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EQUINE DISEASE QUARTERLY

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COMMENTARY

Advances in Understanding of the Pathophysiology of Placental Disease

One of the greatest joys in the horse-breeding industry is the birth of a strong, healthy foal. As such, their caretakers will go to whatever means necessary to ensure a good outcome, including painstaking monitoring for signs of a problem such as early udder development or vaginal discharge. Despite their hard work, some mares still abort seemingly out of the blue, or give birth to a "peanut," a small, runty foal which struggles to gain weight and thrive. Unfortunately, any number of diseases or conditions can cause these outcomes, and it often feels like there is nothing we can do to prevent them.

Fortunately, there have been massive developments in research techniques recently that allow researchers to understand the underlying mechanisms of disease. These range from genetic analyses to RNA-sequencing and advanced proteomics and lipidomics. In brief, DNA is transcribed into messenger RNA (mRNA), which can be translated into protein. Specific proteins (enzymes) alter the use and form of lipids, and each has a slightly different role in our understanding of health and disease. Together, these techniques use the interconnected systems of genetic material and proteins to provide a more holistic understanding of normal and abnormal pregnancy and can be used to identify better ways to diagnose, treat, and even prevent pregnancy complications.

Genetic analysis provides information about a horse's specific DNA. This remains the same throughout a horse's life and can be passed directly to their offspring. Perhaps the best-known genetic testing in horses is the 5-panel test in Quarter Horses, which includes tests for hyperkalemic periodic paralysis (HYPP), hereditary equine regional dermal asthenia (HERDA), polysaccharide storage myopathy (PSSM), and others. If a breeder knows that their mare has one copy of any of these genes, they can avoid complications in their foals if they make sure they breed to a "clean" stallion, or one who does not have a copy of that gene, which makes our horses healthier overall. Additionally, there is work being done to identify the genetic basis for umbilical cord torsion, placentitis, red bag delivery, and others.

RNA-sequencing analyses can assess the amount of mRNA produced in a tissue during disease. Because mRNA expression is the first thing to change during disease, we can often see the body's immediate response to an infectious agent, as well as how that agent causes the body's response to change over time. Additionally, mRNA is relatively simple to measure, allowing us to monitor expression of over 32,000 genes. By monitoring this response, we can better understand what treatments are likely to help, what biomarkers may allow better diagnosis, and how to help prevent the horse from getting sick in the first place.

Lastly, proteins and lipids do the heavy lifting in tissues and are what exert the majority of the biological effects we see. Unfortunately, the technology we have in place for testing large numbers of proteins and lipids is expensive and clumsy, making it difficult to look at changes which are happening at a cellular level. Even so, proteins and lipids are often the best biomarkers because they remain in circulation for relatively long periods of time and reflect actual physiological changes happening in response to the disease. For example, proteomic studies have identified biomarkers for placentitis that change in blood and in amniotic fluid.

From the outside, many of these molecular techniques seem pedantic and far removed from clinical reality; however, these techniques are what allow researchers to better understand the underpinnings of disease and how to diagnose and treat it. RNA-sequencing has recently been used to identify how the mare and unborn foal act to fight both ascending and nocardioform placentitis, including how these diseases affect blood vessel development, nutrient transportation and immune signaling. There have been other studies which have identified the key changes occurring in the placenta during early pregnancy – this work could ultimately lead to improved pregnancy diagnostic tests and better ways to assess pregnancy health.

These molecular level analyses provide keen insight into the workings of the placenta, and how those functions are disrupted during disease. This issue of the *Equine Disease Quarterly* contains articles by Dr. Von Dollen on hydrops and Dr. Swan on leptospiral abortion. Researchers are using various molecular analyses to better understand the pathophysiology of both of these important causes of fetal loss. Although it can be difficult to see exactly how this knowledge benefits horses today, the information from these studies is what will allow researchers to develop better techniques for diagnosis, treatment and prevention of disease. The broad view these techniques offer allows a more complete picture than any previous technology, and is already making a difference in how we diagnose and treat disease. Ultimately, these techniques make major strides towards every breeder's goal of creating pregnancies which result in strong, healthy foals.

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INTERNATIONAL

Fourth Quarter 2021

International Report on equine infectious diseases

The following data was primarily provided by the International Thoroughbred Breeders Federation; International Collating Centre in Newmarket, United Kingdom (UK); the Equine Disease Communication Center of the American Association of Equine Practitioners, and other sources. This report is retrospective and does not claim to be complete. However, it provides an indication of heightened activity of contagious or environment-related diseases among equids. Reports of equine infectious diseases were not received from Central and South America, sub-Saharan and equatorial Africa, and from most parts of Asia (with Japan being an exception).

Strangles was reported from various regions of North America and Europe, including the British Isles.

Several influenza outbreaks were reported from regions of North America, and from various parts of Europe, mainly UK, France, and Germany. EHV-4 respiratory disease was detected in groups of weanlings in Kentucky, USA, as well as from isolated cases, mostly from young stock, in North America, Europe (British Isles, central Europe, and the Mediterranean basin), and the Republic of South Africa.

Individual cases of EHV-1 abortion were reported from the Midwest region of the United States of America (USA), British Isles, continental Europe and from Japan. As expected with the cooler season in the northern hemisphere, outbreaks of equid herpesvirus-associated myeloencephalopathy (EHM) were reported from several regions of North America (Eastern Canada and the Mid-Atlantic, Midwest, and West regions of the USA). EHM was typically detected in one or more horses, while several other in-contact animals only had fevers and respiratory signs. EHM outbreaks were also reported from central Europe and from the Mediterranean basin (Italy).

With the end of 'mosquito season' in the northern hemisphere, there were few cases of West Nile virus encephalitis in North America (30-50 cases), and few cases in Europe; three cases from central Europe (Germany), one horse from the Balkan region, and 11 reports from countries around the Mediterranean Sea. Eastern equine encephalitis virus infection was detected in 15 horses this quarter, including states in the Midwest region of the USA (Michigan and Wisconsin).

Miscellaneous:
Approximately 20 laboratory submissions tested positive for *Taylorella equigenitalis* in the UK. One sample was PCR positive for equine coronavirus in Austria. Five submissions were PCR positive for *Lawsonia intracellularis* in the UK. Three abortions were attributed to *Leptospira* spp. (two in Canada and one in Japan), and two aqueous fluid samples from central Europe tested PCR positive for *Leptospira* sp. One horse tested positive for Potomac horse fever (*Neorickettsia risticii*) in the West region of the USA. Ukraine reported a single equine case of anthrax. The southern part of Africa reported a case of equine encephalosis and several cases of piroplasmosis; no cases of African horse sickness were reported during this quarter. Australia reported a case of Hendra virus infection in a single horse.

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NATIONAL

Hydrops Allantois and Hydrops Amnion in the Mare

While rare, dropsical conditions in the mare are a remarkably unfortunate gestational complication. Hydrallantois (or hydrops allantois) and hydramnion (or hydrops amnion) refer to the pathologic accumulation of excessive fetal fluid within the allantoic or amniotic compartments of the placenta, respectively. A mare affected by hydrallantois or hydramnion will typically present after six months of gestation with a relatively rapid (on the order of days to weeks) onset of increased abdominal size. Normal fluid volume in the allantoic cavity at term is approximately 10-15 L, while in cases of hydrops allantois, this can increase to well over 100 L. For amniotic fluid, the normal volume is approximately 3-5 L at term, with volumes exceeding 50 L reported in some cases of hydramnion. Hydrallantois is more common in the mare than hydramnion, but diagnosis and management are largely similar between the two conditions. A diagnosis relies upon clinical history, physical examination, transrectal palpation, and transabdominal ultrasound. Palpation per rectum classically reveals a domed, fluid-filled uterus, with the fetus unable to be palpated. When the uterus is evaluated by transabdominal ultrasound, a large volume of fluid dominates the field of view (>20 cm in depth), with the fetus suspended within this fluid. In some cases, the fetus cannot be visualized due to the extreme volume of fluid.

There are isolated case reports of delivery of a viable foal, if the hydrops condition develops in late gestation. However, the most common outcome is induced abortion of a nonviable or deceased fetus, in order to salvage the mare. Mares affected by hydrallantois or hydramnion are at increased risk for the development of prepubic tendon rupture, body wall herniation, uterine rupture, and hypovolemic shock during abortion/parturition. Due to these risks and the poor prognosis for fetal viability, management of hydrops conditions is generally accomplished through controlled drainage of fetal fluids and fetal extraction with concurrent supportive care of the mare. The client and clinician should anticipate that the mare will likely retain her placenta.

The underlying factors, which cause hydrops conditions in the mare are not precisely known, but recent research has identified significant differences in expression of genes influencing vascular development in normal placentas versus placentas from mares affected by hydrops conditions. The fetuses from hydrops cases often display gross congenital abnormalities, which have been hypothesized to contribute to the development of the condition through altered fluid dynamics in the fetus (e.g., swallowing and umbilical blood flow). Zoonotic *Leptospira* bacteria have been isolated from the fetus and placenta of a small number of hydrops cases. Therefore, personal protective equipment is recommended to be donned when working with hydrops cases. Recurrence of hydrops conditions in subsequent pregnancies is not a recognized risk. In one retrospective analysis of thirty cases of hydrops at a referral hospital, 95% of affected mares produced a normal foal in their subsequent pregnancy. Despite the poor prognosis for fetal survivability, hydrallantois and hydramnion are associated with a good prognosis for a mare's future broodmare career, if intervention is sought early. Potential heritability of hydrops is a subject of slight controversy, with differing reports found in the literature. A definitive study to answer this question would be ambitious to achieve, given the rarity of hydrops occurrence.

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KENTUCKY

Biosecurity on Equine Breeding Farms: Lessons Learned from Rotavirus B

During the 2021 foaling season in central Kentucky, area farms experienced an outbreak of neonatal foal diarrhea. A novel equine rotavirus group B pathogen was implicated in the disease based on compelling genetic sequencing evidence coupled with the clinical scenario of a highly contagious pathogen that causes diarrhea in foals under 4 days of age. A PCR test for the pathogen was quickly developed at the University of Kentucky, and testing information was shared with labs in the US, England, Ireland, France, Argentina and Japan. Unfortunately, the development of an efficacious vaccine has lagged behind, and disease control has focused on prevention through the implementation of increased biosecurity protocols.

Obtainment of a correct diagnosis must never be underestimated, but this can be hard to comprehend when there are no available cures or protective vaccines. However, a confirmed diagnosis is vital in order to guide mitigation strategies, promote for vaccine development, and leverage for increased biosecurity measures and compliance.

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Workflow, management culture, and practices should be adapted to meet the needs of a particular location. Over and above determining the correct cleaning process, disinfectant choice, application technique, and protective equipment is the process of understanding how a farm and its crew work, not only physically but as a team. Biosecurity is as much about leadership as it is about the actual protocol itself. Time and again we see the heartbreaking results of a farm crew worn down by long hours tending sick animals and adhering to a protocol, only to find one member of the team who does not "buy in." One breakdown in that team can be ruinous to the control of an outbreak and disastrous for morale.

Farm visits are a challenge in the face of an outbreak as we limit vehicular and foot traffic in vulnerable areas, such as the foaling barn. However, it is on these visits where observing how people interact with each other and their charges can be the key to success.
Development of a biosecurity protocol is a team effort where all members contribute thoughts on how to make a workable plan. This can be very hard to do, and suggestions from a third-party observer can often be beneficial. Observers can help identify an optimal starting point to help get everyone on the same game plan and communicate the practicalities of a successful biosecurity plan. Division of a workforce into units to care for groups of geographically isolated horses can be a helpful component of the plan.

Some practical tips for increasing biosecurity include wearing gloves, using disposable footwear and foot baths with appropriate disinfectant, practicing good hygiene between stalls, which includes changing gloves, washing hands and being free of organic material, and limiting visitors.

Disinfectants will not work in the face of organic material (e.g. feces, dirt, bedding). By simply removing organic material, the pathogen load can be decreased by up to 90%. Stalls and floors typically require scrubbing with a detergent prior to application of a disinfectant. Similarly, foot baths with disinfectant will only work when they are clean and changed regularly.

Items used in multiple stalls should be discouraged, but at a minimum they should be disinfected between animals and at the end of the day. All movements in the barn should proceed from clean to dirty, and foals showing signs of diarrhea should receive extra care in biosecurity. Aerosolization of infectious particles may occur with pressure washing or with the use of leaf blowers, so these practices should be avoided if there are animals in the barn or if the stall is expected to be occupied shortly after cleaning. Rotaviruses may survive up to nine months in the environment and therefore farms should not spread contaminated bedding or manure onto their pastures.

The goal of any biosecurity program is to reduce the exposure of animals to disease, requiring a multifaceted approach and collaboration between farm personnel, visitors, and veterinarians. All farms should have basic biosecurity practices in place each foal season and a plan to rapidly increase biosecurity in the event of an infectious disease event. Until an efficacious vaccine is available for rotavirus group B, farms will continue to rely heavily on biosecurity practices to slow the spread of this highly contagious disease.

A rotavirus group B infected foal with diarrhea. Photo Courtesy of Dr Laurie Metcalfe, Rood and Riddle Equine Hospital.

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Equine Amyloidosis

Amyloidosis is a rare disease in which abnormal protein, known as amyloid, is deposited within tissue and disrupts normal organ function. Multiple types of amyloidosis occur in humans and domesticated animals, but only reactive systemic amyloidosis (AA) and immunoglobulin-derived amyloidosis (AL) have been described in horses. Reactive systemic amyloidosis develops secondarily to chronic and non-specific antigenic stimulation. Immunoglobulin-derived amyloidosis occurs due to immunoglobulin light chain deposition during plasma cell dysfunction. Both AA and AL amyloid can be deposited systemically in multiple tissues or locally within a single organ. The University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) USALIMS database was searched for cases of amyloidosis to better understand this disease in horses.

Eleven cases of amyloidosis were diagnosed at the UKVDL from 2010 to 2021. Seven cases were diagnosed in horses that died or were humanely euthanized, and four cases were diagnosed in surgical biopsy submissions. Five cases were identified in Thoroughbreds, two in mixed breeds, and individual cases in a Tennessee walking horse, Saddlebred, Quarter horse, and warmblood. Horses ranged in age from less than a year up to 16 years. Six cases were diagnosed in mares, four in geldings, and one in a colt.

Animals that were submitted for postmortem examination presented with clinical histories of being found dead (two cases), acute colic (three cases), chronic unresolving abdominal abscessation (one case), or proliferative bone lesions (hypertrophic osteopathy; one case). Amyloid was microscopically identified in multiple tissues (systemic distribution) in all seven horses that died or were euthanized. Amyloid was deposited in the liver and spleen (three cases); liver, spleen, and kidney (two cases); liver, spleen, and lymph node (one case); and liver and kidney (one case). Four cases with liver involvement resulted in hepatic rupture and hemoperitoneum; two of these died naturally and one was euthanized. Co-morbidities in these horses included nodular pulmonary fibrosis, aortic mineralization and aneurysm, chronic peritonitis, bone disease (osteopathy), abdominal *R. equi* abscessation, mesenteric rent with small intestinal entrapment, and chronic lymphangitis (big leg). Of note: Chronic lymphangitis was evident in three of the seven necropsy cases and was the only co-morbidity identified in more than one animal. A chronic inflammatory condition was not overtly identified in one case; this horse died due to a mesenteric rent with small intestinal entrapment.

Four cases of amyloidosis were diagnosed from surgical biopsy submissions. These included three cases of nasal (nasopharyngeal) amyloidosis and one case of cutaneous (skin) amyloidosis. Horses with nasal amyloidosis typically present with clinical signs of epistaxis (nose bleed), difficulty breathing, or decreased athletic performance. Examination of the nasal mucosa can reveal nodular to diffuse thickening of the nasal mucosa due to amyloid deposition. Surgical excision is the treatment of choice for nasal amyloidosis. Horses with cutaneous amyloidosis commonly develop multiple papules, nodules, or plaques in the skin of the head, neck, shoulders, and pectoral regions. Skin lesions are firm, non-painful, and do not itch. There are no proven effective treatments for cutaneous amyloidosis. Cutaneous and nasal forms are not typically seen with the systemic form and do not develop from chronic inflammation, like the systemic form. A surgical biopsy should be considered if nasal or cutaneous amyloidosis is suspected.

Microscopically, amyloid from all cases consisted of extracellular homogenous eosinophilic material that distorted tissues. The material had an apple-green appearance under polarized light, consistent with amyloid. Granulomatous inflammation was associated with the amyloid in the cutaneous and nasal forms. None of the cases were assessed by proteomic techniques, electron microscopy, or immunohistochemistry.

In summary, equine amyloidosis is an uncommon cause of systemic, cutaneous, and nasal disease. Any horse breed, sex, or age can be affected. The systemic form is associated with chronic disease processes, and the liver, spleen, kidney, and lymph nodes are frequently affected. Nasal and cutaneous forms are not associated with other ongoing diseases and have a good clinical prognosis.

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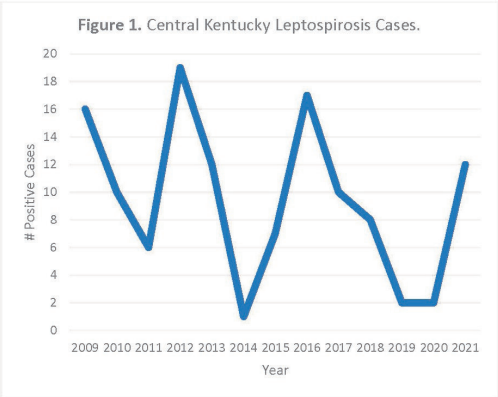
Equine Leptospiral Abortion in Central Kentucky

Leptospires are zoonotic bacteria of worldwide distribution. Adult horses acutely infected with *Leptospira* develop clinical signs associated with acute liver and kidney failure. Additionally, a strong association has been identified with equine recurrent uveitis and leptospiral infection. In general, clinical signs of infection coincide with the bacteria's natural tropism to target and replicate within the bile tract, kidneys, blood, placenta, and eyes. Animals are most commonly infected through contact with water or soil that has been contaminated with urine from an infected carrier animal, either through drinking a contaminated source or through open wounds. Leptospires can persist in the environment for weeks to months post exposure, which makes disease prevention and environmental control difficult. Infection of pregnant mares can result in abortion, stillbirth, or birth of a weak foal.

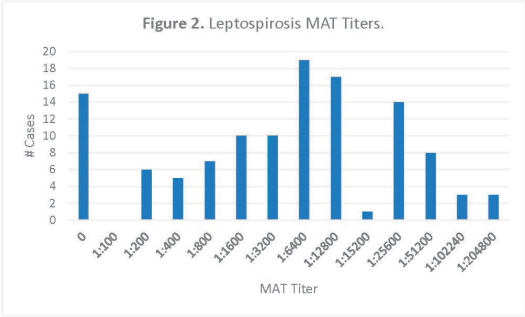
Since 2009, the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) has diagnosed 122 cases of equine leptospirosis in fetuses or neonates. This represents roughly 1.8% of the total number of fetal cases submitted to the UKVDL during this time period. On average, the UKVDL diagnoses nine cases of leptospiral abortion a year, but yearly cases can range from one to 17. An increased incidence of leptospiral abortion can occur in years with heavy rainfall in the late summer and fall. Therefore, the incidence of leptospiral abortion and nocardioform placentitis can be inversely related. Of the 122 cases, 80% were diagnosed in Thoroughbreds and 6.5% were diagnosed in Standardbreds, which directly mirrors the breed distribution in central Kentucky.

Leptospirosis commonly causes abortions during mid



to late fetal gestation. Submissions to the UKVDL ranged in gestational age from 5 to 11 months, with an average gestational age of 8.59 months. Grossly, fetal and placental lesions can vary. When present, the most common gross lesions associated with abortion are icterus, an enlarged mottled yellow liver, petechial hemorrhages within the placenta and/or fetal mucous membranes, and placentitis. Microscopic lesions include placentitis, hepatitis, and nephritis. The organism is typically visible within the fetal and placental tissues using a silver stain or immunohistochemistry.

Leptospira is a large genus of bacterial organisms of which *Leptospira interrogans* is the most important in humans and animals. *Leptospira interrogans* has multiple serovars and specific serovars more commonly cause clinical disease in different domesticated animal. In horses, the serovars that have been reported to cause abortion are Pomona, Grippotyphosa, Icterohaemorrhagiae, Kenewicki, and Bratslava. Currently, Pomona is the most frequent



serovar associated with abortion in Kentucky.

At the UKVDL, a combination of diagnostic tests is used to aid in the diagnosis of leptospiral abortion. Currently, the two most commonly utilized tests by UKVDL veterinary pathologists are the microagglutination test (MAT) and real-time polymerase chain reaction (q-PCR) assay. MAT testing is commonly performed on fetal heart blood or pericardial fluid and yields a titer indicating the fetus was exposed to leptospires during gestation. MAT is a highly sensitive test with relatively low specificity. In adult animals sequential MAT titers are utilized and a greater than fourfold increase in serial titers indicates recent infection in an unvaccinated animal. In fetal tissues, any MAT titer is considered significant. Of the 122 cases, 96.7% of the cases had a positive MAT titer and 90.4% of these cases had a positive MAT titer for either serovars Pomona or Grippotyphosa. Fetal titers ranged from 1:200 to 1:204,800 with the most frequent titer being 1:6,400. Most commonly, MAT titer results will be confirmed with either histologic lesions and a qPCR test. In 2012, the UKVDL implemented a qPCR assay for the detection of leptospires. PCR is a highly sensitive and specific diagnostic assay in which a positive result indicates the presence of the bacterial DNA within the sample. Of the 122 fetal abortions, 44 of these cases were qPCR tested and 95% of these cases tested positive via PCR analysis.

In conclusion, leptospiral abortions represent an

important cause of equine abortion in central Kentucky. Cases of leptospiral abortion are detected each year and farm personnel should be aware that the aborted fetal tissues and mare urine are infectious to other horses and pose a health risk to people coming in contact with them.

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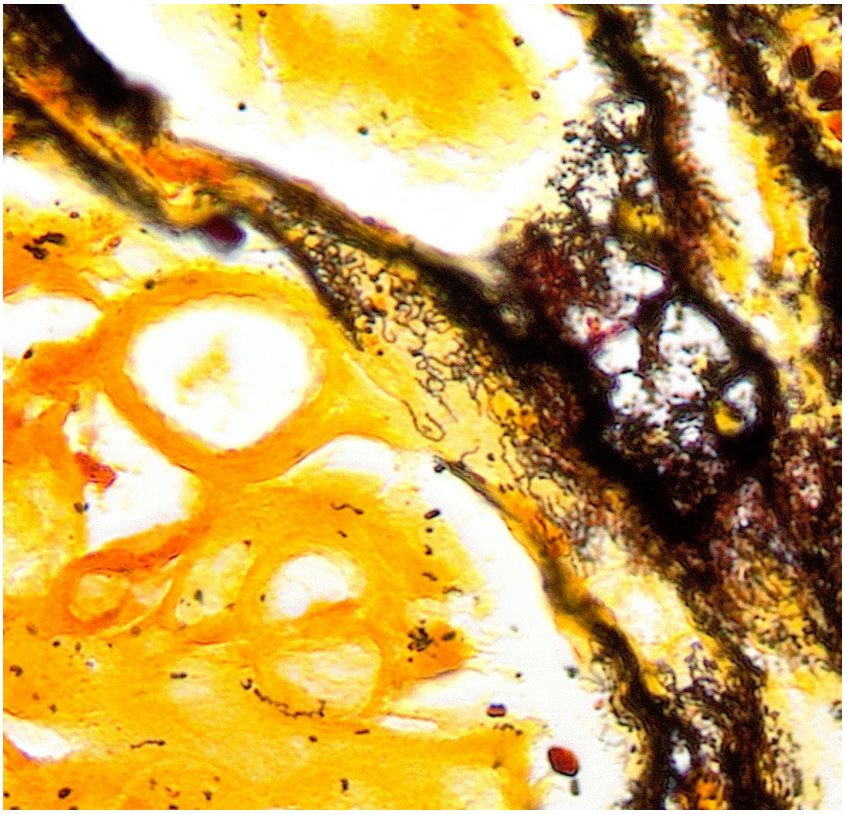

Figure 3. Numerous leptospires are located within the placental stroma. Leptospires are black and have a corkscrew to whip shape.