LABORATORY SERVICES Newsletter
ANIMAL HEALTH LABORATORY GUELPH KEMPTVILLE RIDGETOWN

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In this issue:

What's happening at the AHL?

What's happening at the AHL?

Dr. Pat Collins began on July 2 in her new role as General Manager of the Laboratory Services Division, replacing Dr. Bruce Archibald, who resigned last fall. **Dr. Jim Pettit** had been in this position in the interim. Dr. Collins holds a PhD in biochemistry and is a certified clinical chemist. She has held a variety of positions in medical and environmental laboratories, and most recently was Director of Business Development at MDSWest, Clinical Laboratories, in Vancouver.

Dr. Hazel Alexander, AHL veterinary molecular biologist, left the AHL effective May 28, and has been replaced by **Dr. Hugh Cai**, who has worked for us for the past year in a contract position.

Y2K readiness. All AHL computer systems and laboratory equipment have been thoroughly checked and should be fully operational on January 1, 2000.

Dr. Nonie Smart left the AHL effective July 28 to return to the Ottawa area and pursue other interests. Nonie was the VLSB/AHL veterinary bacteriologist for the past seven years, and she will be missed. This position has been filled effective August 19 by **Dr. Marie Archambault**, a 1993 DMV graduate from St. Hyacinthe (MSc 1995, defending her PhD this fall). We welcome Marie on board.

Raccoon rabies. The mid-Atlantic raccoon rabies strain was identified by CFIA in two raccoons in late July in the Prescott and Brockville areas of eastern Ontario. For current information on the point infection control program, see the Ministry of Natural Resources website at www.mnr.gov.on.ca/mnr/rabies

Please note that the **AHL 'incoming collect' courier numbers** are:
Purolator - 0966901.
DHL - 960-374526
FedEx - 1157-2066-0

Feedback for the AHL? Please feel free to call, fax, or E-mail us at any of our labs.

CATTLE

Terbufos toxicity in a dairy herd

Dr. Herman Boermans, OVC; Dr. Brent Hoff, Mr. Nick Schrier, Dr. Marg Stalker, AHL

In May of 1999, 30 Holstein heifers and dry cows from a herd of 66 animals developed acute, severe clinical signs that included drooling and nasal discharge, dyspnea with increased bronchial sounds, ruminal hypermotility or paresis, depression, and death; pupillary constriction and muscle fasciculations were not reported. Tentative diagnoses included toxicosis from a cholinesterase (ChE)-inhibiting pesticide of the organophosphate (OP) or carbamate class. **Twenty-one cattle died over the course of investigation and treatment.**

Abnormal findings in two heifers presented to the AHL for necropsy included scattered focal transmural hemorrhages in small intestine. Canola seeds found in the rumen were tentatively identified as seed grain treated with CounterR 15G (containing the organophosphate terbufos) that had been disposed of on the farm property. Brain ChE activity from these two heifers was reduced by 80% compared to an unexposed cow; confirming ChE inhibition as the cause of the clinical signs.

Clinically affected cattle responded to atropine treatment; however, the response in more severely affected animals decreased over time. Since terbufos is not degraded within the rumen, administration of activated carbon was recommended to decrease further absorption. However, the supply of both atropine and activated carbon was limited, and the large number of potentially exposed animals made adequate therapy of all impossible (the OP antidote 2-PAM was not available). To assist in treatment decision-making, whole blood ChE activity was analyzed in the remaining 46 live animals to determine which animals had consumed the treated seed and the severity of their exposure. From the ChE results, it was determined that 36 cattle had not consumed the treated seed, 6 cattle had ingested the seed but at such a low level that only short term therapy was necessary, whereas 2 cattle had ingested sufficient terbufos to require prolonged treatment. Concern was also raised as to possible chemical contamination of the milk supply, however since none of the affected cattle were near calving and organophosphates are rapidly broken down after absorption, the likelihood of contamination of the milk supply was determined to be low.

To confirm the source of the toxicosis in this case, samples of rumen content were analyzed for terbufos and results confirmed by mass spectrometry. Tissue analysis is not recommended for organophosphates and carbamates because of rapid degradation of the compounds both pre- and post-mortem. Chemical analysis of gut content or source material should not be used alone in the diagnosis of poisoning by the cholinesterase-inhibiting classes of insecticides, as the analysis takes days to perform at significant cost, thus limiting the diagnostic usefulness to confirmation of exposure.

Cholinesterase analysis, which can be performed rapidly and at relatively low cost, should be used to demonstrate cholinesterase inhibition in affected animals in order to expedite diagnosis and therapy. If ChE levels are found to be normal, the need for costly chemical analysis is avoided and alternative lines of diagnostic investigation should be undertaken. Correct sample selection (whole blood in live cattle, and brain in dead animals) and proper sample handling (refrigeration or freezing if

possible) must, however, be followed in order to make a successful diagnosis. Carbamate poisoning in particular may be missed if tissue samples are permitted to remain at room temperature as regeneration of the cholinesterase enzyme may occur.

We are continuing to monitor blood ChE activity in surviving affected animals in this herd. Since the ChE enzyme is located primarily within red blood cells in cattle (in contrast to dogs and cats, where it is found mainly in the serum), return of bovine cholinesterase activity depends on red cell turnover and replacement. One month after poisoning, the blood cholinesterase levels of affected cattle had increased about 25%. It is expected to take up to several months for levels of activity to return to normal.

New BVD antigen detection ELISA

Dr. Susy Carman, AHL

The Animal Health Laboratory now offers a new rapid **BVD antigen detection ELISA**. This new test, developed at Ithaca, NY, has been approved by both the USDA and the CFIA **for the identification of cattle persistently infected with BVD virus, using sera from animals greater than 3 months of age**.

When compared to virus isolation, this new ELISA is reported to have a relative sensitivity of 100%, with relative specificity of 99.5%, and kappa of 0.94 using sera from persistently infected animals over 3 months of age. Indeed this test performed well at the AHL during our parallel testing with virus isolation (2 passages in primary bovine spleen cells).

However, this test has poor sensitivity (40%) for animals with lower levels of viremia and **should not be used for the detection of animals acutely infected with BVD virus**. As well, this test is not appropriate for animals less than 3 months of age, in which high levels of maternal antibody may interfere with the assay.

The preferred specimen is **serum**, however plasma can be used if serum is not available. **The fee is $10.00 per test to evaluate serum or plasma.**

As with our other antigen detection ELISAs, this ELISA can also be used as a rapid screening test to evaluate tissues for viral antigen. **The fee to process and test tissues is $15.00/test.**

We normally perform this test on Mondays, Wednesdays, and Fridays. However if you need to test large numbers of animals for export within a tight time-frame, please call the laboratory in advance so that we can be prepared to test your specimens as soon as they arrive.

For more information on this new BVD antigen detection ELISA, please call Dr. Susy Carman at 519-824-4120 ext 4551.

SWINE

Transmissible gastroenteritis: Identifying an accurate serological test

Drs. Susy Carman, Gaylan Josephson, Beverly McEwen, Grant Maxie, AHL; Tim Blackwell, John Martin, OMAF

Transmissible gastroenteritis (TGE) is a highly infectious coronaviral enteric disease of swine. It is

important to be able to identify TGE virus (TGEV) infected herds to prevent the spread of the disease to negative herds. Previously the most commonly used serological test to identify TGEV antibody was the virus neutralization (VN) test. However, TGEV antibody has been shown to cross-react with porcine respiratory coronavirus (PRCV) in VN tests. A TGEV/PRCV differentiating ELISA using blocking monoclonal antibodies has been developed by Svanova Laboratories in Sweden. The objectives of our study were to determine a) the sensitivity and specificity of this ELISA to identify sera with and without antibody specific to TGEV; b) if the test could be used alone or needed be used in parallel with the VN test; and c) if the test could be used for individual animal testing or if it should be used only on a herd basis.

Initially, sera from pigs known to be free from antibody to all coronaviruses, and from pigs experimentally infected with either TGEV or PRCV were tested. In these sequentially bled pigs, the ELISA did not consistently determine the TGEV status correctly until 21 days post-infection. For the PRCV-infected pigs, 9/10 animals were correctly identified, but 1 serum was incorrectly declared to be positive for TGEV.

Subsequently, 1783 serum samples (median of 15 samples per commercial herd) were collected from 70 herds considered not to be infected with TGEV and from 30 herds with a clinical history of TGE within the previous 2 years, and which had been confirmed by a positive TGEV fluorescent antibody test. Of the 30 herds with a clinical history of TGE, all but 2 herds had at least 1 animal with antibodies to TGEV. As well, 22 of these herds had pigs which tested positive for PRCV. Of the 70 herds presumed to be free of TGEV, 43 were free from all coronavirus antibody; 26 had antibody only to PRCV; 1 herd believed to be free of TGEV had 8/15 animals with antibody to TGEV. Pigs from TGEV-infected herds with low VN titers were more likely to have a positive PRCV ELISA result. In addition, when ELISA results and VN titers were compared, PRCV-positive ELISA results were more commonly associated with VN titers < 1:128.

When all 100 herds were included in the statistical evaluation, the ability of the Svanova ELISA to detect TGEV-positive herds (when at least 1 positive animal was present) was high (sensitivity = 0.933; specificity = 0.943). However, on an individual animal basis, the sensitivity was low (0.641), with a high specificity (0.989). The ability of the Svanova ELISA and VN to detect coronavirus antibody on an individual animal basis showed good agreement, with kappa = 0.84 (relative sensitivity = 0.979; relative specificity = 0.964). This agreement improved to kappa = 1.0 when the ELISA was evaluated on a herd basis.

When age was considered for all pigs from the 100 herds, the Svanova ELISA was most sensitive in identifying TGEV-infected herds when suckling pigs were evaluated (sensitivity = 0.83), as compared with sows (0.757), nursery (0.657), boars (0.25), gilts (0.233), and grow-finisher (0.133) pigs.

From this study, we conclude that the Svanova TGEV/PRCV differential ELISA is sensitive and specific when used on a "herd basis" and does not need to be used in conjunction with a VN test to identify TGEV-infected herds.

Other collaborators: Thank you to all of the **Canadian swine practitioners** who forwarded sera making this study possible; **Mioara Antochi**, AHL; **Ken Eernisse** USDA, APHIS, NVSL, Ames IA; **Drs. Gopi Nayar**, Veterinary Services, Manitoba Agriculture, Winnipeg, MB; **Pat Halbur**, Iowa State University, Veterinary Diagnostic Laboratory, Ames, IO; **Gene Erickson**, Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC.

Differentiation of PRRS virus using restriction fragment length

polymorphism (RFLP)

Drs. Gaylan Josephson, Hugh Cai, Hazel Alexander, Susy Carman, AHL

The porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded RNA virus with eight overlapping open reading frames (ORFs) from which genomic information is transcribed. The ORFs 5, 6 and 7 code for the major viral structural proteins - ORF 5 and 6 encode envelop proteins; ORF 7 encodes a highly basic nucleocapsid (N) protein. This N protein, known to evoke the greatest immune response, is well conserved among PRRS isolates from both North America and Europe (1).

The reverse transcriptase polymerase chain reaction (RT-PCR) technique has greatly increased our ability to identify all strains of PRRS virus in porcine tissues. For this test, a primer pair specific for the highly conserved ORF 7 is used. These primers will identify both field and vaccines strains of PRRS virus.

Following the detection of PRRSV, it is important to determine if the PRRSV identified is a field strain or a vaccine strain. In 1995, it became possible to differentiate between field strains of PRRSV and the RespPRRS/Repro vaccine virus (including the parent virus VR-2332), using RT-PCR and primers specific for ORF 5. ORF 5 is more variable among different genotypes of the virus, giving the possibility of genotyping by comparing the differences of this open reading frame (1). Following a RT-PCR reaction which uses a primer pair directed to ORF 5, specific restriction enzymes (Mlu I, Hinc II and Sac II) are used to cut the amplified product into genomic fragments. These fragments have variable lengths, resulting in restriction fragment length polymorphism (RFLP), and hence migrate at different rates in agar gels. Using this technology, a unique pattern is produced by each restriction enzyme for each PRRSV. These pattern types are given numerical codes. Hence the resulting RFLP pattern consists of three numbers, using the order Mlu I, Hinc II and Sac II (2).

PRRSV, being an RNA virus, mutates at a relatively high rate, and previous reports indicate that the virus undergoes genetic drift (3) while replicating in pigs. ORF 5 only contains 4-5% of the PRRSV genome and may undergo more or less change than other regions of the genome. With this genetic variability, and with digestion of ORF 5 amplicans using all 3 restriction enzymes, a large number of different patterns can exist. Mlu I can potentially produce 2 RFLP cut patterns, digestion with Hinc II can result in 8 different digestion patterns, and digestion with Sac II can produce 4 cut patterns (2).

For "vaccine" strains, the RFLP code for RespPRRS/Repro vaccine and its parent virus is unique, being 2-5-2 (2). However, the1-4-4 pattern for Prime Pac PRRS is not unique - this cut pattern can occasionally be found among field strains. (Note: research for the selection of the 3 restriction enzymes to differentiate vaccine and field viruses was done prior to Prime Pac PRRS entering the market.) "Field" strains can have many patterns such as: 1-7-4, 1-2-4, 1-3-2, 1-3-4, etc. Some strains appear to be "intermediate", having patterns that are close (either a single loss or gain of a restriction site) to the RespPRRS/Repro vaccine pattern. These are believed to have resulted from genetic drift within the vaccine virus ORF 5 sequence (2). Examples of intermediate patterns are: 1-5-2, 2-1-2, 2-2-2, 2-4-2, 2-5-4, 2-6-2, and 2-?-2.

Below is a summary of the RT-PCR and RFLP typing of PRRS viruses from diagnostic specimens submitted to the Molecular Biology Laboratory, AHL, Guelph on 136 occasions from January 1998 through to May, 1999. These samples originated from 101 different premises. On several premises, more than 1 RFLP cut pattern was identified over a period of time, including both field and vaccine strain-like patterns (e.g. 1-1-1, 1-1-2, 1-1-4, and 1-3-4 or 1-3-2 and 2-5-2). On farms where the vaccine-like strain pattern 2-5-2 was the first pattern identified, it remained the only restriction pattern noted on subsequent identifications from the same premises. The results of studies performed in Iowa are included for

comparison. The reason for the differences between Guelph and Ames in the identification rates for field vs. vaccine strains is unknown.

Comparison of results of RFLP typing of PRRSV by AHL and by NADC, Ames

	AHL, Guelph	NADC, Ames
Total tested using RFLP	136	221
Field strain	63 (46%)*	72 (33%)*
Vaccine-like strain Resp/PRRS Prime Pac	 50 (36%)* 3 (2%)	 134 (61%)* not done
Intermediate strain Resp/PRRS	 12 (9%)	 15 (7%)
Unable to type	8 (6%)	

* The testing performed by the National Animal Disease Center, Ames, Iowa, was performed in mid-1996 on PRRS virus from Iowa swine; testing at AHL, Guelph was done approximately 2 years later with PRRS virus from Ontario herds.

It must be remembered that the RFLP pattern gives no indication of virulence. As well the results cannot be used to determine which vaccine should be used in a specific production unit. However, evaluation of virus types over time can help in understanding the epidemiology of infection and whether there are multiple strains of virus on the farm. Very importantly, it helps to differentiate between vaccine and field strains of the virus. However, it must also be remembered that the procedure cannot differentiate between 2-5-2 vaccine and the parent field strain of the virus, which may be circulating at a low level.

References

1. Wootton SK, Nelson EA, Yoo D. Antigenic structure of the nucleocapsid protein of porcine reproductive and respiratory syndrome virus. Clin Diagn Lab Immunol 1998; 5: 773-779.

2. Wesley RD, Mengeling WL, Lager KM, Roof MB, Vorwald AC, Clouser DF. Evidence for the divergence of restriction fragment length polymorphism (RFLP) patterns after replication of PRRSV in Pigs. Proc Am Assoc Swine Pract Meeting 1998; 393-398.

3. Dee S. Overview - Third International Symposium on PRRS. International Pigletter 1999; 19: 25-27.

SPECIMEN RECEPTION

Sample submission suggestions

Ms. Linda McCaig

Most clinics are doing an excellent job of packing their samples to arrive here on time and in good condition. However there are still some problems and we offer the following recommendations:

1. **Think of the courier system as a "gorilla" handling all of your samples**. Some parcels arrive crushed, ripped, leaking, and generally in very poor condition. Sometimes samples are missing completely! Boxboard (cereal box type) and PUROPAKS are especially vulnerable to being caught and crushed in the conveyor belts of the central distributing warehouse.

2. Whenever possible, **please use corrugated cardboard boxes to ship samples**. Line the box with several layers of newsprint for absorbency and insulation. All samples should be shipped with freezer packs (gel type only, NO ICE CUBES). All samples should be clearly labeled with indelible pen and wrapped separately. Serum should be separated from the clot after complete clotting has taken place. Histology samples in formalin will only be accepted in screw-top containers (no whirl-pak bags) specifically designed for this purpose. Screw-cap urine bottles are inferior because the jars split and the caps leak. **We will supply your clinic with histo kits free of charge** (fax your request to 519-821-8072). Histo jars should be placed in whirl-pak bags which are folded down several times to make an air-tight, leak-tight package (remember the gorilla).

3. **Fresh tissues should be packed in separate whirl-pak bags labeled with their contents**. These bags should be sealed by folding several times and then should be placed inside another bag to prevent leaking. Feces should ONLY be submitted in screw cap jars NOT WHIRL-PAK BAGS, or VACUTAINER TUBES, or RECTAL SLEEVES. Ice packs (use gel type only, NO ICE CUBES) should be placed inside grocery bags or wrapped in newspaper so that condensation from them does not soak samples.

4. **All paperwork should be placed in a separate plastic bag** to prevent contamination.

5. **Your parcel should be as leakproof as possible**. Courier services are very upset by parcels that leak blood, other body fluids, or formalin. Please keep in mind that an unsuspecting handler could be exposed to any number of zoonotic diseases from your leaking package.

6. **Please fill in COMPLETE clinic name and address**. There are a number of clinics around the province with similar names. Also include the submitting veterinarian's name. You will notice that on our new requisitions we request demographic information (shaded areas on the forms). OMAF is requesting that we collect this information in order for you to receive the OMAF supported rates for testing of food/fiber producing animals.

Animal Health Laboratory Accreditations:
American Association of Veterinary Laboratory Diagnosticians (AAVLD) (lab system)
Thyroid Registry of the Orthopedic Foundation for Animals Inc. (OFA) (thyroid function)
Canadian Food Inspection Agency (CFIA) (EIA)
Canadian Association of Environmental Analytical Laboratories (CAEAL) (metals)
ISO 9002 registered (toxicology)

Mailing list

If you would like to be added to, or removed from, the AHL Newsletter mailing list, please fax your request to **Ms. Helen Oliver** at 519-821-8072 or E-mail to holiver@lsd.uoguelph.ca

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