

PROJECT SUMMARY



RURAL
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Research & Development
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Use of the guinea pig as a laboratory model for Equine Amnionitis and Fetal Loss (EAFL)

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Overview

Equine amnionitis and fetal loss (EAFL) is an unusual form of abortion that was first recognised in Australia in 2004 (Todhunter et al., 2009). Research carried out at The University of Queensland demonstrated that ingestion of whole Processionary caterpillars (PC) (*Ochrogaster lunifer*) and the PC exoskeleton shed during the growth phases of the caterpillar can induce abortion in mares, similar to naturally occurring EAFL (Cawdell-Smith and Bryden, 2009, Cawdell-Smith et al., 2012, Cawdell-Smith et al., 2013). These studies showed that the barbed setae of the caterpillar penetrate the gastrointestinal tract of the mare and can then pass into the uterus and fetal tissues (Todhunter et al., 2014a, Todhunter et al., 2014b).

The use of mares for further investigation into this condition is difficult due to the length of their pregnancies (approximately 340 days) and the associated high cost of maintaining pregnant mares over this period. The guinea pig was identified as a potential biological model for EAFL and this research was initiated to investigate and develop a guinea pig model for potential use in research to further our understanding of EAFL.



Who should be interested in these findings?

A successful biological model for EAFL will result in a greater understanding of the mechanism of this disease as well as allowing investigation of other hairy caterpillars as a possible cause of EAFL. These findings will be of interest to equine veterinarians, stud managers and mare owners. International researchers with a focus on mare abortion will also be interested in this study.

Background

Fetal loss is a cause of significant wastage in the equine industry with approximately 7–10% of Thoroughbred pregnancies lost in Australia each year (Ford, 2012). EAFL was first recognised in 2004 following an outbreak of abortions in the Hunter Valley of New South Wales. EAFL accounted for approximately one third of all equine abortions investigated in the eastern states of Australia in that year (Todhunter et al., 2009). An investigation of farms where most cases of EAFL occurred in that year, identified the Processionary caterpillar (PC, *Ochrogaster lunifer*) or the plant Australian Pennyroyal (*Mentha satureioides*) as possible causes of this condition (Perkins, 2005).



A similar form of abortion, mare reproductive loss syndrome (MRLS), was identified in Kentucky, USA in 2001 and 2002 and this condition resulted in the loss of Thoroughbred foals valued at approximately \$500 million (Sebastian et al., 2006). Investigations of environmental factors associated with these abortions identified the Eastern Tent Caterpillar (ETC, *Malacosoma americanum*) as a potential cause of MRLS and subsequent experiments in which pregnant mares were administered ETC orally resulted in abortion. These findings and the identification of PC on farms affected with EAFL led to the investigation of the PC as the cause of EAFL.

The cost of acquiring and maintaining pregnant mares, their long gestation and their size makes them impractical to use as an experimental model for ongoing research into EAFL. Attempts to identify a laboratory animal model for MRLS were largely unsuccessful with mice, rats and goats trialled (Sebastian et al., 2008). Pigs were also used and abortion was induced in this species following administration of ETC (McDowell et al., 2010). However, the use of pigs provides few advantages over the use of horses.

The guinea pig has been identified as a possible model for EAFL due to the similarity of its gastrointestinal tract to that of the horse.

Aims and objectives

The aim of this study was to investigate the guinea pig as a laboratory model for EAFL.

The objectives were to:

- Determine if the administration of PC exoskeleton to pregnant guinea pigs will result in fetal loss (death and/or abortion).
- Determine the stage of pregnancy when the administration of PC exoskeleton will reliably result in fetal loss in guinea pigs.
- Confirm that fetal loss following administration of PC exoskeleton to guinea pigs results in similar microbiological findings to those found in cases of EAFL.

Methods

The studies were approved by The University of Queensland Animal Ethics Committee.

Three studies were undertaken.

Study 1.

The guinea pigs were administered PC exoskeleton daily for 5 days from mid-gestation (Day 35 –Day 41 of pregnancy). Two guinea pigs were administered PC exoskeleton for 5 days from days 26 and 31 of pregnancy respectively. The guinea pigs in the control group were administered commercial guinea pig feed in a capsule.

Study 2.

In this study, based on the findings in Study 1, the treated guinea pigs were administered PC exoskeleton daily for 5 days from Day 25 of pregnancy. The guinea pigs and their fetuses were necropsied and sampled at set time points unless fetal death was detected, in which case the guinea pig was necropsied at the time of fetal death. The time points for sampling were gestational days 26, 28, 31, 40, 45, 50, 55 and 60. The animals in the control group were treated as for experiment 1.

Study 3.

The guinea pigs were administered PC exoskeleton daily for 3 days and similar to Study 2 were necropsied at set time points. However, sampling of the guinea pigs was undertaken daily from the second day of treatment (day 26 of pregnancy) through to day 35 of pregnancy and then every second day until day 39 with the final guinea pig sampled on day 46 of pregnancy.

In each study, guinea pigs were monitored daily for evidence of oestrus and left with the male for 24 hrs. This was designated as the first day of pregnancy unless the guinea pig returned to oestrus. (*continues overleaf*)

(from previous)

The guinea pigs were matched according to stage of pregnancy and PC exoskeleton was administered to the treated guinea pigs with the dose based on the body weight of the guinea pigs. The PC exoskeleton was macerated and put into gelatine capsules and administered orally. The control animals were treated as described above for Study 1. Guinea pigs were monitored twice a day from the commencement of the trial period and the pregnancies were monitored each morning by transabdominal ultrasound for the presence of fetal heartbeats from day 21 of pregnancy.

In Study 1, only one guinea pig suffered fetal loss. This guinea pig was anaesthetised, sampled and necropsied on the day of abortion. In studies 2 and 3, guinea pigs were anaesthetised, sampled and then necropsied at their set time point unless fetal death was detected beforehand, in which case they were sampled at the time of detection of fetal death.

In all cases where guinea pigs were necropsied, the peritoneal cavity, uterine cavity and all components of the placenta were sampled for bacteriology. In addition, maternal heart blood, amniotic fluid and lung and stomach contents from each fetus were collected for bacteriological examination. All samples were placed on horse blood agar and McConkey's agar bacteriology plates and incubated for 2 days at 37°C under both aerobic and anaerobic conditions. In Studies 2 and 3 samples were also placed into brain-heart infusion broth to enhance bacterial growth. Bacteria were identified using standard bacterial identification techniques with one isolate being further speciated using partial 16S rDNA sequencing.

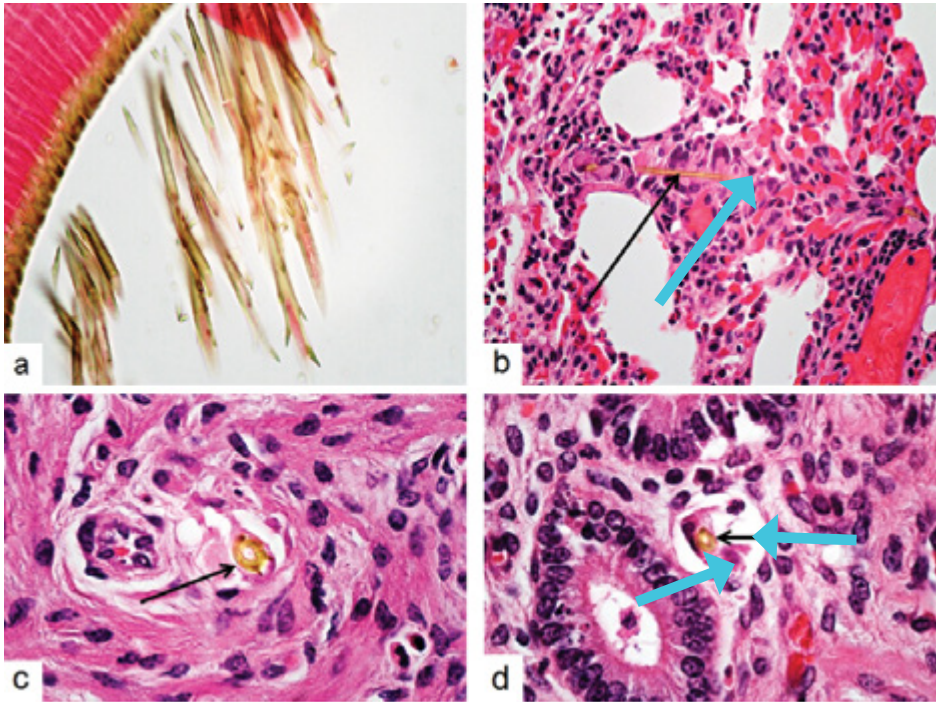
Tissues samples from both the dam and fetuses were collected and fixed in 10% buffered formal saline for histological examination.

Results/key findings

In Study 1, one guinea pig aborted. This guinea pig was first treated on day 26 of pregnancy and aborted on gestational day 31. The bacterial species isolated from the uterus of this guinea pig was of environmental origin, similar to the types of bacteria associated with EAFL. The remaining guinea pigs delivered healthy pups at term as did the animals in the control group. This finding suggested that PC can cause abortion in the guinea pig but only in the early placental stages of pregnancy. This is possibly due to the ability of immunoglobulins to cross the placenta in the guinea pig which is not the case in the horse.

Based on this finding in Study 1, in the other two studies the guinea pigs were challenged with PC exoskeleton from day 25 of pregnancy. In the second study, two of the eight treated guinea pigs suffered fetal death. In the third study, approximately 65% of the treated guinea pigs had abnormal pregnancy outcomes: fetal death occurred in six of the treated guinea pigs and one other had an abnormal placenta. Fetal death was most commonly seen between days 30 and 34 of pregnancy. The other two affected guinea pigs underwent timed euthanasia prior to gestational day 30 and in both cases bacteria consistent with those seen in cases of EAFL were isolated from the maternal and fetal tissues. Bacteria were also isolated from the other affected animals. The sites from which bacteria were isolated included: the peritoneal cavity, uterus, placenta, the junction between the uterus and placenta, amniotic fluid and stomach contents. Fetal death did not occur in any of the guinea pigs in the control groups.

The choice of the guinea pig as a laboratory model for EAFL was based on the similarity between its gastrointestinal tract and that of the horse. Both are hindgut fermenters with a large caecum. The disadvantage of this model is the difference in the type of placenta of the two species. The horse has a diffuse, epitheliochorial placenta with the chorion of the placenta in contact with the entire uterine endometrial surface. The guinea pig had a discoid, haemochorial placenta where the placenta is only in close contact with the endometrium over a small area. However, the guinea pig has multiple young so there is a considerable area of placenta in contact with the endometrium. In addition, the haemochorial placenta of the guinea pig has fewer layers separating maternal blood from fetal blood allowing passage of immunoglobulins from the dam to the fetus. Immunoglobulins do not cross the placenta of the horse. If, as indicated by the experimental studies undertaken in the horse (Todhunter et al., 2014a, Todhunter et al., 2014b), EAFL results from the passage of PC setae into the placenta, the discoid placenta of the guinea pig may reduce the effectiveness of the guinea pig for these studies. *(continues overleaf)*



Experiment 1.

Guinea Pig 7 that aborted on gestational day 32 following treatment with 1g/kg PC daily for 5 days from gestational day 26.

- a) Photomicrograph of a section of PC exoskeleton with setae barbs in situ.
- b) Lungs with setal shaft (arrow)
- c) Uterus with setal fragment in cross section (arrow)
- d) Endometrial gland with setae in cross section (arrow)

(from previous) The passage of immunoglobulins across the placenta in the fetus may also allow control of bacteria by the fetus. However, the results of these preliminary studies indicate that these disadvantages can be overcome by the administration of caterpillar components at day 25 of pregnancy rather than later in gestation.

Histological examination of the tissue samples collected during these experiments remains to be done. The findings from this aspect of the research will add to the knowledge of the mechanical aspects of setal penetration of tissues and the response of the tissues to their presence.

The findings of these studies indicate that the guinea pig is a suitable laboratory model for the study of the role of other caterpillar species in EAFL. The value of the guinea pig as a model for investigation of the pathophysiology of EAFL will be determined when the histological studies are complete.

Implications for stakeholders

The development of this model allows further studies into the mechanism by which PC components cause abortion. More importantly, for stud managers and mare owners it will allow investigation of other species of hairy caterpillars as possible causes of EAFL and may rule out some hairy caterpillars that are unlikely to cause fetal loss. This will allow targeted caterpillar control measures for the management of EAFL thereby reducing environmental and occupational health and safety risks.

Recommendations

Further research should be conducted into the mechanism of EAFL and the risk posed by exposure of pregnant mares to other hairy caterpillars. Ecological studies of the PC are needed to identify strategic measures which will reduce mare exposure. Evaluation of control measures undertaken on stud farms in the Hunter Valley indicate that targeted control of the early larval stages of the PC can reduce the incidence of EAFL (Carrick et al., 2014). This emphasises the value of further developing this aspect of the research.

Publications

Liu, N., Todhunter, K.H., Boe-Hansen, G., Bryden, W.L. and Cawdell-Smith, A.J. (2012) The guinea pig as a model for Equine Amnionitis and Fetal Loss. In: *Proceedings of the 4th Australasian Equine Science Symposium*, 4:61

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