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Animal Health Monitor

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Mission Statement: To provide our clients with important information regarding the work being done by the Animal Health Centre and Regulatory Unit, as well as events and issues relevant

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Editorial by Ursula Viney

Welcome to the newly revamped version of the Animal Health Monitor. As this has been a year of many changes at the Animal Health Centre (AHC) we felt it only fitting to provide our clients with a more structured and comprehensive newsletter in hopes of serving our clients better by sharing more information on services provided by the AHC, trends in disease outbreaks and some of the many projects and initiatives being undertaken here at the AHC.

Like everyone in the world, the Animal Health Centre has been dealing with the effects and challenges of the COVID-19 pandemic for the past year. During that time though, our facility has seen many changes, lots of new faces and restructuring of our operations. As announced by our former Executive Director and Chief Veterinarian, Dr. Jane Pritchard, in Feb 2020, I have assumed the role of Executive Director for the Plant and Animal Health Branch. With Dr. Pritchard's retirement in March 2020 a standalone position of Chief Veterinarian and Manager of the Regulatory Unit was created and filled by Dr. Rayna Gunvaldsen in June 2020. We are in the process of seeking the new permanent Laboratory Director for the Animal Health Centre and hope to make an announcement welcoming a new staff member into that role in the coming months. We are also pleased to welcome Dr. Shauna-Lee Chai as the new Director of the Plant Health Unit in September 2020 and Dr. Kazal Ghosh as our new Veterinary Microbiologist and Section Head of Bacteriology and Serology in October 2020.

The past 14 months have been challenging for us at the Animal Health Centre. The lab has faced ongoing disruptions in receiving testing supplies and equipment due to the pandemic, and felt the pressures of the sudden global demand for PPE in 2020. We've also had to adjust our lab practices to overcome the operational challenges of working in a close environment when physical distancing is required. We are very proud that we have been able to adapt and continue to maintain full diagnostic services to our clients with little or no disruptions. Our team at the AHC have come together to work through these challenges and continue providing the quality work we pride ourselves on. The AHC was also faced with the additional challenge in late 2020 of providing SARS-CoV-2 testing of mink specimens in response to the outbreaks. Our staff have worked hard to adapt workflow and initiate additional safety and containment procedures for us to safely undertake this work while protecting the health and safety of our staff, our clients, and the citizens of BC. We will continue to work closely with our clients and other partners in providing diagnostic services for detection of suspected SARS-CoV-2 infection in our agricultural sector.

We welcome feedback, questions or comments from our clients and I encourage you to contact us at the Animal Health Centre at : PAHB@gov.bc.ca or 604-556-3003. I also look forward to getting to know you better in my new role of Executive Director and welcome you to reach out to me anytime.

All authors are employees of the Ministry of Agriculture, Food & Fisheries unless otherwise noted.

Salmonella Dublin in BC Dairy Cattle

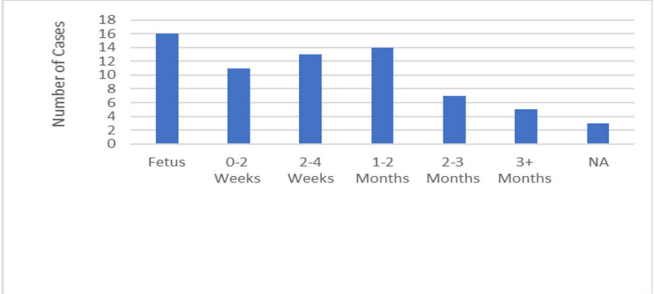
Harveen Atwal (Coop Student), Doris Leung (Veterinary Epidemiologist), Kaylee Byers (Research Scientist), Chelsea Himsworth (Leader for Veterinary Science and Diagnostics)

Salmonella Dublin is a bacterial pathogen that has significant negative economic impacts on the dairy industry due to calf losses, abortion, and reduced milk yield. A Danish study found that losses due to S. Dublin can be as high as $487 CAD/stall annually. Once *S. Dublin* enters a herd, it can spread quickly and be difficult to eliminate. Many animals become lifetime asymptomatic carriers – constantly shedding the bacterium in the feces and milk. Transmission occurs through contact between animals or through the environment, where the bacterium can survive for months. *S. Dublin* can also be transmitted to people in contact with infected cattle or who consume contaminated dairy products.

The incidence of S. Dublin is increasing across North America, including strains that are multi-drug resistant. *S. Dublin* is a reportable/notifiable disease in several Canadian provinces and those provinces have established surveillance and control programs. In BC, there has yet to be an organized, province-wide effort to monitor or mitigate the disease. To fill this knowledge gap, the Animal Health Centre is undertaking a two-year program of research to better understand *S. Dublin* in BC and to develop resources to assist industry in controlling the disease

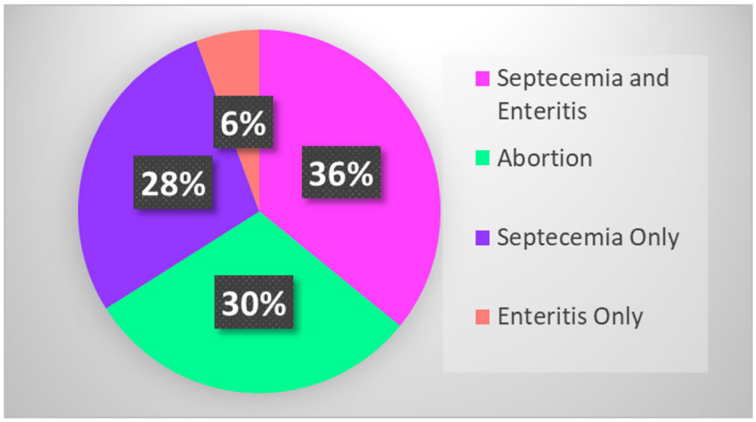
Our first step in this program was an analysis S. Dublin cases submitted to the AHC between 2015 to 2020. A case was defined as one or more cattle submitted from the same farm during the same timeframe. Only whole animals (vs. fecal samples) were included. There were a total of 70 cases from 27 farms in which BC cattle were infected with *S. Dublin*. All cases involved dairy cattle, the majority of which were Holsteins. Most cases were fetuses, followed by calves 1-2 months of age (Figure 1).

Figure 1. Ages of Cases with *S. Dublin* Infection.



The two most common clinical signs were diarrhea (47% of cases) and respiratory issues (36% of cases). Other clinical signs reported in the history included abortion, sudden death, fever, and non-specific signs of illness (e.g., lethargy, not eating nor drinking, un-thriftiness, and malaise).

Figure 2. Salmonellosis form of *S. Dublin* upon Diagnosis of Death.



On post-mortem examination, the primary form of salmonellosis that occurred was a combination of septicemia and enteritis followed by abortion (Figure 2). Most cases only had salmonellosis. However, 36% had other, concurrent diseases (e.g., scours due to other bacteria and viruses), which may have contributed to the cause of death. Trends in the data appear to show an increase in the number of cases from 2016 to 2020 (Figure 3). The number of newly infected farms also increased during that time. Cases were most common the late fall and early winter (figure 4).

Figure 3. Number of *S. Dublin* Cases per Year from 2015-2020.

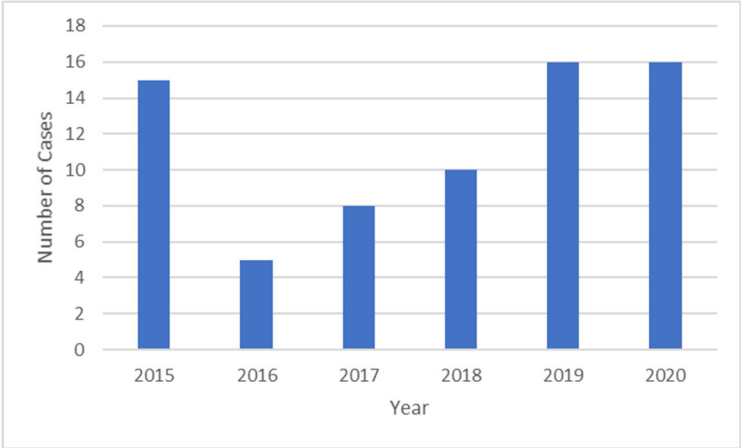
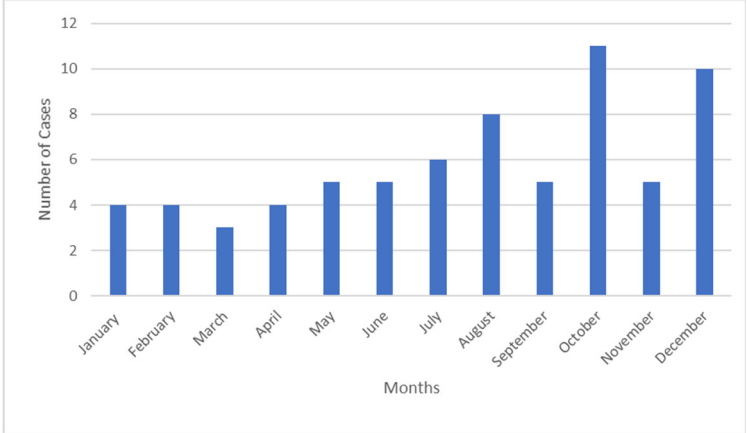


Figure 4. Number of *S. Dublin* Cases per Month from 2015-2020.



Salmonella Dublin in BC Dairy Cattle cont'd

Next steps in the project include retrospective and prospective epidemiological investigations, as well as facilitated meetings to gather feedback from veterinarians and producers. Ultimately, this work will provide the knowledge and resources needed to reduce the spread of S. Dublin in BC, which will improve animal health as well as the productivity and profitability of the BC dairy industry. For further information on this project, please contact Doris Leung at Doris.Leung@gov.bc.ca.

Fatal Navel Ill in Two Neonatal Beef Calves

Ann Britton (Veterinary Pathologist)

Two neonatal beef calves from separate farms were submitted for necropsy to the Animal Health Centre (one 3 day old female and one 4 day old male) in March 2021. Both calves came from a herd reporting multiple previous deaths in neonatal calves this year. One of the calves was not nursing prior to death while the other was exhibiting abdominal pain, depression and a swollen umbilicus. On necropsy, both calves had a swollen umbilicus with evidence of marked bacterial infection. The infection had spread from the umbilicus into the abdominal cavity causing peritonitis and to the circulatory system causing septicemia. One of the calves also had spread to the brain causing meningitis. Both calves were diagnosed with bacterial omphalitis (navel ill) with secondary peritonitis and septicemia.

Navel ill is a preventable condition of newborn calves. The umbilical cord connects the blood circulation of the calf to the placenta during pregnancy and serves to exchange carbon dioxide and oxygen in the fetal blood via the cow. During birth, the umbilical cord breaks and the calf assumes gas exchange on its own via its lungs. Following birth, the umbilical blood vessels will shrink, break down and scar leading to a completely closed circulatory system which is not susceptible to infection from the environment. However, right after birth and for a few days, the blood vessels in the umbilicus are still open and susceptible to bacterial infection. If infected, the calf may experience 3 presentations: a localized infection, an umbilical abscess or spread of infection to the abdomen and circulatory system.

Prevention is key with navel ill. Providing a clean dry area for calving and the neonatal period is extremely important. This minimizes the chances of bacterial infection from a dirty or wet environment. Ensuring the calf receives adequate colostrum the first day of life will further improve the calf's chances for a healthy start to life. And finally, antiseptic treatment of the navel following birth will help keep the bacteria at bay but please note, umbilical disinfection is not a substitute for a clean dry calving area. Providing all three preventative measures will maximize a calf's chances at a good start to life.

Unusual Presentation of Fatal Coccidiosis in a Young Beef Calf

Ann Britton (Veterinary Pathologist)

In April, a 2 month old beef calf on 70 acres of dry pasture in the BC southern interior was suddenly found depressed and dehydrated without signs of diarrhea and subsequently died. At necropsy, the calf had no fecal staining of the tailhead or hind end. The tissues and organs were pale indicative of anemia and the spiral colon was distended with bloody fluid. Direct smear of the colonic content revealed large numbers of coccidia oocysts. On microscopic examination, a severe necrotizing colitis was diagnosed with large numbers of coccidia organisms present in much of the remaining lining of the colon. Coccidiosis was diagnosed.

This is an unusual presentation of bovine coccidiosis due to the time of year (April) and the conditions under which the herd was being kept (dry pasture). Bovine coccidiosis is most commonly found in areas prone to coccidia oocyst accumulation and contamination, usually in barns or small fenced off areas. Young animals are most susceptible. Severely affected animals can become anemic due to intestinal blood loss and die from the combined effects of dehydration and anemia.

Clinical disease is most commonly observed in the fall and winter when animals are housed or kept in small or crowded yards. Infection is spread via the fecal - oral route. Management practices which reduce exposure to feces, reduce crowding of animals, minimize feeding off the ground and minimize fecal contamination of feed and water troughs can be effective in preventing disease. Cold weather may also trigger clinical disease in infected calves, likely via stress induced immunosuppression. Providing good shelter during cold snaps may also help to prevent disease.

Veterinarians and owners should keep coccidiosis in mind when examining sick calves with signs of dehydration, diarrhea (especially if bloody) and/or unexplained depression. A fecal floatation test can be a quick method to determine whether coccidiosis is a factor in the calf's illness.

An Unusual Finding in a Beef Neonate

Stephen Raverty (Veterinary Pathologist)

A field post-mortem was performed on a Hereford perinatal calf in central British Columbia and harvested tissues were submitted to the Animal Health Center for diagnostic evaluation. A near-term calf had been born alive, suckled, and was found dead the following morning. Although a mid to late gestational abortion had occurred a month earlier in the herd, there were no other significant clinical health concerns. The cows had been vaccinated with Express + VL5 and Vision8 and were fed silage and alfalfa brome hay. The owner was not aware of any mold within the silage.

On gross examination, there was enlargement of the right mandibular lymph node with multifocal erythema and subcutaneous hematomas throughout the torso and mild focal submucosal hemorrhage in the abomasum. Tissues were collected and preserved in formalin for histopathology and chilled stomach contents were provided for microbiology. The most significant microscopic findings in the lungs were large regularly spaced and uniformly sized dense nodules adjacent to and impinging upon the lumen large caliber airways (Figure 1). The nodules were well circumscribed, non encapsulated, peripherally displaced and compressed adjoining lung parenchyma, and occasionally entrapped a few small caliber airways and blood vessels. The cells were round with indistinct cell membranes and a small amount of homogenous to finely granular eosinophilic cytoplasm. The nuclei were circular to ovoid with stippling and variably distinct 1-3 nucleoli. The mitotic index was 0-3 per high powered field and there were moderate amounts of dispersed karyorrhectic and pyknotic debris. Small numbers of lymphocytes and histiocytes infiltrated the neoplasms. Similar nodular accumulations were detected in portal tracts in the liver, submucosa of the abomasum, and expanded and effaced large areas of lymph nodes and spleen.

Based on the gross and microscopic findings in this case a diagnosis of juvenile multicentric bovine lymphoma was rendered. This neoplasia is not associated with bovine leukosis virus (BLV) and typically features T-cell and occasionally B-cell involvement. There are 3 primary clinical presentations, including 1). Cutaneous, in cattle between 2 and 3 years old, 2). A thymic or juvenile form, most commonly recognized in beef calves, including Herefords, and 3). A multicentric or calf form, which typically presents within the first 6 months of life, however, congenital involvement has been previously reported. In contrast to the thymic or juvenile forms of non-BLV associated neoplasms, affected calves may also feature leukemia, myelophthisis, and bone marrow infarction. The pathogenesis of neoplastic transformation in these cases has not yet been fully resolved and clinical signs may wax and wane with progressive tumor growth and damage to vital organs. Congenital sporadic bovine lymphoma is a rare and apparently spontaneous condition that may have a predilection for beef breeds. At present, the natural history of this neoplasm is poorly understood and there are no reported control or preventative measures.

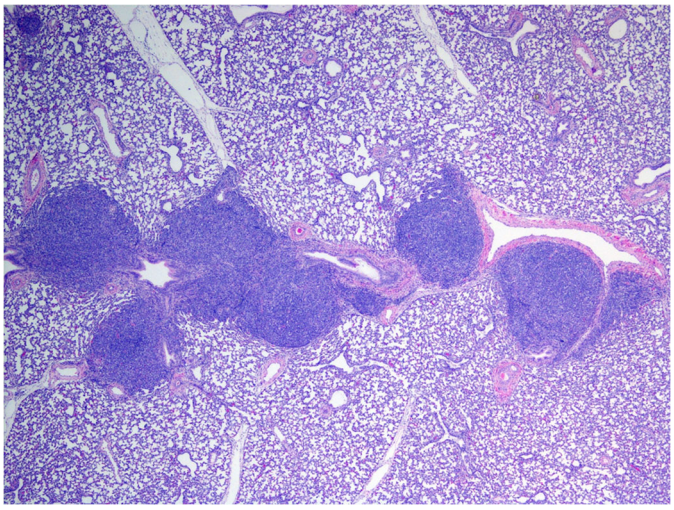


Figure 1, Section of neonatal lung with multiple, individual to confluent nodular masses.

Cryptosporidium parvum in Cases of Calf Scours: Comparison of Diagnostic Methods

Harveen Atwal (Coop Student), Erin Zabek (BSO/Section Head/Laboratory Operations), Julie Bidulka (Lab Science Officer), Alecia DuCharme (Pathology Student), Michael Pawlik (Veterinary Pathologist), Chelsea Himsworth (Leader for Veterinary Science and Diagnostics)

Cryptosporidium parvum is a protozoan parasite that causes diarrhea (called cryptosporidiosis) in calves aged 1-4 weeks old. *C. parvum* can occur on its own or in association with other pathogenic bacteria and viruses; however, the availability of vaccines for other enteropathogens has resulted in *C. parvum* becoming one of the most important causes of calf diarrhea.

Common methods to detect *C. parvum* in the feces of live calves include direct smears, PCR, and antigen detection methods such as immunochromatographic and immunofluorescent assays. The Animal Health Centre (AHC) currently uses the Cold Kinyoun Acid Fast stain direct smear for the routine detection of *C. parvum* and PCR upon request. The objective of this study was to evaluate this workflow by trying two other diagnostic modalities - Crypt-a-Glo fluorescent antibody test and Xpect lateral flow immunoassay – and to determine the degree of agreement among all four assays for the detection of C. parvum in calves with diarrhea.

Seventy-four fecal samples from 1-6-week-old calves with diarrhea were submitted to the AHC between April to December 2020. Each sample was tested using the four different assays and the Fleiss kappa value was calculated to assess statistical agreement among all four tests.

Out of the 74 samples compared, 62 were in agreement across all 4 tests. For 12 samples there were discordant results among one or more of the tests (Table 1). The Fleiss kappa value was 0.813. Kappa values greater than 0.75 indicate excellent agreement. Given that the performance of all four assays was roughly equivalent and given that the Cold Kinyoun Acid Fast stain direct smear is inexpensive, quick and easy to perform, the AHC will continue using this assay for routine detection of *C. parvum*.

Table 1. Comparison of Sample results. (+) represents a positive result for the presence of Cryptosporidium in cattle feces while (-) represents a negative result.

Number of samples	Cold Kinyon Acid Fast Stain Direct Smear	**Xpect**	Crypt-a-Glo	**PCR**
20	+	+	+	+
42	-	-	-	-
4	-	-	-	+
1	+	+	-	+
4	+	-	-	-
1	-	+	+	+
1	-	-	+	+
1	+	+	+	-

Encephalitis in a Feral Rabbit

Stephen Raverty (Veterinary Pathologist)

On March 7, 2021, a 975gm feral male rabbit was observed in lateral recumbency in a local parking lot. On approach, the rabbit attempted to hop, but would only bound in tight circles and eventually collapse on its side. The rabbit was presented to a local veterinary clinic and on initial assessment, the neck was hyperextended, there was vertical nystagmus and the hind-end was matted with feces. Due to the grave clinical presentation and poor prognosis, the animal was humanely euthanized and submitted for post-mortem examination.

At necropsy, the animal was in good body condition and post-mortem state with adequate subcutaneous and visceral fat stores. There was fecal matting throughout the perineum. On internal exam, the most significant gross finding was multifocal acute pulmonary congestion and edema. Tissues were collected for histopathology, bacteriology, parasitology, and molecular studies to screen for rabbit hemorrhagic disease virus. Microscopically, in the cerebellum and brain stem, there was marked multinodular nonsuppurative encephalitis with multifocal gliosis, lymphohistiocytic infiltrates and epithelioid macrophages (Figure 1). In a few small caliber blood vessels, there is mild lymphocytic and occasional eosinophilic cuffing. Additional recuts and special stains disclose small numbers of gram-positive spores within vascular endothelia morphologically consistent with encephalitozoonosis. Based on the extent and severity of involvement the brain, the lesions would have contributed significantly to antemortem neurologic signs. Mild multifocal nonsuppurative interstitial nephritis with scattered tubular cyst formation and occasional protein casts were noted. No bacteria were recovered from the lung or brain and fecal parasitology disclosed large numbers of coccidia with moderate accumulations of strongyles. Molecular studies of pooled tissues was negative for rabbit hemorrhagic disease virus.

Encephalitozoonosis is due to Encephalitozoon canaliculi, a gram positive obligate intracellular microsporidian closely related to fungi, that can infect a range of mammalian species, including lab and companion animals, wildlife species and humans. A closely related parasite has also been recognized in reptiles, fish and invertebrates. Infection is generally acquired through ingestion of contaminated prey, water, and plants, and has also occurred transplacentally, and via inhalation of infectious spores. In most cases, infections are inapparent and histopathologic findings are incidental. The parasites have a predilection for blood vessels in the brain and kidney but will also localize to renal tubules with shedding primarily via urine and feces. Rabbits typically present with head tilt, ataxia, and vestibular signs; whereas, in cats and dogs, young animals may feature stunted growth, renal failure, muscle spasms, neurologic signs, and death. Several serology and skin tests are available to screen rabbits; however, results would only indicate past exposure and not confirm active infection.

Currently, there are few effective treatments for rabbits and in rabbitries, prevention is strongly recommended with good sanitation and possible serologic screening of breeding stock with removal of reactive animals.

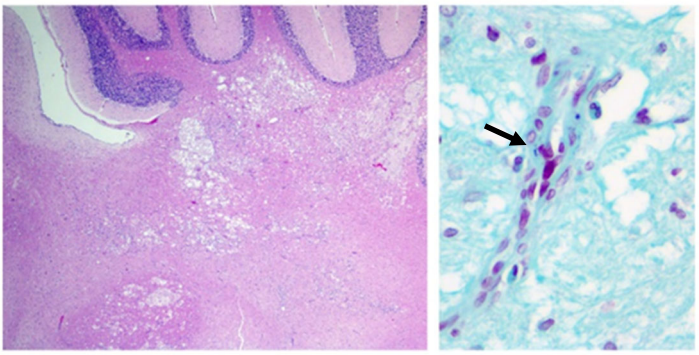


Figure 1. Nonsuppurative encephalitis in a rabbit. The image on the right features 3 Gram positive spores in a vessel wall (arrow).

Asian Giant Hornet in BC

Paul van Westendorp (Apiculture Specialist)

In September 2019, the Asian Giant Hornet (AGH) *Vespa mandarinia* was first identified in North America. Two specimens were collected by a Nanaimo beekeeper after the hornets were visiting his apiary. Additional sightings were reported in the general area for several weeks that resulted in an intensive search for the nest. A team of local beekeepers located the hornets' nest in Robins Park of central Nanaimo where it was subsequently eradicated.

In November 2019, a single AGH specimen was found in central White Rock and in early December, two additional specimens were confirmed in Blaine, WA. The detection of these specimens so late in the year indicated that AGH had established itself in BC and Washington State. The sightings on Vancouver Island and the mainland demanded comprehensive surveillance programs in 2020.

AGH is the largest hornet species of the genus *Vespa* with a body length of 5 cm and wingspan of nearly 7 cm. Its native distribution includes coastal China, Japan and the Korean peninsula. Its closely related sister species *Vespa soror* is distributed farther south including Taiwan and S.E. Asia. Both species have a preference to construct ground nests in heavily forested habitats. Because ground nests have greater exposure to potential predators, these two species have developed strong defence responses when their nest is disturbed. 30-50 human fatalities are reported in Japan each year as a result of accidental nest disturbance.

AGH is the apex-predator of the insect world. As with all apex predators, it is widely distributed but with low prevalence. At this early stage of establishment in BC, locating nests pose significant challenges. Cluster sightings are needed to locate nests. During the 2020 Fraser Valley surveillance program, only six specimens were collected by the public covering an area in excess of 350 sq. km. Not a single specimen was collected in the dozens of bottle traps distributed throughout the western Fraser Valley along the Canada-US border. No AGH was collected or sighted on Vancouver Island in 2020.

While surveys are planned for the Fraser Valley and Vancouver Island in 2021, emphasis will be placed on public participation to report sightings. When there is a cluster of confirmed sightings, a field crew will conduct a detailed search of the area. When a nest is located, the area will be cordoned off and a team of Ministry staff will carry out the eradication.

In May 2019, a large hornet was collected at Vancouver Harbour. The specimen was identified as a *Vespa soror* queen. Even though no nest or other sightings were recorded, this confirmation and that of *Vespa mandarinia* several months later, indicates that introductions of Vespa species in the future are likely to occur again.

While climatic conditions may be conducive to AGH's establishment in Coastal BC, other factors including vegetative cover, insect prey, inbreeding potential and human predation may jeopardize its success.

An Update on Antimicrobial Sensitivity Testing at the Animal Health Center

Kazal K. Ghosh (Veterinary Microbiologist)

We have recently reviewed and updated our antimicrobial susceptibility testing panels based on current Clinical and Laboratory Standards Institute (CLSI) guidelines and a published article titled "Developing an evidence-based approach for antimicrobial resistance reporting for British Columbia diagnostic animal health laboratory data" by Burns et al. 2018. There is now a total of 8 new panels which are being grouped according to animal species. We also have reviewed and updated the results interpretation criteria based on current CLSI guidelines. BIOMIC® V3, a digital plate reader has been added in the bacteriology laboratory section for automatic plate reading as well as interpretation of test results if available. We would like to thank Jaime Battle, Giselle Hughes, and Daniel Knowles from the bacteriology laboratory for making all the necessary changes to implement these new panels soon.

New panels name	Mammalian/Bovine General Bovine Respiratory Bovine Mastitis Avian Companion Porcine Fish Exotic and VanAqua
Test method	Kirby-Bauer Disk Diffusion
Results interpretation	Results will be interpreted as Susceptible, Intermediate, Resistant, or Not available (S, I, R, or NA) for each antimicrobial. S (Susceptible): A bacterial strain is said to be susceptible to a given antimicrobial agent when the zone diameter is at or above the susceptible breakpoints suggests a high likelihood of therapeutic success. I (Intermediate): The sensitivity of a bacterial strain to a given antimicrobial agent is said to be intermediate when the zone diameter is within the intermediated range suggests an uncertain therapeutic effect. R (Resistant): A bacterial strain is said to be resistant to a given antimicrobial agent when the zone diameter is at or below the resistant breakpoints suggests a high likelihood of therapeutic failure. NA (Not Available): If there are no criteria available for any particular antimicrobial, then we will be reporting it as not available.

Salmonella Dublin ELISA testing at the Animal Health Centre

Kazal K. Ghosh (Veterinary Microbiologist)

The Animal Health Centre (AHC) is currently offering ELISA for the detection of antibodies to Salmonella Dublin. This test is suitable for large-scale screening of serum and bulk tank milk samples. We would like to thank Roberta Yemen and Tracy Roddick from the Serology laboratory for verifying and adding this test.

Species	Bovine
Specimen type	Serum or Bulk Tank Milk
Sampling requirements	Submit 2ml of Serum or bulk tank milk samples. Excessively hemolyzed samples are not acceptable for this test. For detailed sampling instructions please click here.
Shipping information	Ship the samples to the laboratory with ice packs. If samples cannot immediately be transported to the laboratory, refrigerate the serum at 2–7°C for up to 3–5 days or freeze at 20°C for long-term storage. When submitting more than 20 samples at a time, please send an MS Excel file with animal IDs by e-mail to PAHB@gov.bc.ca. Enter animal IDs in a single column identified as "Animal ID" and the AHC report will contain IDs as entered in this column. Place samples in the same order in the rack/box as in the MS Excel file.
Price	$10 per sample
Schedule	Tuesday of each week
Results interpretation	The result is expressed as Percent Positivity. If the Percent Positivity value is less than 35, the sample is negative which suggests Salmonella-specific antibodies are absent in that test sample. If it is greater than 35, the sample is positive which suggests Salmonella-specific antibodies are present in that test sample. Results should be interpreted in the context of all available clinical, historical, and epidemiological information relevant to the animal or herd under test.

Shipping Formalized Tissues to the Animal Health Centre

Chelsea Wood (Head Pathology Technician)

When shipping formalized tissue to the Animal Health Centre, please follow Transportation of Dangerous Guidelines. Any samples being sent should follow a triple packaging system:

Equipment Required:

- Twist top container
- Sturdy tape such a glass tape, electrical tape, duct tape or film
- Absorbent material such as paper towel
- Two zip lock bags

Steps:

1) The sample should be placed in a leakproof Primary Receptacle such as a twist top container along with formalin. Make sure that the container is clearly marked with what percentage of formalin it contains.
2) Ensure to tape around the lid of the twist top container to prevent it from coming loose or leaking. Use tape such as glass tape, electrical tape or duct tape for maximum adhesion. Parafilm works well for this as well.
3) The twist top container is then placed inside a secondary layer of packaging. A sturdy Ziplock bag may be used for this. Ensure to include within this layer enough material to absorb all the formalin, should the primary receptacle crack or leak. Paper towel or absorbent pads may be used for this.
4) Place this package inside of another zip lock bag and seal before placing into a box or other suitable shipping container.
5) Once placed in the final shipping container, ensure directional arrows are used to mark the upright direction of the package during shipment. If there is extra space in the shipping container, add something such as bubble wrap or news paper to take up the extra space, protect the sample and prevent it from moving around during transport.