Pilot studies on mare reproductive loss syndrome

Stuart Burns,[a] Lenn Harrison,[b] Peter Sheerin[c]
[a]Burns Equine Veterinary Service, Paris, KY
[b]University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY
[c]Rood and Riddle Equine Hospital, Lexington, KY

Abstract

Past investigations utilized administration of either whole eastern tent caterpillars or their integument, to pregnant mares to study mare reproductive loss syndrome. Five pilot studies using various approaches were conducted to reproduce clinical signs of this syndrome. It is suggested that a stable ester toxin, mandelonitrile benzoate, present in cuticle (formed after ingestion of leaves with prunasin) is implicated.

Keywords: Caterpillar, mandelonitrile benzoate, prunasin, abortion

Introduction

An unusual abortion storm in pregnant mares in Ohio river valley region of US in 2001 and 2002 was recognized as mare reproductive loss syndrome (MRLS). Clinical signs were early and late abortions, pericarditis, and panophthalmitis. Approximately 9% of 2001 foal crop was lost to late pregnancy abortions and 26% of 2002 crop was lost to early gestation abortions. By September 2002, Jockey Club reported a 15% decline in foal registrations compared to same interval in 2001.[1] Economic loss to equine industry was estimated ~ $500M.[2] There were no pathognomonic lesions identified in late pregnancy abortions and tissue autolysis at time of conceptus expulsion in early pregnancy losses prevented detection of significant lesions.[2] A total of $2,313,552 was provided over 2002 - 2003 by multiple equine industry agencies, including Kentucky Thoroughbred Association, Kentucky Thoroughbred Owners and Breeders Association, Agriculture Development Board, Grayson-Jockey Club Research Foundation, University of Kentucky and USDA Agriculture Research Service.[3] Researchers from University of Kentucky Gluck Center, University of Kentucky Veterinary Science Department, University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) alone, or in cooperation with local equine veterinary practitioners, published research findings.

Collectively, research identified that eastern tent caterpillar (ETC; *Malacosoma americanum*) had a central role in causing MRLS and 2 hypotheses were advanced to explain pathogenesis. These hypotheses suggested either consumption of ETCs with bacteria-carrying setae (septic setae) or a possible toxin, were responsible for abortion. Since feeding sterilized (irradiated[4] or soaked in 95% ethanol for 2 hours[5]) caterpillars induced abortion, alimentary tract bacteria were considered responsible by colonizing affected tissues to cause abortion.[6,7] However, other researchers[8] suggested that bacteria that were isolated had little tendency to induce inflammation in colonized tissue. When fed irradiated ETCs, 3 of 6 pregnant mares aborted at longer than expected time, without clinical signs, suggesting absence of an infectious agent in this syndrome.[2,9] Thus, septic setae and toxin hypotheses both remain unproven[2] and exact cause of MRLS is undetermined.[10] Overall objective was to determine the right hypotheses using 5 pilot studies. Each study was designed to answer a single primary question of relevance, keeping animal numbers in perspective,[11] and using evidence-based principles.[12,13]

Experiments 1 and 2 were approved by University of Kentucky Institutional Animal Care Use Committee and Experiments 3 to 5 were conducted in compliance with USDA guidelines (refer previous paper in this issue). An independent ultrasonographer was engaged to eliminate any potential investigator bias and provide outside confirmation that horses participating in these protocols were in good health and receiving appropriate care.

Experiment 1

Transmission of toxic irritants produced by Lepidopteran caterpillars via aerosol exposure has been reported.[14,15] Chemical agents responsible for “urticating hairs” associated with these caterpillars

may vary within 12 common families associated with caterpillar toxins. Usual signs of toxicity include urticating dermatitis, renal failure, asthma, intracerebral hemorrhage and osteochondritis.[14] Caterpillar envenoming is associated with specialized setae, spines or urticating hairs,[14] which by themselves, are inert. These setae may be covered with, or contain within a hollow shaft, the offending toxic chemical(s) (Diaz 2006, personal communication). Objective was to determine if an unidentified toxic agent associated with ETC could reproduce abortions, clinical signs and/or tissue changes associated with MRLS following exposure to air containing an ETC toxin and/or setae.

Materials and methods

Four pregnant (180 - 300 days) mares were maintained in a paddock that provided free choice grazing and an open water source. Each mare was exposed to vapors produced by live, freshly fed ETC placed in a metal strainer with a mesh size of 1.7 mm, covered with a double layer of wire screen of 1.5 mm mesh size. These containers were held in a canvas nosebag feeder (well tolerated by mares) placed over the mare's muzzle, thus ensuring that most inhaled air would also have been in contact with ETC (Figure 1). Freshly fed, live ETC were used for each exposure, which lasted 6 hours per day for 10 days. Each mare was examined by transrectal ultrasonography every 48 hours to confirm pregnancy. Temperatures, heart rates and respiration rates were recorded once a day.

Figure 1. Canvas nose bag containing ETCs wire mesh container

Results

Three of 4 mares were unaffected by exposure to any vapors or aerosolized setae associated with ETC. All remained normal and all 3 mares delivered live, normal foals. An unexpected variable was introduced on day 7, when the 4th mare immersed her entire nosebag containing ETC in an open water source to drink. This behavior was repeated on day 8 and the mare aborted 72 hours later. Enterobacter spp. were isolated from placenta and all fetal organs. Expelled placenta was normal in size and weight, but had tan coloration, suggesting placental separation before abortion[2] and/or diffuse intracapillary thrombosis caused reduction in placental perfusion.

Discussion

Findings suggested that ETCs did not provide aerosolized setae or a vapor containing a chemical toxin and setae and/or toxin associated with their cuticle did not volatilize sufficiently to allow aerosol transmission. Abortion in 1 mare was caused by consumption of water contaminated by ETC, suggesting that setae and any bacteria associated with ETC, or a toxin associated with cuticle of ETC, were stable in water and it could have been a transfer medium that moved setae, bacteria or toxin from ETC cuticle to mare. Open water system might have been a risk factor in this abortion.[16] Water transmission in this protocol did not eliminate the possibility of setae involvement, as pore size of the container screen containing ETC setae would permit transfer of setae through screens to allow contact with oral or nasal mucosa of test animal. Bacterial isolates were associated with 90% of all MRLS abortions.[8]

Histopathological findings in this aborted fetus were limited to a fetal pneumonia and mild placentitis, reported in 65 and 10% of MRLS abortions, respectively.[8] These findings are commonly associated with late pregnancy fetal loss in MRLS.

Conclusion

Abortion, bacterial isolates and tissue changes associated with MRLS can be induced by consumption of water contaminated by ETCs. Transmission of the causative agent of MRLS via contaminated aerosols could not be demonstrated or did not occur, so attention should be directed to water as a transmitting medium.

Experiment 2

Inhalation of possible vapors and/or setae produced by ETCs did not induce MRLS, but water contaminated by ETCs induced MRLS. Protocol used in Experiment 1 could not determine whether abortifacient was a water-stable toxin or due to exposure to ETCs.[17] To determine if water contaminated by ETCs, but not containing setae, could induce MRLS abortion, clinical signs and/or tissue changes associated with MRLS. Objective was to determine if setae were a necessary component of MRLS, or if syndrome could be reproduced in their absence by exposure to water containing an unidentified toxin, but no setae.

Materials and methods

Five pregnant (273 - 310 days) mares were maintained together in a paddock similar to that in Experiment 1. Freshly fed live ETCs (500 grams) were placed at room temperature in 2.5 liters of distilled water for 30 minutes. Water was filtered through 6 layers of sterile gauze to remove any particulates, including setae, that may have originated from intact ETCs. Each day water was prepared fresh and examined microscopically for setae. Mares were gavaged once daily for 10 days with 500 ml of water and examined by transrectal ultrasonography every 48 hours. Temperatures, heart rates and respiration rates were recorded once a day.

Results

Four mares remained unaffected and had normal temperature, pulse, and respiration. On day 9 of exposure, 1 mare developed tachypnea that persisted for 4 days. Three days after the 10th exposure, this mare aborted with an uncorrectable dystocia and she was euthanized. Necropsy findings included congestion of all tissues, plus 300 ml of amber and slightly cloudy fluid in pericardial sac. Fluid analysis (total protein: 300 mg/dl, WBC: 1320, specific gravity: 1.024, pH: 8.0, increased glucose and low viscosity) suggested vascular permeability and leakage of peripheral blood components into pericardial space. Placental surface was a brownish color, similar to that observed in Experiment 1 abortion. There were no gross lesions on the amnion or allantois. Histopathological findings in fetus were a mild neutrophilic fetal pneumonia and a mild amnionitis. No bacteria were isolated from fetal tissues.

Discussion

Dystocia,[18] prepartum placental separation, tachypnea and pericarditis in this mare together with fetal pneumonia (observed in 65% of MRLS abortions) and amnionitis (observed in 80% of MRLS abortions), were reported earlier in MRLS.[8] This is the only reported experimentally induced MRLS abortion accompanied by pericarditis.

Conclusion

MRLS can be induced by a water stable toxin passing from ETC cuticle into water in contact with its surface. This protocol indicated that gastrointestinal exposure of setae-free ETC contaminated water could induce MRLS, thereby suggesting ETC setae are not obligatory to cause MRLS.

Experiment 3

Experiments 1 and 2 demonstrated that water contaminated by ETCs could induce MRLS. Experiment I protocol could not eliminate the possibility of setae contamination with possible tissue penetration. Experiment 2 provided a challenge dose of water contaminated by ETCs, but filtered to remove all particulates, including setae. Setae-free challenge caused 1 of 5 mares to suffer an MRLS abortion accompanied by pericarditis. Objective was to confirm that exposure of oral mucosa alone to fluid contaminated by contact with ETCs allows transmission of an unknown toxin in sufficient quantities to induce abortion, clinical signs and/or tissue changes associated with MRLS.

Materials and methods

Five pregnant (45 - 65 days) mares were maintained similar to earlier experiments. Mares were challenged with 100 ml of ETC contaminated saline sprayed into their oral cavity once daily for 5 consecutive days. This challenge fluid was prepared by placing 35 grams of dead ETCs into 35 ml of sterile saline, followed by centrifugation at 1600 x g for 10 minutes. To increase concentration of an unknown cuticle toxin, resulting supernatant was filtered through Waterman quantitative filter paper and frozen in 15 ml aliquots in plastic vials. Mares were then examined every 48 hours for 20 days by transrectal ultrasonography for changes in fetal fluid echogenicity and fetal heartbeat. Fluid echogenicity was scored 1 to 5 as described,[19] with higher numbers indicating increased echogenicity.

Results

Two of 5 mares developed echogenicity Score 3 in their fetal fluids on day 13, remaining 3 pregnancies maintained anechoic fluids. However, none of 5 mares aborted within 50 days after last exposure and all subsequently delivered normal foals.

Discussion

Anechoic fetal fluids are normal in equine pregnancies from days 10 - 85 of pregnancy, with any echogenicity during this interval regarded as an indication of fetal insult.[19] There was a 100% correlation between detection of increased echogenicity of fetal fluid preceding early pregnancy losses during 2001 and 2002 MRLS outbreak. Not all mares exhibiting fetal fluid echogenicity aborted (73% maintained their pregnancies).[20] Nevertheless, observation of fetal fluid echogenicity was considered an indicator for presence MRLS etiological agent.[4,2] Two pregnancies sharing this change in echogenicity of fetal fluids suggest that causative agent can cross oral mucosa without penetration by setae and initiate an inflammatory response in amnion, causing increased vascular permeability, allowing leakage of thrombin and fibrinogen to form insoluble strands of fibrin, followed by sloughing of some amniotic epithelium into the amniotic fluid, increasing echogenicity. There is a dose-response relationship between tissue response and abortion.[2]

Failure to proceed to abortion was likely related to dose and duration of exposure. Limited supply of challenge fluid resulted in only 5 days of treatment. Also, use of dead ETCs may have reduced amount of toxin recovered. Body motion of live ETCs may be a significant factor in moving toxic agent from its storage site at setae base (Figure 2) to cuticle surface and getting washed from surface to enter challenge fluid.

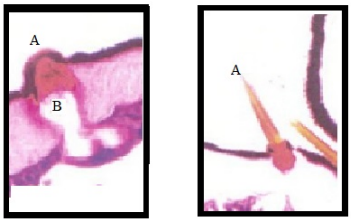

Figure 2. Cross section of tent caterpillar cuticle; (A) setae and (B) storage site.

Experiment 4

Experiment 3 suggested that agent causing MRLS was present in supernatant collected after ETCs were centrifuged with an equal weight of water. Based on University of Kentucky Veterinary Diagnostic Laboratory records, at least 10 species of bacteria were recovered from 682 late pregnancy abortions and only 1.6% of these isolates accounted for oral commensals, alpha streptococci and actinobacillus.[21] Source of nonhemolytic streptococci[22] and actinobacillus[23] in aborted fetuses was from their dam's oral mucosa. Unique DNA of cultured isolates from each mare's oral mucosa matched identically to similar organisms retrieved from her aborted foal. Type of bacteria isolated from late pregnancy aborted fetuses is dependent upon types of bacteria colonizing mucosal surface exposed to toxic agent. Consuming contaminated forage or water results in highest level of exposure to streptococci and actinobacilli, considered normal, commensal organisms in oral mucosa. When this mucosa is bypassed by delivering challenge dose of whole ETCs via a nasogastric tube, enteric bacteria are more readily involved, as intestinal mucosa becomes location of primary contact with toxin. When delivery of challenge agent via intravenous infusion resulting abortion could be only due to agent delivered. Objective was to determine if sterile abortion would result when contact exposure of the oral/intestinal mucosal is bypassed by intravenous presentation of the toxin associated with MRLS.

Material and methods

Challenge agent was prepared by placing 100 g of live ETCs in 100 ml of sterile water; these caterpillars had been denied access to cherry tree leaves for 24 hours, as Fitzgerald[24] reported that ETC foregut will be clear of mandelonitrile, a degradation product of prunasin, within 7 hours after last ingestion of cherry tree leaves. Thus, by 24 hours, there would be little or no contamination of the challenge fluid by foregut regurgitation, so that source of any persisting toxin would likely be from surface of ETC's cuticle. Mixture of ETCs and water was agitated gently for 10 minutes to stimulate activity of live ETCs and to wash off any material on cuticle surface. This contaminated water was then poured off, leaving ETCs in mixing vessel. This supernatant was filtered through 6 layers of sterile gauze to remove all particulate matter and frozen immediately in 25 ml aliquots in glass vials. A thawed sample was cultured and very few colonies of mixed growth bacteria were observed (considered nonpathogenic *Bacillus* spp. based on morphology and biochemical analyses). Intradermal injection (0.2 ml) of this challenge fluid was administered to a gelding to assess its ability to initiate an inflammatory response. The injection site was biopsied 96 hours later; there was inflammation and mild collagen necrosis within upper and middle dermis that included infiltrating neutrophils and macrophages, many of which were necrotic. Adjacent blood vessels had mild perivascular accumulations of neutrophils, macrophages and eosinophils.

We planned to send samples of ETC contaminated challenge fluid and sterile amniotic and allantoic fluid samples recovered from aborted conceptus (if any) to Institute of Biological Chemistry at Washington State University for identification of possible etiological agents.[25]

Four pregnant (days 60, 65, 85, and 100) mares were maintained on pasture, similar to earlier experiments. Last 2 pregnant mares (controls) received intravenously 100 ml sterile saline. First 2 mares received challenge agent intravenously (50 ml on day 1 to evaluate their tolerance to infusion, followed by 100 ml per day for 5 more days). Mares were scanned transrectally at 48 hour intervals for fetal fluid echogenicity grading[19] and fetal heartbeat.

Results

Two control mares were unaffected. Their fetuses maintained a heartbeat and fetal fluids appeared normal. Both treated mares (60 and 65 days of pregnancy), tolerated intravenous administration of challenge agent, with no indications of discomfort; they maintained normal body temperatures, pulse, and respiration rates during 6 days of treatment. Fetal fluids remained anechoic for first 5 days of challenge. On day 6, 1 mare developed uterine edema in nongravid uterine horn and had grade 2 fetal fluid echogenicity. She aborted between days 6 and 8 while at pasture and abortus was not found. Second treated mare was then examined at 24 hour intervals. Her fetal fluids remained anechoic for 8 days after

following first treatment. Uterine edema was observed in nongravid uterine horn on days 9 and 10. On day 11, fetal fluid became grade 2 echogenic and fetus died. Six hours after a fetal heartbeat could no longer be detected, mare was anesthetized to harvest conceptus, fetal membranes and uterus, as "fresh tissues" for bacterial culture and gross and histopathological examinations; however, fetus and its membranes delivered spontaneously during administration of general anesthetic (Figure 3). No gross lesions were observed in fetus or membranes and no bacteria were cultured from either source. Sloughed squamous cells from inner surface of amnion and clotted strands of fibrin were observed during cytological examination of amniotic fluid and amniotic portion of umbilical cord was inflamed (funisitis). Fibrin and sloughed amniotic cells caused fetal fluid echogenicity. Endometrial stroma had hemorrhage, as noted in 60% of MRLS abortions in 2001 and 2002.[8] Base of the endometrial cups was infiltrated with activated neutrophils (Figure 4) demonstrating an inflammatory response to a systemic irritant. Edema was observed in nongravid uterine horns both aborted mares.

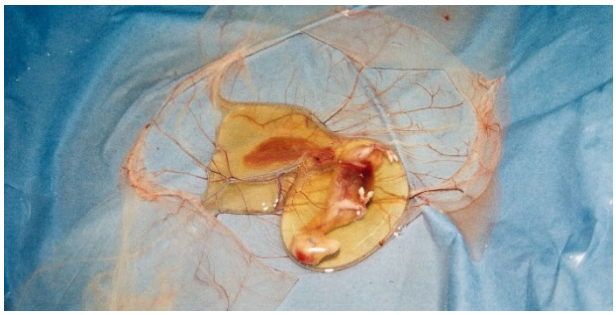

Figure 3. Aborted fetus 6 days after intravenous treatment

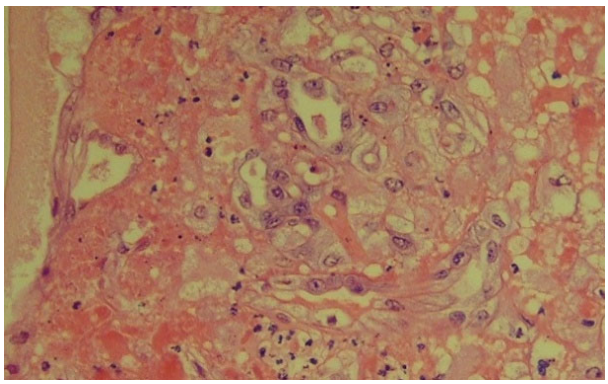

Figure 4. Activated neutrophils at base of endometrial cup (Antczak DF and Allen WR [published with permission])

Chromatographic analysis revealed that challenge agent, amniotic and allantoic fluids contained 358, 438 and 236 components, respectively; challenge agent and amniotic fluid shared 23 compounds in common, challenge agent and allantoic fluid shared 30 components and allantoic and amniotic fluids shared 72 components.

Discussion

Sixteen early-stage pregnant aborting mares did not undergo luteolysis and had plasma progesterone concentrations of 4 - 5.9 ng/ml at time of fetal death.[20] Plasma progesterone was not measured in our mares. Fetal fluid echogenicity preceding abortion in treated mares occurred only 24 - 48 hours before fetal death or abortion compared to 72 hours or more observed in many clinical cases of MRLS. This suggests that toxic agent was lethal before greater echogenicity could develop in fetal fluids.

However, histological signs of inflammation were similar to clinical cases of MRLS. Although revealed by chemical signature, the identity of each molecule remains unknown and it is tempting to speculate that the 23 compounds shared by the challenge fluid and the amniotic fluids may have included the toxin that caused MRLS.

Experiment 5

Some degree of tissue inflammation is a consistent feature of MRLS. However, most frequent isolates of oral commensal bacteria isolated from MRLS aborted fetuses are not capable of initiating such an inflammatory response or they would do so while in contact with oral mucosa. A previously unidentified toxic agent that can be washed from cuticle of ETC is able to initiate an inflammatory response and early pregnancy loss resembling MRLS. This agent is related to diet of ETCs which feed on black cherry (*Prunus serotina*) leaves that contain prunasin that degrades into glucose and mandelonitrile, an intense, but relatively unstable, tissue irritant. Mandelonitrile degrades to hydrogen cyanide and benzaldehyde. When 4 mares were given 3 mg/kg of mandelonitrile orally, a peak of cyanide in the blood, derived from the mandelonitrile degradation, was as high as 2025 ng/ml which occurred within 3 minutes after ingestion, but decreased to 250 ng/ml within 30 minutes. Seven pregnant mares fed 2 mg/kg of mandelonitrile in apple sauce twice daily for 14 days had peak blood concentrations of cyanide immediately after ingestion of mandelonitrile that stabilized at 315 ng/ml (range, 215 to 594 ng/ml) within 30 minutes. This dose and duration of exposure to mandelonitrile in circulation was unable to induce MRLS.[26] Mandelonitrile is present in ETC foregut after feeding.[27] It combines with mandelonitrile radicals, benzyl nitrile or possibly benzoic acid, to form a remarkably stable ester, mandelonitrile benzoate (MB), present on ETC cuticle surface.[28] Freezing ETCs produced a crystalline substance on their cuticle that could be shaken off the surface. These crystals were suspended in methylene chloride and the resulting solution injected directly into a GC/MS apparatus which confirmed the presence of MB. MB is an intensely irritating cyanogenic compound. Its stability does not allow release of a sufficient quantity of free cyanide to be detectable by colorimetric methods. This stability is useful to ETC, as it prevents absorption of free cyanide that would be toxic to ETC while retaining repellant properties of benzaldehyde that protects ETC from insect and vertebrate predation. Because of this stability, plasma cyanide concentrations in early pregnant mares could not be detected following their exposure to gavaged ETCs, even though many mares aborted. Objective was to determine if pregnant mares would develop clinical signs of MRLS (e.g. fetal fluid echogenicity and/or abortion) after oral exposure to mandelonitrile benzoate. This agent is produced by ETC and is derived from stabilization of mandelonitrile present in the ETC foregut after ingestion of prunasin-rich Black Cherry tree leaves.

Materials and methods

MB was prepared using a modified method of the procedure described.[29] This preparation would be expected to contain equal amounts of r and s isomers of mandelonitrile. It is unknown whether both isomers have equal biological activity. However, the r isomer is the only form in Black Cherry tree leaves[20] so that the preparation of MB may have only half the biological activity of naturally produced mandelonitrile benzoate. It was examined for its potential to initiate an inflammatory response by injecting 0.2 ml of MB solution intradermally into a test gelding. Mandelonitrile benzoate is insoluble in water and 95% ethanol, but is soluble in DMSO. Three 0.2 ml intradermal injections each of water, DMSO alone and MB dissolved in DMSO were administered to a gelding and injection sites were biopsied 96 hours later. Water and DMSO alone injection sites shared a tissue response that was essentially normal for a skin section. MB/DMSO injection site, however, had inflammation and necrosis within mid- and deep-dermis; this included infiltrating neutrophils and macrophages, many of which were necrotic. Adjacent and deeper blood vessels had moderate perivascular accumulations of neutrophils, macrophages and eosinophils nearly identical to those observed in the tissue response to intradermal injections of washings from the ETC cuticle in Experiment 4.

Three pregnant (days 45, 65, and 180) were maintained on pasture in similar conditions to other experiments. They were exposed by oral spraying of 10 ml of a solution containing 1 g of MB twice daily

for 14 days, similar to dose of mandelonitrile fed in applesauce.[26] Mares were examined by transrectal ultrasonography every 48 hours to assess fetal fluid echogenicity and fetal health (heartbeat); last mare (day 180) was examined transrectally with a 5.0 MHz transducer and transabdominally with a 3.5 MHz transducer every 48 hours, all beginning on first day of exposure to MB oral spray.

Results

Mare 1 (45 days of pregnancy) fetal fluids became grade 1 echogenic on day 13. This level of echogenicity remained unchanged for next 12 days and no further changes were observed. This mare did not abort. Mare 2 (65 days of pregnancy) fetal fluid echogenicity became grade 2 on 6th day of exposure. This progressed to grade 3 by day 13 and grade 4 by day 20 after first exposure. On day 24 (still at grade 4 echogenicity) fetus was observed to be at internal cervical os, through which fetal membranes were protruding. Fetus still had a heartbeat, but based on dilated cervix, position of fetus and membranes and prolonged interval of increasing fetal fluid echogenicity, abortion was imminent. Fetus and placenta were removed by vaginal delivery rather than risk losing or contaminating specimen.

There were no gross lesions on fetus or placenta. Fetal liver and spleen had marked diffuse echymotic hemorrhages, as observed in 60% of MRLS abortions and focal, minimal, endodermal, squamous metaplasia was observed on amniotic portion of umbilical cord, indicating early funisitis, observed in 80% of MRLS abortions. No other histological lesions were noted. No bacteria were isolated from any fetal tissue or fluid and tests for leptospirosis and EHV1 virus were negative. Cytological examination of allantoic fluid revealed abundant RBCs, sloughed chorionic cells and a few neutrophils, consistent with increased vascular permeability.

In Mare 3 (180 days of pregnancy), fetal fluid echogenicity was at grade 2 (prior to first exposure to MB), regarded as normal for mares beyond 85 days of pregnancy. Echogenicity increased to grade 3 on day 7 and to grade 4 by day 9. Fetal activity decreased by 50% within 48 hours after first exposure and continued to decline until day 4 of treatment, but then began to recover by day 6. Fetal heart rate was 124 on first day of exposure, decreased to 112 at 48 hours after first treatment and declined further to 104 by day 7. It increased again to 112 by day 11 and remained stable thereafter for the duration of treatment. Conceptus was not aborted, although fetus appeared to have incurred some stress.

Discussion

Mandelonitrile benzoate caused increased fetal fluid echogenicity, considered indicative od the presence of the agent that causes MRLS.[2,20] MB caused histological and cytological changes in day 65 conceptus that were identical to those in MRLS abortions in 2001 and 2002. Hence, tissue response to exposure to MB and ETC cuticle washings was almost identical, as was response to intradermal exposure to both agents. Based on these findings, we inferred that MB may be the etiological agent of MRLS.

Conclusion

Collectively, these experiments supported the suggestion that MRLS can be induced without any contribution from ETC setae or bacteria. It is possibly a chemical toxin derived from prunasin which is ingested by ETCs and in which its metabolites are processed in the foregut of the ETC to form the very stable toxin, mandelonitrile benzoate. This toxin is ingested by mares consuming contaminated forage or water. It acts as a tissue irritant, initiating inflammatory response that eventually induces abortion. However, findings need to be replicated in a larger population of test mares using a MB challenge containing only the r isomer of mandelonitrile.

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Conflict of interest

Authors have no conflicts of interest to report.

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