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Swine pathology and diagnosis

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Case Studies

by Kurt Rossow

Case 1

History

Formalin fixed and unfixed brain, lung, liver, heart, kidney, and intestine along with sera and EDTA blood were submitted from six, four- to five-week-old pigs with a clinical history of paddling, stiff gait, hunched back, dog sitting, opisthotnos, and diarrhea. The development of clinical signs was slow and progressive over seven days. One hundred percent of 1000 pigs were affected and mortality was 5%. There was no response to antibiotic treatment. The pigs were on a new feed trial.

Gross lesions

Two lungs were tan and firm. Colon content from one pig was soft and yellow.

Diagnostic tests

Histopathology: Tissues were submitted for routine processing.

Bacteriology: Cultures made from lung, liver, and brain from each pig. *E. coli* and Salmonella were cultured from each small intestine and mesenteric lymph node. Salmonella isolation and darkfield examination were performed on each colon.

Electron microscopy: A pooled fecal sample was submitted.

Immunohistochemistry: From each pig, lung was tested for PRRSV and small intestine was tested for TGEV.

Molecular diagnostics: Pooled sera and tissues were tested for PRRSV by polymerase chain reaction (PCR).

Parasitology: Two pooled fecal samples were submitted for flotation.

Serology: Sera were submitted for PRRSV ELISA and PRV G1.

Toxicology: Six livers were submitted for ICP (Inductive Coupled Plasma Spectrometry). Five brains were submitted for sodium analysis.

Virology: Pooled tissues were submitted for PRRSV and PRV isolation. Pooled bronchial swabs were submitted for influenza testing by Directigen Flu A technique.

Results

Histopathology: Two pigs had bronchopneumonia and villous atrophy. Brain, liver, kidney, heart and colons did not have lesions.

Bacteriology: There were no significant bacterial isolates.

Electron microscopy: Rotavirus was identified in pooled feces.

Immunohistochemistry: No PRRSV was identified in lungs. No TGEV was identified in small intestine.

Molecular diagnostics: PRRSV was identified in pooled sera by PCR. No PRRSV was identified in pooled tissue by PCR.

Parasitology: Fecal flotation was negative.

Serology: Four of five sera were PRRSV ELISA negative. All sera were PRV G1 negative.

Toxicology: Five of six pigs had toxic liver arsenic levels. Each of the pigs had an elevated liver zinc level. Brain sodium levels were normal.

Additional submissions

Cervical spinal cord from an affected pig was submitted.

Diagnosis

Spinal cord lesions and liver arsenic levels were compatible with "3-nitro" toxicity.

3-nitro-4-hydroxyphenylarsonic acid ("3-nitro") is an organoarsenical added to swine feed as a growth promotant. 3-nitro is more toxic compared to other organoarsenicals and is recommended at lower levels in the feed. Episodes of toxicity are commonly induced by stress. Generalized muscle tremors and high-pitched squealing progressing to sternal recumbency and death characterize clinical toxicity. Some pigs may use their snout to maintain balance. Tremors diminish when pigs are recumbent. Some pigs may develop ataxia and paraparesis or paraplegia within a few days.

Diagnosis is dependent on demonstration of characteristic lesions in the cervical and lumbar spinal cord and demonstration of elevated arsenic levels in liver and/or feed.

Case 2

History
Formalin fixed and unfixed lung, liver, brain, small intestine and cecal contents were submitted from one two-day-old pig and two five-day-old pigs with a clinical history of diarrhea in two-day-old pigs and diarrhea and coughing in five-day-old pigs.

Gross lesions
Tissues from the two-day-old pig did not have lesions. Lungs from one five-day-old pig were diffusely gray/pink. Apical and middle lung lobes from the second five-day-old pig were red and firm. Intestinal contents from each five-day-old pig were watery and yellow.

Diagnostic tests
Histopathology: Tissues were submitted for routine processing.

Bacteriology: Lung, liver and intestine from each pig was submitted for culture.

Electron microscopy: Pooled feces were submitted for testing.

Immunohistochemistry: Lung was submitted for PRRSV testing.

Molecular diagnostics: Pooled tissue was submitted for PRRSV PCR.

Parasitology: Small intestine from the two five-day-old pigs was submitted for direct examination.

Virology: Small intestine was submitted for TGEV and rotavirus fluorescent antibody test. Pooled bronchial swabs were submitted for influenza testing by Directigen Flu A technique. Tissue was submitted for PRRSV, PRV and PRV isolation.

Results
Histopathology: The two-day-old pig had small intestinal lesions suggestive of *Clostridium perfringens* type A. One five-day-old pig had interstitial pneumonia and atrophic enteritis with coccidial merozoites. The second five-day-old pig had necrotic bronchitis and interstitial pneumonia. Villi in the small intestine were atrophied.

Bacteriology: There were no significant bacterial isolates.

Electron microscopy: Pooled feces were negative for virus.

Immunohistochemistry: PRRSV was identified in lung of each five-day-old pig.

Molecular diagnostics: PRRSV was identified in tissue homogenate by PCR.

Parasitology: Small intestine from each pig was positive for coccidia.

Virology: The Directigen Flu A test was positive and influenza virus was isolated from lung. PRRSV was isolated from pooled tissue homogenate cultured on porcine alveolar macrophages but not on MARC-145 cells. PRV isolation was negative. FA examination of small intestine was negative for TGEV and rotavirus.

Diagnosis
The one five-day-old pig was diagnosed with swine influenza pneumonia. The two five-day-old pigs were diagnosed with PRRSV pneumonia and coccidial enteritis.

Additional testing
The influenza isolate was submitted to the National Veterinary Services Laboratory and identified as swine influenza virus type A, subtype H3N2.

Case 3

History
Two live boars and one dead boar were submitted with a clinical history of pneumonia and death loss. In addition, nasal swabs from nine other boars were submitted. Boars were markedly dyspneic and febrile at arrival.

Gross lesions
The middle lung lobe of one boar is purple and firm. Remaining lung is red, heavy, and wet throughout. Middle lung lobes from the second live pig are red, heavy, and wet throughout. Lung from the dead boar is mottled red and wet throughout. The diaphragm is torn and the stomach is herniated into the mediastinal space.

Diagnostic tests
Histopathology: Tissues were submitted for routine processing.

Bacteriology: Brain, lung, liver, lymph nodes, and blood were submitted for culture.

Immunohistochemistry: Lung from each pig was submitted for PRRSV testing.

Molecular diagnostics: Tissue homogenate was submitted for PRRSV PCR. Pooled bronchial swabs were submitted for *Mycoplasma hyopneumoniae* PCR.

Serology: Sera were submitted for PRRSV ELISA, SIV H1N1 HI, and PRV G1.

Virology: Pooled bronchial swabs from the boars and pooled nasal swabs were submitted for Directigen Flu A testing. Pooled tissues were submitted for PRRSV and

PRV isolation. Sera were submitted for PRRSV isolation. Tonsils were submitted for PRV fluorescent antibody test.

Results

Histopathology: Lungs in both live boars were characterized by necrotic bronchitis.

Bacteriology: No pathogens were isolated from tissues or blood.

Immunohistochemistry: No PRRSV was identified in lungs.

Molecular diagnostics: No PRRSV was identified in tissue homogenate by PCR. No *Mycoplasma hyopneumoniae* was identified from bronchial swabs by PCR.

Serology: One of two boars had a positive PRRSV ELISA. SIV H1N1 titers were 80 and 160. PRV G1 tests were negative.

Virology: Influenza virus was identified from bronchial and nasal swabs by Directigen Flu A technique. Influenza virus was isolated from lung. No PRRSV was isolated from tissue or sera. FA examination of tonsil was negative for PRV. No PRV was isolated from tissue.

Diagnosis

Bronchitis, influenza virus

Additional testing

The influenza virus was submitted to the National Veterinary Services Laboratory and identified as swine influenza type A, subtype H3N2.

Swine influenza virus H3N21,2

Acute outbreaks of severe respiratory disease affecting pigs of all ages in multiple southern Minnesota swine herds began during the early winter of 1998. Coughing, fever, inappetence, and dyspnea characterized the clinical syndrome in all ages of pigs; in addition, sows/gilts in all stages of gestation were aborting. Herds well immunized with H1N1 swine influenza vaccine also experienced similar severe clinical disease. Swine influenza virus isolates submitted to the National Veterinary Services Laboratory were identified as type A influenza virus, subtype H3N2. Herds infected with the H3N2 influenza virus commonly experienced additional respiratory disease problems several weeks after the initial outbreak such as porcine reproductive and respiratory syndrome virus, pseudorabies virus, and *Mycoplasma hyopneumoniae*.

In the early fall of 1998, a H3N2 influenza virus was isolated from pigs with respiratory disease in North Carolina and concurrent with clinical disease in Minnesota, H3N2 influenza isolates were identified in Iowa and Texas. An isolate from each state was forwarded directly or by the NVSL to Dr. Robert Webster at St. Jude Children's research hospital in Memphis, Tennessee. The four H3N2 isolates were identified antigenically and genetically. The hemagglutinin gene of the H3N2 isolates originated from a human H3N2 circulating in 1995. However, the North Carolina isolate evolved from reassortment of human and swine influenza viruses while the other three H3N2 viruses represented reassortment of human, avian, and swine influenza genomes. H3N2 influenza virus has historically been uncommon in North America. An H3N2 influenza virus was isolated from pigs in Quebec, Canada in 1991 and was similar to 1975 human influenza strains. Within the United States, there had been only one H3N2 isolate, A/swine/Colorado/1/77.

Antibody to the haemagglutinin (HA) protein correlates with resistance to infection and disease. In humans, rechallenge with homologous virus demonstrates a strong resistance to reinfection for at least for years. Immune cross protection between influenza variants within a subtype is generally considered to be good. However, infection with one subtype of influenza confers little or no immunity to other subtypes, which explains why pigs with H1N1 antibodies had clinically severe disease. The patterns of the HA antibody development are the same for both subtypes of influenza virus. IgG antibodies appear two to three weeks after infection, peak at four to seven weeks after infection and fall over the first six months after the peak titer. Influenza titers will generally remain stable for two to three years without further antigenic stimulation.

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Foot lesions as the primary manifestation in a case of selenium toxicosis in weaned pigs

by Rodney K. Frank and Michael J. Murphy

Summary

Selenium toxicosis was diagnosed as the cause of foot lesions and poor performance in a group of 180 five- to eight-week-old weaned pigs. The primary lesions were hoof wall separation from the foot at the coronary band, mild emaciation, and slight alopecia. Liver selenium values of affected pigs ranged from 13.5–25.8 ppm wet weight (normal level<1.2 ppm). EDTA blood selenium values varied from 1.42–2.26 ppm (normal range 0.2–1.2 ppm). A base mix containing approximately 70X the label claim of 1 ppm selenium was identified as the source of excess selenium. The most common clinical sign in six other cases of selenium toxicosis in growing and

finishing pigs with selenium toxicosis diagnosed at the Minnesota Veterinary Diagnostic Laboratory was paresis/paralysis. The typical lesion seen when spinal cord was examined was malacia of the ventral horns of cervical and lumbar spinal cord. Based on findings in the present case, selenium toxicosis should be considered when foot lesions (hoof wall separation) are seen with poor growth in weaned pigs, even when paresis/paralysis is not a primary clinical sign.

Introduction

Selenium toxicosis has been produced in pigs by administration of inorganic and selenium supplements, selenium accumulating plants, and injection. Primary clinical signs of selenium toxicosis in postweaning pigs include alopecia, paresis/paralysis, abnormal hoof formation at the coronary band, depressed feed consumption, and poor performance.[1,4,5] Grossly, foot lesions of selenium toxicosis are characterized by hoof wall separation from the foot (corium) beginning at the coronary band.[3,4] Microscopically, lesions described for selenium toxicosis are focal symmetrical poliomalacia of the cervical and lumbar intumescences,[1,3,7] skeletal and myocardial muscle degeneration,[6] and hepatocyte degeneration or necrosis.[1]

Case history

Tissues from a group of 180 five- to eight-week-old pigs experiencing foot lesions, rough hair coats, and slow growth were submitted to the University of Minnesota Veterinary Diagnostic Laboratory (MVDL) in June of 1999. The pigs had been vaccinated with a commercial vaccine against *Mycoplasma hyopneumoniae*. When clinical signs first appeared, the producer injected the pigs with tylosin and iron dextran and medicated water with gentamycin. Gross lesions for two pigs necropsied by the submitting veterinarian were limited to hoof wall separation and mild serous atrophy of fat around the heart base.

Feed was mixed on the farm using a portable grinder-mixer. Six hundred (600) pounds of a commercial base mix was added to 1400 pounds of corn to make 2000 lb of feed.

A farm visit was made the following week. Approximately ten pigs were still affected. Foot lesions varied from slight separation of the hoof at the coronary band to marked separation and swelling of the feet. Affected pigs were thin, had slight alopecia, and walked stiffly. Pigs that were weaned subsequent to suspecting a feed-related problem and given a different feed had not experienced the problem. One of the pigs that was severely affected was euthanized, transported to the MVDL and a complete postmortem examination was performed. Six EDTA blood samples were collected from severely affected pigs and six blood samples were collected from the subsequently weaned pigs that had not experienced the problem.

Livers and feet from the two pigs were submitted to the MVDL. Livers were analyzed for arsenic, cadmium, cobalt, copper, iron, magnesium, manganese, molybdenum, lead, selenium, and zinc by inductive coupled plasma spectrometry analysis. Livers, blood samples, and feed were also analyzed for selenium concentration by gas chromatography.

The computer database at the Minnesota Veterinary Diagnostic Laboratory was queried to compare the present case with previous cases having a diagnosis of selenium toxicosis.

Results

Selenium analysis results

Livers contained 13.5, 25.8, and 15.3 ppm selenium on a wet weight basis. Liver selenium values of less than 0.4, 0.4 to 1.2 and greater than 1.25 ppm are interpreted by the MVDL as reduced, adequate and elevated, respectively, in swine. The base mix contained 69.5 ppm selenium (the label claim was 1.0 ppm). Estimated selenium concentration in final feed based on the addition of 600 pounds of concentrate per ton of feed was 20.85 ppm. EDTA blood selenium values for affected pigs were: 2.1, 1.63, 2.16, 1.42, 2.26, 1.70, and 1.76 ppm (m=1.861 ppm). EDTA blood selenium values for unaffected pigs were 0.78, 0.47, 0.41, 0.75, 0.62, and 0.58 ppm (m=0.602 ppm).

Gross pathology

The carcass submitted intact to the MVDL was thin and had mild hair loss. All four feet had moderate to severe separation of the hoof keratin from underlying tissue on the cranial aspect of each claw beginning at the coronary band.

Histopathology

For submitted tissues, livers had scattered single cell hepatocyte necrosis, hepatocyte karyomegaly, and mild to moderate portal hepatitis. Coronary band lesions consisted of a surface covered by necrotic cells and neutrophils with mild fibroplasia immediately beneath. In sections with an intact keratin layer, a thick layer of filamentous bacteria covered the inner surface of the keratin. No lesions were seen in heart or kidney.

For the submitted carcass, no lesions were seen in kidney, skeletal muscle, heart, ileum, spinal cord, or brain. The pig had a moderate lymphoproliferative interstitial pneumonia (consistent with lesions of mycoplasma pneumonia).

Bacteriology

No aerobic bacterial growth was obtained from the livers of the initial two tissue sets submitted. From lung, liver, and spleen of the submitted carcass, a scant growth of non-pathogens was isolated by direct culture. A *Salmonella* sp. (Group B4, 5) was isolated from enrichment

broth. From two of the feet, an abundant growth of beta-hemolytic *Staphylococcus* sp., mixed *Streptococcus* spp. and non-hemolytic coliforms was isolated.

Other cases of selenium toxicosis from the MVDL database

Information for other cases of selenium toxicosis in swine from submissions to the MVDL are presented in **Table 1**. Six of six groups of growing and finishing pigs had clinical signs of paresis and paralysis. Foot lesions were not reported as one of the clinical signs seen by any of the submitting veterinarians for these cases. Spinal cords were examined for three of these cases and in each case poliomyelomalacia of the ventral horns of the gray matter was seen. In one of the other cases for which spinal cord was not examined, encephalomalacia of the brainstem was seen. Liver selenium values for the pigs varied from 1.4 to 6.3 ppm on a wet weight basis. Feed was identified as the source of excess selenium in each of these cases but one.

Discussion

This case is interesting in that poor performance and foot lesions were the primary clinical signs of selenium toxicosis and paresis/paralysis were seen in only a few of the pigs. Experimentally-induced findings in pigs supplemented with toxic doses of selenium (20 or 40 ppm selenium in the diet) for 17 days included inability to coordinate walking, inability to stand, abnormal hoof formation at the coronary band, reduced body weight gains, and reduced feed consumption.[4]

Higher selenium exposure levels than those reported in other selenium toxicity studies may explain the predominance of foot lesions over posterior paresis/paralysis. Liver selenium values for the present case were 13.5, 25.8, and 15.3 ppm. These values are higher than those reported for other cases of selenium toxicosis in swine seen at the MVDL (**Table 1**) and for concentrations reported for other studies.[1,4,7] Mean liver selenium levels for pigs fed 20 and 40 ppm of selenium for 37 days were 6.483 and 5.796 ppm wet weight, respectively.[4] In another experimental

Table 1: Summary of information for cases of selenium toxicosis in pigs for submissions to the Minnesota Veterinary Diagnostic Laboratory from January 1, 1992 to December 31, 1998

Age	No. in affected group	Number dead/ Number affected	Clinical signs	Necropsy findings	Liver selenium (ppm wet weight)	Source of excess selenium	Final diagnoses
2 d – 1 w	190	50/122	Sudden death Rough hair coat Losing hair	Submandibular edema Emaciation	3.1, 3.3, 5.1	Iron formulation	Selenium toxicosis
6-8 w 9-12 w	200	6/12	Alert downers (paresis/paralysis)	Diffuse pneumonia	2.5, 2.8, 6.3	Feed	1. Selenium toxicosis 2. PRRS pneumonia
12-15 w	300	7/15	Posterior paresis Afebrile	Chronic pneumonia (2/4) Histopath: poliomyelomalacia	2.8, 2.6, 4.4, 4.2	Feed	1. Selenium toxicosis 2. Chronic bacterial pneumonia
8-12 w	75	25/50	Dullness (attitude) Laying around	Diaphragmatic hernia (1/4) Pneumonia (4/4) Histopath: Poliomyelomalacia (4/4); Suppurative pneumonia (4/4)	3.5, 2.2, 2.8, 3.3	Concentrate	1. Selenium toxicosis 2. Pneumonia
6-8 w	?	15/?	Alert downers (paresis/paralysis)	Reddened lungs Histopath: Poliomyelomalacia Interstitial pneumonia	1.4	Feed	1. Selenium toxicosis 2. Interstitial pneumonia
4 m	220	4/15	Weakness Paresis/paralysis Anorexia Emaciation	Histopath: Encephalomalacia (brainstem)	5.9	Feed	Selenium toxicosis
5.5 m	150	3/6	Rear leg weakness Stiffness Anorexia	No significant lesions (spinal cord not examined)	2.1, 2.7	?	Selenium toxicosis
1 y	500	0/1	Poor semen quality	Tissues submitted Histopath: Mild pneumonia Testicular degeneration	4.2	?	1. Selenium toxicosis 2. Pneumonia – PRRS virus, *Pasteurella multocida*
1 y	125	3/0	Paresis/paralysis Dyspnea Death (in 3-4 h)	Tissues submitted Histopath: Thrombosis in ileum (Spinal cord not examined)	4.9, 2.5	Feed	Selenium toxicosis
1 y	23	1/3	Staggering/ataxia Paresis/paralysis Anorexia Sudden death	Scoliosis (lumbar spine) Histopath: Poliomyelomalacia	5.5		Selenium toxicosis

study in which pigs were administered selenium in capsules, maximum selenium concentration for liver was reported to be 8.7 ppm for a daily selenium dose of 2.6 mg per kg body weight. In another case report of selenium toxicity for which both spinal cord lesions and foot lesions were reported, liver selenium levels for two pigs were 4.5 and 1.5 ppm and dietary selenium in complete rations were 9.7, 12.8, 26 and 27 ppm.[1]

Slaughter withdrawal time is a concern in pigs exposed to high levels of selenium. In a recent study of a herd experiencing selenium toxicosis, the mean blood selenium half-life was estimated to be 12 days.[2] Sixty days, the withdrawal time recommended by the Food Animal Residue Avoidance Database, was considered adequate to allow safe human consumption of tissues from the exposed pigs. Based on these data, pigs in the present case should have tissue selenium levels at an acceptable level for human consumption by slaughter age.

Conclusion

A diagnosis of selenium toxicosis should be considered for immature swine with any combination of clinical signs of posterior peresis/paralysis, separation of hoof from the foot beginning at the coronary band, alopecia, depressed feed consumption, and poor performance. For mature swine, boar infertility and posterior paresis/paralysis should be considered.

Acknowledgments

We thank Tom Arendt, University of Minnesota Veterinary Diagnostic Laboratory, for conducting all of the chemical analyses.

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Rep-PCR: A tool for strategic decision making on disease control

by C. Pijoan, M. Torremorell, M. Calsamiglia, S. Mendoza, and A. Ruiz

Strategic decisions for on-farm disease control require some evaluation of the epidemiology of the problem at hand. Until now, this has been difficult to do and has relied on clinical appraisal together with some information on the isolates obtained from diseased animals. This information has traditionally relied on classifying isolates based on phenotypic characteristics such as serotype or antibiotic sensitivity profiles.

A few years ago, discrimination between isolates was much improved with the advent of genetic analysis of the isolates. Usually this has been performed by digesting the DNA with restriction enzymes (called DNA fingerprinting) and then analyzing the resulting band pattern. Most practitioners are now familiar with the use of such techniques in analysis of viral isolates, especially PRRS virus, where restriction analysis (RFLP) has been widely used to differentiate and classify farm isolates, therefore getting some idea of their possible origin, diversity, and virulence.

However, the technique works less well with bacterial isolates because their larger genome results in many bands that are difficult to analyze well. Other techniques, such as ribotyping and pulsed field electrophoresis, have been used successfully, but they are difficult to do, expensive, and slow. Recently, a new technique called Rep-PCR has been described as a useful alternative for bacterial genomic typing. Our laboratory has standardized the conditions for Rep-PCR typing of both *Streptococcus suis* and *Haemophilus parasuis* which we have used as an important research tool for epidemiological studies. The technique is now offered through the diagnostic laboratory to all practitioners.

Case 1

This was a 1000-sow farm on two sites that had *S. suis* mortality in the nursery in excess of 5%. During a period of two years, we obtained systemic ("brain") isolates from this farm and compared them. All isolates proved to be the same strain even though the farm had doubled inventory to 2000 sows, established a three-site SEW production system, and instituted a variety of treatments and vaccine programs. This suggests that vaccination, of either sows or piglets, even using the same serotype, did not affect the presence of the predominant virulent strain for that farm. Therefore this technique proved useful in identifying the strain that was causing mortality and showing persistence of a virulent *S. suis* strain in a swine farm.

Case 2

This was a multisite system with multiple-origin two site production with excessive mortality attributed to *H. parasuis*. An analysis of isolates from this system obtained through the last years showed the presence of two distinct virulent strains. An analysis of the dates at which these strains were isolated demonstrated that isolate N was first present in the population and later isolate O replaced it. At that time, a vaccine with the isolate N but not O was used. The reasons why strain O arose are not known, but one explanation might be that vaccine pressure resulted in the emergence of a new strain. If this is the case, this also suggests that, in contrast to *S. suis*, *H. parasuis* vaccination does have a limiting effect on the presence of virulent strains within farms.

In summary, Rep-PCR is useful for the following:

- Identifying the virulent and prevalent strains in a given farm
- Establishing whether there is more than one prevalent strain
- Establishing the possible source of these strains
- Obtaining the appropriate isolate for autogenous vaccines and antibiotic sensitivity testing

In order to do this test, the following measures should be followed:

- At least two—but preferably many more—isolates from the farm should be submitted
- Isolates must be from systemic sites (not respiratory)
- Isolates must be obtained either at different times (single source farm), from different sources, or both