a skin infection typically localized to the fingers and hands and usually preceded by a skin abrasion or cut. The lesion is actually a cellulitis and is quite painful (Fig. 2). Systemic effects, such as fever and septicemic complications, are uncommon. Those occupations at higher risk include fish handlers, veterinarians, farmers, slaughter plant workers and butchers. Some of the colloquial names for this condition include fish handler's disease, seal finger, and whale finger.

Farm workers and veterinarians should protect themselves by wearing gloves when handling dead turkeys.

Consult with your veterinarian prior to commencing treatment to understand if treatment is warranted, as the disease may resume after treatment is stopped. Penicillin is the drug of choice when treating an outbreak of erysipelas. Water-soluble penicillin is approved as an aid in the treatment of erysipelas in turkeys in Canada.

Vaccination using a killed bacterin should be considered if the risk of infection is high. AHL

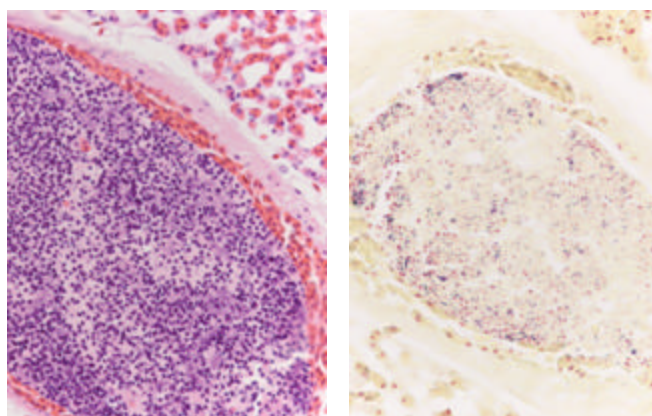


Figure 1. (Left) Pulmonary thrombus containing numerous gram positive rods, H&E stain. (Right) Same thrombus with Brown and Brenn Gram's stain.



Figure 2. Example of human erysipeloid. (Courtesy of Dr. Marie Archambault, University of Montreal.)

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- McKean JD. Swine Erysipelas. Swine Health Fact Sheet 13. Iowa State University Extension. Jan 1990.
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HORSES

Equine abortion summary, 1998 - 2006

Beverly McEwen, Susy Carman, Durda Slavic

More than one-third (37%) of equine abortions in 2005/2006 were due to infections (Table 1). ***Equid herpesvirus 1 (EHV-1) remains the most frequent single abortifacient pathogen identified, accounting for 18% of all abortion diagnoses.*** The main cause of non-infectious abortion is umbilical torsion.

The submissions in 2005/2006 were received from 33 owners, however, owner identification was not given in 28 cases, making diagnoses at the herd-level problematic. Three of the EHV-1 abortions were received from 1 premises. Sixteen breeds were represented, with Thoroughbred and Standardbred submissions dominating at 27% and 25% respectively. *AHL*

Table 1. Equine abortion, pathology diagnoses, AHL fiscal years, 1998 - 2006.

	Fiscal year:	98/99	99/00	00/01	01/02	02/03	03/04	04/05	05/06
Total abortion cases submitted		64	89	89	97	92	84	75	68
Frequency of abortion of total equine submissions		1.3%	1.7%	1.5%	1.5%	1.1%	0.9%	0.8%	0.8%
<i>Equid herpesvirus 1 (EHV-1)</i>		9	8	13	10	11	15	8	12
Proportion of EHV-1 equine abortions		14%	9%	15%	10%	12%	18%	11%	18%
Infectious causes of abortion, non-viral, combined		17	25	16	21	23	14	10	13
Proportion non-viral infectious abortion		27%	28%	18%	22%	25%	17%	13%	19%
<i>Streptococcus zooepidemicus</i>		1	5	1	4	6	3	1	4
<i>Streptococcus equisimilis</i>		2	0	0	1	2	0	1	0
<i>Staphylococcus aureus</i>		0	3	0	0	0	0	0	0
<i>Klebsiella</i> sp.		0	0	1	0	0	0	1	0
<i>Leptospira</i> sp.		3	0	0	1	1	0	0	0
<i>Nocardia</i> sp.		0	0	1	0	0	0	0	0
Placentitis		8	7	6	11	13	8	0	5
Miscellaneous bacteria/fungi		2	8	0	0	0	2	2	1
Mycotic		1	0	1	2	0	1	1	1
Lesions compatible with bacterial		0	2	6	2	1	0	4	2
Non-infectious causes of abortion, combined		15	21	24	26	18	18	31	15
Proportion non-infectious causes of abortion		23%	24%	27%	27%	20%	21%	41%	22%
Umbilical torsion		8	12	8	15	11	13	19	7
Placental edema		4	3	8	0	1	0	0	2
Placental infarction		0	0	0	0	0	1	0	0
Placental mineralization		1	0	0	4	3	0	5	1
Placental adenomatous/cystic hyperplasia		0	1	0	0	2	1	0	0
Fetal goiter		0	0	1	1	1	1	2	1
Dystocia/stillbirth		2	5	7	6	4	3	4	3
Twins		0	0	0	0	0	0	1	1
No lesions/idiopathic		24	38	36	42	39	37	28	31
Proportion no lesions/idiopathic		38%	43%	40%	43%	42%	44%	37%	46%

* Number of diagnoses may add to more than the number of cases submitted because more than 1 diagnosis was made in some cases.

SWINE

Porcine circovirus 2 - associated disease diagnoses decline in the later part of 2006

Susy Carman, Beverly McEwen, Josepha DeLay, Hugh Cai, Jim Fairles

Porcine circovirus 2 (PCV-2)-associated disease (PCVAD) continued into late 2006, with 303 new cases presented from Jan-Oct 2006. PCVAD as a percent of total swine submissions increased from 8.9% in 2005 to 10.9% for the first part of 2006 and declined to 8.4% for the later part of 2006 (Fig. 1). Because these data are impacted by submission biases to the diagnostic laboratory, they cannot be regarded as population prevalence estimates.

The PCV-2 PCR-RFLP typing for all PCR testing requests continued to show a **significant change from RFLP type 422 seen in previous years to RFLP type 321** (Fig. 2) with RFLP type 321 seen in 109/135 cases. However RFLP type 422 is still present within Ontario swine,

with RFLP type 422 seen in 15/135 cases. Both RFLP types 422 and 321 have been demonstrated in the same herd.

This consistent change in RFLP typing patterns continues to reflect a constant change in the gene sequence recognized by two restriction enzymes. Ontario RFLP type 321 viruses continue to have greater than 99% sequence homology with each other, and to have 98% sequence homology with those reported for the UK, France and China. These Ontario RFLP type 321 viruses have only 91.6% sequence homology to the previously dominant Ontario RFLP type 422 viruses, and are only 92-93% similar to those previously reported from the USA. AHL

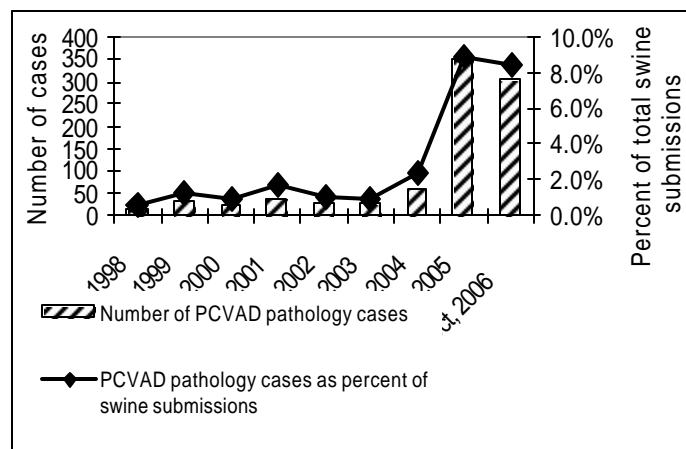


Figure 1. Number of PCVAD pathology cases, and PCVAD pathology cases as percent of total swine submissions, 1998-2006.

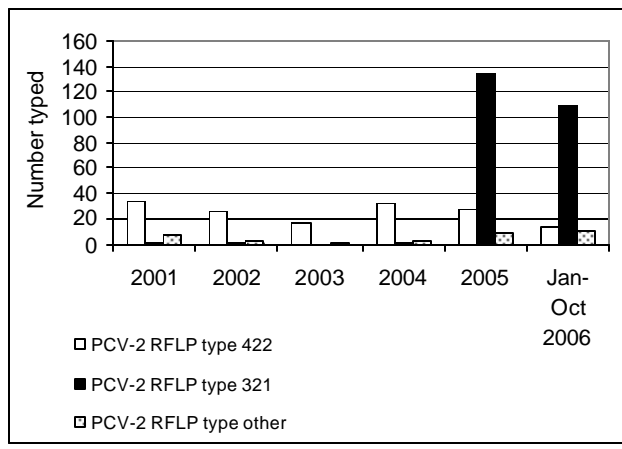


Figure 2. PCR-RFLP typing of PCV-2, 2001-2006.

Ascarid larval pneumonia in 5 swine herds

Peter Lusia, Beverly McEwen

Over the past year, we have diagnosed ascarid larval pneumonia in 5 herds of 50-300 pigs. Where reported, clinical signs were high morbidity (up to 40%) with coughing and “puffing”, and death of some pigs. Gross necropsy findings included firm, wet lungs with consolidation of scattered lobules in all lobes, and cranioventral consolidation in some cases. “Milk spots” in livers and/or mature ascarids in gastrointestinal tracts were present in some, but not all, pigs.

Bacteriology was negative in 1 case; *Streptococcus suis* and/or *Pasteurella multocida* and/or *Bordetella bronchiseptica* were isolated from 3 cases. Swine influenza virus PCR was negative in 4 of 4 cases. PRRSV PCR was positive

in 1 of 4 cases and *Porcine circovirus-2* PCR was positive in 2 of 4 cases.

On histopathology, there was acute tracheitis with ascarid larvae in one case, bronchointerstitial pneumonia with ascarid larvae in all cases, and interlobular fibrosis containing eosinophils in the liver in most cases.

Ascarid larval migration can be a cause of pneumonia outbreaks in herds without adequate anthelmintic programs. Mature ascarids and/or “milk spots” may or may not be present, and bacterial and/or viral agents may or may not be present in fatal cases. AHL

Immunohistochemistry for influenza A viruses

Josepha DeLay

The histopathology laboratory at the AHL offers IHC for influenza A viruses (Fig. 1), including swine influenza virus, as an individual test and as part of the pig respiratory panel with *Porcine circovirus 2* (PCV2) and PRRSV. Because of the high number of swine pneumonia cases with multiple etiologies, we recommend that practitioners continue to request this 3-part panel when selecting IHC tests on pig lung.

We are currently validating a new polyclonal anti-influenza A antibody that appears to have higher sensitivity than our initial monoclonal antibody in identifying influenza viral antigen in lung sections. Initial trials indicate that this new antibody reacts with both H1N1 and H3N2 swine influenza viruses. However, IHC cannot differentiate between the various types of influenza virus, and virus isolation is strongly recommended on all suspected cases of swine influenza to facilitate virus typing. *AHL*

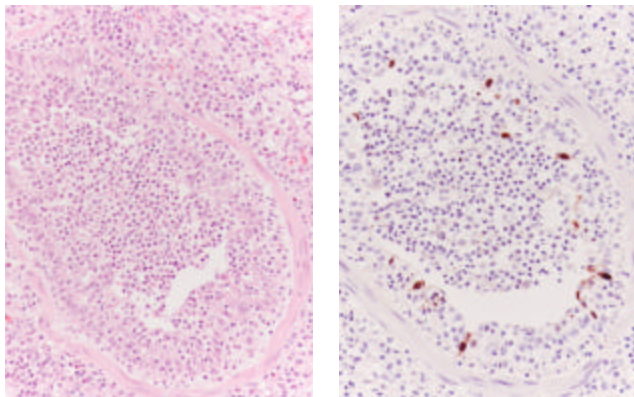


Figure 1. Swine lung with necrosuppurative bronchiolitis, caused by swine influenza virus. (Left) H & E section. (Right) Same bronchiole; positive immunohistochemistry using anti-influenza A antiserum. (Best appreciated in color on our website.)

COMPANION ANIMALS

Candidatus Mycoplasma haematoparvum infection in a dog

Hugh Cai, Brent Hoff, Patricia Bell-Rogers

Hemotropic mycoplasmas in cats and dogs, formerly known as *Haemobartonella*, are associated with anemia. In 2005, the AHL implemented a PCR assay for the detection of *Mycoplasma haemofelis* and *Candidatus Mycoplasma haemominutum* from feline blood samples (the *Candidatus* designation is given to incompletely characterized species). In the past 18 months, the AHL has tested 120 feline blood samples; 13 were positive for *M. haemofelis*, 19 were positive for *M. haemominutum*, and 2 were positive for both organisms. The assay also identifies *Mycoplasma haemocanis* and *Candidatus Mycoplasma haematoparvum* from canine blood samples.

Recently, using the PCR assay and DNA sequencing confirmation, we identified a *Candidatus Mycoplasma haematoparvum* from a mature, female, mixed breed dog admitted to the OVC Veterinary Teaching Hospital because of **failure to gain weight and progressive hemolytic anemia**. Examination of the hemogram revealed a macrocytic, hypochromic, markedly regenerative anemia with a PCV of 19%. The leukogram was characterized by mature neutrophilic leukocytosis. No serum biochemical abnormalities were noted. Light microscopy revealed **spherical or ring-shaped organisms individually or in chains** (Figure 1). This dog had been splenectomized some time ago, but this organism has also been reported in dogs undergoing chemotherapy. Despite an intense immune response and even with antibiotic treatment, infected animals probably remain chronic carriers after clinical signs have resolved. *AHL*

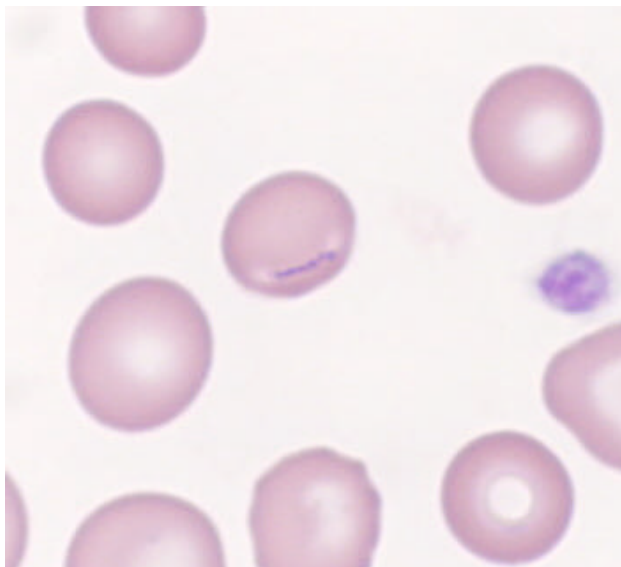


Figure 1. Chain of *Candidatus Mycoplasma haematoparvum* organisms in a canine red blood cell.

References

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- Sykes JE, et al. '*Candidatus Mycoplasma haematoparvum*', a novel small haemotropic mycoplasma from a dog. *Int J Syst Evol Microbiol* 2005;55:27-30.

Cytologic detection of *Eucoleus aerophilus* infection in a dog

Hilary Burgess, Kristiina Ruotsalo

A 4-year-old, spayed-female Standard Poodle was presented to the Ontario Veterinary College with a 3-week history of a moist, productive cough that was first noted following a stay at a boarding kennel. Initial blood work revealed a moderate leukocytosis composed of a mature neutrophilia and monocytosis, and electrolyte changes that were consistent with previously diagnosed hypoadrenocorticism. Radiographs revealed a diffuse interstitial pattern in both left and right cranial lung lobes. The dog was unresponsive to antimicrobial treatment (Clavamox), and became increasingly lethargic and inappetent prior to referral.

A bronchoalveolar lavage was performed and submitted for cytologic evaluation. The nucleated cell count was moderately increased at $4.2 \times 10^9/L$. Cytological examination of concentrated slide preparations revealed copious amounts of mucus in which large numbers of non-lytic neutrophils were entrapped. Eosinophils were also found in low numbers along with occasional macrophages. **Numerous extracellular, barrel-shaped ova with bipolar opercula were noted.** These ova were identified as *Eucoleus aerophilus* (formerly known as *Capillaria aerophila*), based on their size (estimated to be 50-80 μm), and the slightly asymmetrical placement of the opercula.

The subfamily *Capillariinae* is large, including 300 described species. Currently the species found in the dog and cat have been placed in three genera: (1) *Eucoleus* for those found in the airways, (2) *Aonchotheca* for the worms found in the intestinal tract, and (3) *Pearsonema* for those that occur in the bladder. *Eucoleus aerophilus* is a major parasite of foxes and can be found embedded in the superficial epithelium of the trachea, bronchi and bronchioles. The lifecycle of *E. aerophilus* can involve direct transmission as well as transmission through an earthworm facultative intermediate host. The prepatent period is 3-5 weeks, at which time the female nematode lays her eggs to be coughed up, swallowed and passed in the feces. Once the eggs are deposited in the soil, ~40 days are required for the ova to become infectious. Infectious ova are hardy and can survive in harsh environments up to 1 year.

Diagnosis is based on identification of the 59-83 μm long by 26-40 μm wide, often asymmetrical, bipolar eggs

in the feces or tracheal mucus. *E. aerophilus* can easily be confused with *Trichuris vulpis* eggs in fecal samples; however, *Trichuris vulpis* eggs are larger and symmetrically bipolar.

Because the life cycle of this parasite can be direct, **sanitation is imperative for disease control and for outbreak prevention in group housing situations.** Although, not a common occurrence, *Eucoleus* has been reported in humans, and appropriate precautions should be taken when exposed to fecal matter from an infected animal. AHL

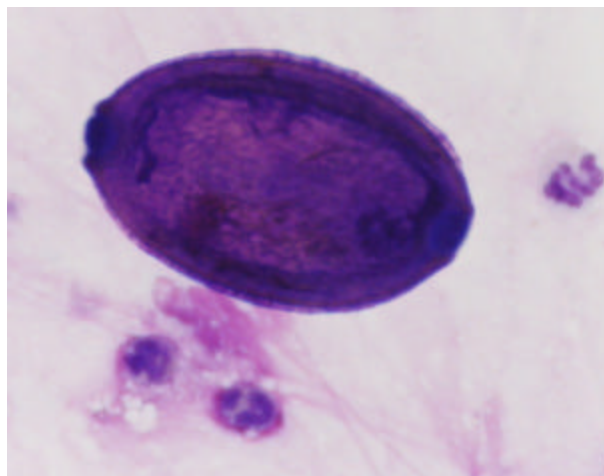


Figure 1. *Eucoleus aerophilus* (formerly *Capillaria aerophila*) ovum, barrel-shaped with bipolar opercula, in the bronchoalveolar lavage fluid from a dog. Note size relative to neutrophils in this field.

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- Barrs VR, et al. Pulmonary cryptococcosis and *Capillaria aerophila* infection in an FIV-positive cat. Aust Vet J 2000;78:154-158.
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Dec/06 crossword puzzle answers:

Across: 1. HERPES, 3. ERYSIPELOID, 6. STALKER, 7. POMONA, 9. TRICHURIS.

Down: 1. HAEMATOPARVUM, 2. INFLUENZA, 4. CIRCOVIRUS, 5. POSITIVE, 8. CCWHC.

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