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Elevated serum amyloid A levels in cases of aborted equine fetuses due to fetal and placental infections

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ABSTRACT

Determination of fetal serum amyloid A (SAA) concentrations in aborted fetuses can provide valuable information regarding the infectious and/or inflammatory process of abortion in horses. To investigate the relationship between fetal SAA levels and the infectious/inflammatory disease process in feto-placental tissues, a SAA ELISA was used to test heart serum samples of 89 equine fetuses that were necropsied and diagnosed in the following groups: a multiorgan disease process diagnosed with an identified microorganism (14 cases, group 1); only placentitis diagnosed with an identified microorganism (nine cases, group 2); only placentitis diagnosed with no microorganism identified (six cases, group 3); and no infectious or inflammatory disease process diagnosed (60 cases, group 4). Serum amyloid A concentrations in equine fetuses were elevated from 10.5 to ≥40 mg/L in 10 of 14 cases in group 1; and from less than 2.5 mg/L to greater than 40 mg/L in seven of nine cases in group 2. In group 3, SAA concentrations were found to be less than 2.5 mg/L in five of six cases. In group 4, SAA concentrations were less than 2.5 mg/L in 55 cases, whereas in five cases SAA concentrations were elevated. Statistical significant differences were found between the concentrations of SAA in fetal horse blood and the presence of infectious and/or inflammatory disease process in the feto-placental tissues when a causative microorganism was identified. These results suggest that testing SAA concentrations in fetal heart blood may aid in further understanding the causes of abortions in horses.

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1. Introduction

Serum amyloid A (SAA) is one of the major acute phase proteins and a highly conserved protein synthesized predominantly by the liver. Serum amyloid A is triggered by infection [1–5], inflammation [2,6], stress [7,8], neoplasia [9,10], trauma [11], and toxins [12]. Free SAA mediates the chemotaxis of monocytes, granulocytes or T-cells, down-regulates fever, and inhibits prostaglandin E2 release as well as oxidative burst [13]. Serum amyloid A is also suggested as an important protective modulator of the inflammatory state and thought to play a role in innate immunity [14].

Serum amyloid A determination is well recognized and used in human medicine for diagnosis, prognosis, and assessment of health [4–6,9]. Increased concentrations of SAA have been detected with inflammation, infections, and surgical trauma in adult horses and with bacterial infections, arthritis, and septicemia in foals [1,15]. Recent studies showed that SAA concentrations were elevated in mares with ascending placentitis and suggested that SAA could be used as a diagnostic marker [16,17].

The causes of equine abortion have been previously studied [18–20]. It was found that infectious causes accounted for 25.9% of abortions in horses, but a diagnosis could be not be established in 16.9% of equine abortions

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[18]. Serum amyloid A was reported as elevated in preterm lambs experimentally induced by ventilation and endotoxin [21], but to our knowledge, SAA concentrations in fetal heart blood have not been reported in clinical abortion cases in domestic animals or humans. In this study, we determined the fetal heart SAA concentrations and investigated the association with infectious, inflammatory, and other causes in aborted equine fetuses.

2. Materials and methods

2.1. Materials

Eighty-nine, late-to full-term equine fetuses, submitted to the University of Kentucky Veterinary Diagnostic Laboratory between September 1, 2011 and April 11, 2012 were included in the investigation. Necropsies were performed by several different veterinary pathologists at the University of Kentucky Veterinary Diagnostic Laboratory using a standardized laboratory protocol for ascertaining the cause of the abortion. Formalin-fixed tissues were stained with hematoxylin and eosin for histopathological examination. Representative samples of liver, lung, gastric contents, and placenta were submitted for virus isolation and bacterial culture. A microscopic agglutination test on the fetal heart blood and fluorescent antibody test on placental and kidney tissues were performed to confirm leptospirosis. Fungal culture was undertaken in some cases at the discretion of the veterinary pathologist. Virus isolation and fluorescent antibody test for equine herpes virus-1 (EHV-1) were performed on fetal tissues and placenta. Equine herpes virus-1 positive cases were confirmed by a real-time polymerase chain test assay. For SAA testing, available amount of fetal heart blood was collected at the time of postmortem examination, placed into a red-top and/or serum tube and centrifuged at 2300 × *g* for 5 minutes; serum was separated and stored at −80°C until use. All testing were performed at the University of Kentucky Veterinary Diagnostic Laboratory, fully accredited by the American Association Veterinary Laboratory Diagnosticians (AAVLD).

2.2. Experimental method

Based on the diagnosis in the final pathology report, the 89 equine fetal abortions cases were assigned to one of four groups: Group 1: multiorgan infectious or inflammatory disease diagnosed, with an identified microorganism (14 cases); Group 2: placentitis only, with an identified microorganism (nine cases); Group 3: placentitis only, without an identified microorganism (six cases); and Group 4: an infectious or inflammatory process was not identified (60 cases). A commercial ELISA kit was used to assess the association between fetal SAA levels and infectious and/or inflammatory process in these abortion cases.

2.3. ELISA for SAA

A commercially available multispecies SAA enzyme-linked immunosorbent assay (ELISA, Multispecies SAA ELISA kit; Tridelta Development Ltd. Kildare, Ireland), with the measuring range of 2.5 mg/L to 40 mg/L was used to measure the fetal heart SAA concentration for the 89 aborted equine fetuses. As per the manufacturer's recommendation, the sera were diluted 2000-fold, and assay was performed according to the manufacturer's instructions.

2.4. Measuring the postmortem delay by SAA ELISA

Three hemolyzed sera samples from Group 1 (cases 7, 8, and 9) which had a high fetal heart blood SAA concentration were selected to investigate the potential effect of a delay between the time of the death/abortion and postmortem collection of the fetal heart blood. The samples underwent three freeze-thaw cycles, were incubated at 4 °C, 24 °C, and 37 °C, and the SAA concentration at Days 0, 1, 2, and 3, using the same multispecies SAA ELISA (Tridelta Development Ltd.), was measured.

2.5. Statistics

Mann–Whitney rank sum test was used to determine if there was an association between fetal heart SAA concentrations and the presence of inflammatory or infectious disease in the designated groups and to determine if there was an effect of delay between the abortion and postmortem collection of the fetal heart blood on the fetal heart SAA concentration. Given that the measuring range for the multispecies SAA Elisa kita was 2.5 to 40 mg/L, serum samples with a fetal heart SAA concentration less than 2.5 mg/L were assigned a value of 2.5 mg/L, whereas those

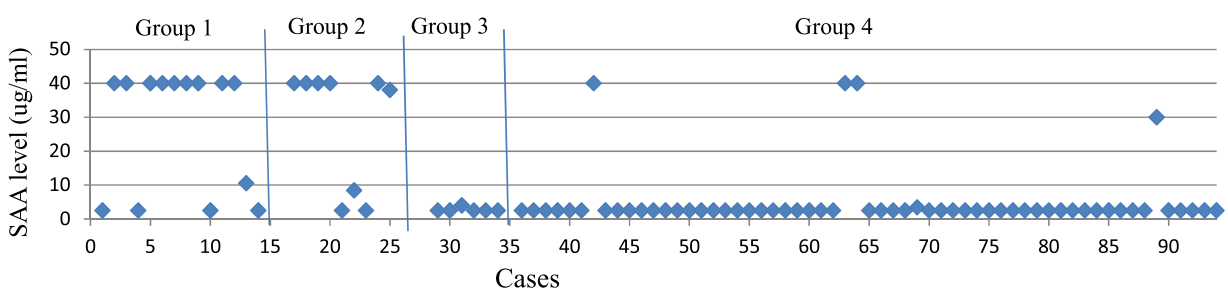

Fig. 1. SAA levels in fetal heart blood in equine abortion cases. Group 1: Multiorgan infectious disease process with identified microorganism. Group 2: Only placentitis with identified microorganism. Group 3: Only placentitis without identified microorganism. Group 4: No infectious and/or inflammatory disease process identified. Because the detection limit of the ELISA is between 2.5 mg/L and 40 mg/L, samples above or below these detection limits were reported as 2.5 mg/L or 40 mg/L.