Equine Amnionitis and Foetal Loss

— The role of caterpillars —

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The role of caterpillars

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Foreword

Reproductive efficiency is an important determinant of the success of the equine breeding industry. This is most apparent with thoroughbred breeding where extensive statistics are kept by the Australian Stud Book. Over the last 40 years there has been a gradual improvement in equine reproductive efficiency due to the efforts of scientists and veterinarians. In 2004, cases of unusual abortions were reported in the Hunter Valley region of New South Wales and it was subsequently shown that these cases contributed a third of all foetal losses where an autopsy was performed.

Following extensive investigations, and a detailed epidemiological study of the cases, it appeared that caterpillars or a poisonous plant could be the major aetiological agent. Interestingly, a similar syndrome occurred in the United States between 2001 and 2002 and the causal agent was shown to be a caterpillar. The research described in this report has shown that caterpillars and caterpillar exoskeleton can reproduce the syndrome initially described in the Hunter Valley. The results of this project will benefit all those who breed horses, and highlights the need for owners and breeders to be vigilant in the management of their stud enterprises.

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This report, an addition to RIRDC’s diverse range of over 1900 research publications, forms part of our Horse R&D Program, which aims to support research into disease prevention, diagnosis and treatment, as well as animal breeding and genetics.

Most of RIRDC’s publications are available for viewing, downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

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Executive Summary

What the report is about

Reproductive loss is a major cause of wastage in the thoroughbred breeding industry and probably accounts for a loss of about 7 - 10% of pregnancies per annum. While there are many causes of reproductive loss or abortion in mares, in recent years this loss has been reduced, especially with improved management and the vaccination against the equine herpes virus. However, in 2004 a series of abortions were described in the Hunter Valley region of NSW that showed an unusual and consistent pattern of clinical and pathological signs. The condition, which came to be known as equine amnionitis and foetal loss (EAFL), had not been previously reported. The case definition of EAFL showed similarities to descriptions of abortions associated with a condition called mare reproductive loss syndrome (MRLS) reported in Kentucky, USA in 2001 and 2002. The major causal factor that has been shown experimentally to be associated with MRLS is exposure of pregnant mares to Eastern Tent Caterpillars (Malacosoma americanum). These caterpillars do not occur in Australia, but other similar caterpillars do, and the question thus arose – are caterpillars involved in the pathogenesis of EAFL?

Who is the report targeted at?

The primary target group of this report is the thoroughbred breeding industry, especially equine stud veterinarians and stud managers. The results of this project will also be relevant to all of those who breed horses. The information will allow the development of on-farm strategies that will reduce the incidence of EAFL.

Aims

The primary aim of this project was to determine or elucidate the role of caterpillars in the pathogenesis of EAFL, specifically to determine if exposure of pregnant mares to processionary caterpillars (Ochrogaster lunifer) is associated with increased risk of abortion.

Methodology

Processionary caterpillars and their shed exoskeletons were collected from various sites on the Darling Downs, Queensland. The samples collected were stored frozen until used in experiments. Mares between the ages of 4-12 years were used in the experiments and exposure to caterpillars, or shed exoskeleton, was by nasogastric intubation. Prior to intubation the caterpillars and shed exoskeleton were blended in distilled water to form a slurry. The clinical response of mares to the caterpillars and times to abortion were recorded. All aborted foetuses underwent a full autopsy with samples cultured for bacteriology and tissues samples preserved for histopathology.

Key findings

Intubation of mares in mid-pregnancy with preparations of either whole caterpillars or shed caterpillar exoskeleton induced abortion. Mares showed very few signs of the impending foetal loss and appeared clinically normal. The gross pathology of aborted foetuses was similar to that observed in field cases of EAFL. Bacteria isolated from the aborted foetuses were enteric and environmental bacteria not associated with infections in the horse. Exposure of mares in the pre-placentation stage of pregnancy (< 35 days) did not result in abortion. However, during the early-placentation stage (days 45-60) exposure of mares to shed exoskeleton preparations did result in foetal loss. The results of these studies provide experimental evidence that exposure to processionary caterpillar materials can induce pregnancy loss in mares, and that shed caterpillar exoskeletons alone can also cause abortion. Gross pathology and bacteriology were similar to that seen in cases of EAFL.
**Implications and recommendations**

The results of this project unequivocally demonstrate that exposure to processionary caterpillars may result in abortion in mares. For many in industry, this concept has been difficult because it is generally assumed that it is unlikely that horses would consume processionary caterpillars. However, the results demonstrate that shed exoskeleton can also induce EAFL. It is conceivable in field situations that the small amount of exoskeleton required to induce abortion could be inadvertently consumed by a horse while grazing. Moreover, the light nature of this material means that it could be carried over reasonable distances by wind and expose horses in pastures in which there may be no evidence of the presence of processionary caterpillars.

The establishment of the association of processionary caterpillars with the induction of foetal loss in mares provides a focus for the development of strategies to reduce risk from this form of abortion. In particular, attention should be given to developing farming and grazing strategies that reduce the likely exposure of pregnant mares to processionary caterpillars and perhaps other similar caterpillars which may also induce abortion.

There is scope for further research on the mechanism of action of the caterpillar materials and how they allow gastrointestinal and placental transfer of microorganisms, as well as for the investigation of other methods that may reduce or eliminate the factors that cause EAFL in pregnant mares.
Introduction

Causes of foetal loss or abortion are commonly divided into those of infectious, non-infectious or unknown nature. Reported causes of abortion in mares are changing over time, largely in response to continued advances in understanding the pathogenesis of abortion and subsequent improvements in control and prevention of particular conditions (Acland, 2003). For example, the importance of equine herpes virus abortion due to EHV-1 has declined over the past three decades due to availability of vaccines and more effective biosecurity based on understanding of the epidemiology of the disease. Twinning has also declined in importance as a cause of abortion since the widespread adoption of ultrasonography for early pregnancy testing. Placentitis due to a variety of causes has emerged as perhaps the most important cause of pregnancy loss in the last decade (Perkins, 2008).

Two previously unreported syndromes of pregnancy losses in mares that occurred in the USA (2001-2002) and Australia (2004), have similar clinical and pathological characteristics and in both instances caterpillars have been implicated as a causal factor. The interrelationships between potential causal factors for these conditions remain to be fully elucidated. It is not clear whether such causal factors may be the same in the two regions. Before reviewing the Australian syndrome, it is informative to discuss what occurred in the USA.

Mare Reproductive Loss Syndrome (MRLS)

Mare reproductive loss syndrome (MRLS) was observed on breeding farms in Ohio and central Kentucky during 2001 and 2002 (Dwyer, 2003; Riddle, 2003). The clinical syndromes associated with MRLS were abortion, foetal loss, opthalmitis and pericarditis. The abortion syndrome was divided into mares that suffered late foetal loss (LFL) and those that suffered early foetal loss (EFL).

In the cases of LFL, affected mares showed few or no impending signs of abortion. Abortions occurred in the last several weeks of gestation and mares showed signs such as abdominal pain commonly associated with delivery of a near-to-term foetus rather than signs associated with a disease that might have caused the abortion (Brown, 2003). About one third of cases presented with premature placental separation as red-bag deliveries and many of the deliveries were complicated by malposition of the foetus. Foals that were delivered alive were often compromised and required intensive care (Brown, 2003; Dwyer, 2003).

Foetuses from affected mares were of normal size and weight and were generally fresh. Aeration of the lungs in some foetuses indicated that the foal had made respiratory efforts and was alive at the time of delivery. The main pathologic findings were funisitis (inflammation of the umbilical cord) of the amniotic portion of the umbilical cord, amnionitis, foetal pneumonia, bacterial infection and haemorrhages (Brown, 2003; Byars and Seahorn, 2003). The bacterial infection was an ante-mortem event and most of the pathological lesions could be attributed to bacterial infection. The most common bacteria isolated from foetal tissue, stomach contents or placental samples, included non-beta haemolytic *Streptococci* spp. and *Actinobacilli* spp (Donahue et al., 2003; Williams et al., 2004).

Most early foetal loss (EFL) occurred between days 40-80 of gestation (range 32 to 140 days). Mares showed no outward signs of foetal death, with the most common finding being the presence of a dead foetus on ultrasound examination with echogenic allantoic and amnionic fluid. In some mares a vaginal discharge was the only presenting sign (Riddle, 2003).

Both opthalmitis (Latimer, 2003) and pericarditis (Seahorn et al., 2003) were seen as unusual diseases associated with the occurrence of the MRLS outbreak.
Induction of MRLS

Known causes of abortion in horses were ruled out through field and laboratory investigation. Monitoring of pasture and soil factors (plant cyanogens, phytoestrogens, nitrate, minerals, ergot alkaloids and other mycotoxins) and climatic indices on both MRLS-affected and unaffected Kentucky farms during 2002 did not identify any association between measured parameters and either increased or decreased risk of MRLS (Powell et al., 2003). However, an association was demonstrated between the presence on affected farms of the Eastern Tent Caterpillar (ETC; Malacosoma americanum) and the incidence of MRLS (Cohen et al., 2003a,b; Dwyer et al., 2003). Experimental studies involving exposure of mares to ETC demonstrated increased incidence of abortion in mares receiving ETC compared to control animals (Powell et al., 2004). In brief, abortions that resembled naturally occurring MRLS cases, were induced by feeding ETC on pasture and mixed with sweet feed, as well as by administering ETC by nasogastric intubation. Feeding the outer skin or exoskeleton also caused abortions (Bernard et al., 2004; Sebastian et al., 2003a,b; Webb et al., 2004).

Equine Amnionitis and Foetal Loss (EAFL)

During the winter of 2004 a number of abortions were reported from thoroughbred studs in Australia that involved an unusual and consistent clinical and pathological appearance. Cases were reported in New South Wales, Queensland and Victoria. Abortions were detected from mid-pregnancy to term, and mares typically did not show any signs of illness or discomfort prior to abortion. The term equine amnionitis and foetal loss (EAFL) was applied to the condition to reflect the typical presenting signs.

Most affected farms reported one case of EAFL, and the highest number of cases diagnosed on one farm was three. This is likely to be an under-representation of the incidence, given the likelihood that not all abortions were detected or investigated. In addition, many investigations involved partial field necropsy specifically targeting equine herpes virus diagnosis, and in these cases it was not possible to diagnose other causes of abortion, including EAFL.

EAFL cases showed an unusual pattern of inflammation of the placental membranes, with inflammatory changes appearing to initiate within the amnion and amniotic portion of the umbilical cord and then extending to involve the allantois (particularly the extra-embryonic coelomic space or allantoic cavity). In a small number of chronic cases the inflammatory changes appeared to extend through the allantois to involve portions of the chorion, particularly around the site of the attachment of the umbilical cord. A range of unusual and normally non-pathogenic bacteria were recovered on culture of samples from foetal stomach, lung or amnion/umbilical cord. Cultured organisms included bacteria considered to be commensal isolates from the mouth and gastro-intestinal tract, nasal passages and organisms more commonly isolated from soil and pasture. These organisms have been loosely identified as environmental organisms and include Cellulomonas sp., Microbacterium aggregans, Arthrobacter sp., Curtobacterium sp., non-beta haemolytic slow-growing Streptococci, other coryneform bacteria, and some gram negative non-fermenting rods (Todhunter et al., 2008).

A range of gross presentations were identified. Acute cases included aborted foetuses that showed little gross evidence of inflammatory changes, though histological examination of foetal and placental tissues and microbial culture revealed the typical pattern of funisitis and amnionitis described above, and recovery of one or more of the unusual bacterial isolates. Sub-acute cases demonstrated moderate to severe gross evidence of funisitis and amnionitis with variable involvement of the allantois. Chronic cases showed severe necrotising inflammatory changes in the umbilical cord and amnion with thick adherent plaques of necrotic material. These changes had often extended through the allantois to involve the chorion, and the chorionic changes resembled those observed with chronic ascending placentitis.
A positive diagnosis of EAFL required the following conditions to be met (Perkins, 2005):

- a complete autopsy including examination of foetus, chorioallantois and amnion and incorporating gross examination, examination of tissue samples (histopathology) and microbiology (culture or virus isolation);
- absence of any evidence of other known causes of equine abortion on gross pathology, histology or culture/virus isolation;
- typical gross and/or histopathological changes in amnion, amniotic portion of umbilical cord and allantois;
- absence of gross and histological evidence of significant chorionitis, particularly cervical pole and body chorionitis;
- in chronic cases potential display of focal severe chorionitis usually in the body of the chorion, commonly associated with more extensive, severe chronic changes in the amnion and amniotic umbilical cord and allantois;
- growth of environmental bacteria from culture of foetal stomach contents/ lung or from uncontaminated amnion. This requirement was not considered essential to a diagnosis because of difficulties in obtaining positive culture results from some cases.

**Factors associated with the occurrence of EAFL**

Many possible interrelated factors that might contribute to the occurrence of an outbreak of EAFL were identified, as shown in Figure 1.

Investigations of environmental, soil and pasture parameters did not identify any abnormalities. Similarities between the clinical and pathological features of EAFL and MRLS led to consideration of caterpillars as a possible risk factor for EAFL. A number of farms in the affected regions reported large numbers of processional caterpillars (*Ochrogaster lunifer*) on eucalypt trees either on or bordering their properties. One farm with multiple EAFL cases reported mares showing signs of urticaria after sheltering under trees that were heavily infested with processional caterpillars. Some properties reported large numbers of white cedar moth caterpillars (*Leptocneria reducta*) and a third urticarial caterpillar – the mistletoe brown-tail moth caterpillar (*Euproctis edwardsi*) – had previously been reported in the geographic regions where abortions had occurred. Australian native Pennyroyal (*Mentha satureioides*) was reported to be present in large amounts on one affected property, and this plant has been described by Everitt (1981) as a possible cause of abortion in domestic livestock species.
Summary

Although MRLS was first described in 2001 and EAFL in 2004, there is now anecdotal evidence to suggest that both conditions had occurred previously but had not been recognised. In contrast to MRLS, there is no evidence to indicate that EAFL might be associated with increased incidence of early pregnancy loss or other conditions such as pericarditis and ophthalmitis. Since Australian mares are usually bred between September and December and EAFL cases were observed between April and October, the most likely explanation for an absence of early pregnancy loss cases is simply that there are few mares that are early pregnant in Australia between April and October. ETC do not occur in Australia, though a number of other similar caterpillar species do occur. The conditions of the syndromes showed sufficient similarities to warrant consideration of commonalities in the pathogenesis (Perkins et al., 2007).
Experimental Studies

All experimentation was done with approval of the University of Queensland Animal Ethics Committee (AEC Approval Numbers SAS/384/08/RIRDC and SAS/499/08/RIRDC).

Experiment 1

Objectives

To determine if oral administration of macerated whole Processionary Caterpillar (PC) (*Ochrogaster lunifer*) induced abortion in mares in mid-gestation.

Methodology

Selection of mares

Sixteen mares were purchased in December 2005 and were housed in the Equine Unit on the University of Queensland, Gatton Campus. On arrival all mares underwent a routine induction procedure in which they were identified, weighed, vaccinated for strangles and tetanus, and treated with an anthelminitic prior to a 10 day period of isolation. On completion of the quarantine period the mares were all mated with the same stallion and their ovulation dates recorded. Once the mares had been scanned as pregnant for more than 45 days gestation they were turned out in a paddock together for six months. During this period they were monitored daily and treated for parasites.

In July 2006 all of the mares were scanned for pregnancy. Eight mares were then selected based on gestational age, which ranged from 194 to 206 days on the first day of treatment. The mares were then matched for weight and randomly allocated into two groups of four mares.

Collection of Processionary Caterpillars

The PC builds nests which either hang from the branches or are adherent to the trunk of some species of eucalypts and acacias (Figures 2 and 3). In late March to April the nests are easily seen in the trees. The caterpillars start to leave their nests in mid- to late-April, when they stop feeding and migrate into the soil to undergo pupation. Early in April 2006, caterpillar nests were collected from a number of properties on the Darling Downs of Queensland. Following collection the nests were stored at 5°C overnight, and on the following day the nests were opened and the caterpillars weighed into 100 g lots and stored in sealed bags at -20°C. At the same time, caterpillar frass (excrement) and exoskeleton that had been shed by the caterpillars during the growth stages was collected, separated and stored at -20°C.
Figure 2: Processionary caterpillar nest in a eucalypt tree. The nest in the centre of the picture is losing its integrity and will soon fall out of the tree. To the left and below this nest are the remnants of another nest that has already fallen apart.

**Preparation of caterpillars for administration**

Four lots of 100 g of whole caterpillar were selected at random from the freezer. Each 100 g of caterpillars was placed in a laboratory blender with 500 mls of distilled water and blended into a slurry. The blender was washed between the blending of each lot. The slurry of each prepared dose was stored in a separate container and used within one hour of preparation.
**Treatment of Mares**

Mares were brought into stables seven days prior to the commencement of the study and started on a diet of commercial concentrate and hay. On the day prior to the first day of treatment (Day -1) a full clinical examination including temperature, heart rate, respiratory rate, pulse, mucous membrane colour and refill, and gut sounds was performed on each mare between 8.00 am and 9.00 am. Appetite and faecal output was also recorded. At the same time, blood samples were collected. One sample was processed for haematology and biochemistry (using standard laboratory procedures) and the other was centrifuged and the plasma collected and frozen at -20°C for progesterone determination with a radioimmunoassay. A rectal ultrasound was performed on each mare and the examination recorded. The clinical examination and blood sampling were repeated between 5.00 pm and 6.00 pm. These examinations and blood collections were repeated daily for the duration of the trial.

Following the ultrasound examination of the mares on the first treatment day (Day 0), each mare in the treatment group received 100 g of macerated whole caterpillar by nasogastric intubation. The control group were intubated and dosed with distilled water only. Mares were treated daily for five days. All procedures were performed with the control group prior to handling the caterpillars and permitting treatments on the experimental group. Each mare was kept in the same stable throughout the trial to eliminate the risk of exposing the control mares to any caterpillar components.

**Autopsy procedure**

All aborted foetuses underwent an autopsy following a standard protocol (Todhunter et al., 2008).

**Results**

On the first and second days of treatment, three of the four mares that received caterpillars showed evidence of an allergic response with skin wheals (Figure 4) and facial swelling. These signs were no longer apparent by the following day. The fourth mare did not develop wheals but did show an increase in respiratory rate immediately after treatment. By the third day of treatment none of the mares showed any allergic signs after treatment.
Figure 4: Urticarial reaction on mare’s neck following treatment with processionary caterpillar slurry.

On Day 3, one of the treated mares showed a change in the fluid surrounding the foetus (Figure 5) and the foetus showed no movement. This mare aborted her pregnancy the following night, 84 hours after the initial treatment. On Day 7 the foetus of a second mare was detected as being dead on ultrasound examination, and it was aborted the following afternoon. A third mare aborted on Day 11, one day after her foetus was detected as being dead on ultrasound examination.

Figure 5: Ultrasound of pregnancy prior to initial treatment (left) and on third day of treatment (right). Note increased echogenicity of amniotic fluid in the view on the right in which the foetus is dead. This foetus was aborted the following day.

Autopsy of each of the foetuses showed gross pathology consistent with that seen in the field cases of EAFL (Figures 6, 7 and 8). Bacteria similar to those seen in clinical cases were isolated using
standard laboratory procedures from foetal organs, most predominantly stomach and lung. These bacteria were normal gut flora that are usually non-pathogenic and included *Streptococcus bovis*, *Lactobacillus salivarius*, *Microbacterium spp*, *Corynebacterium spp*, *Aeromonas hydrophila* and *Actinobacillus equuli*.

The fourth mare that was treated was euthanased due to ongoing colic on Day 7. Her foal was alive at the time of euthanasia but on autopsy showed gross pathology similar to the other three aborted foetuses, and bacterial culture of foetal organs grew similar bacteria.

Throughout the trial, none of the mares showed any evidence of bacterial infection. None experienced a febrile episode. Haematology was inconclusive, with no significant difference between white cell counts in the control and treated mares. Biochemistry was initially unremarkable, but two of the mares suffered gut stasis in the latter part of the trial and this was reflected in the biochemical profiles. The first mare to abort showed no alteration in her clinical parameters. Progesterone levels dropped in all mares at approximately the same time as foetal death.

None of the control mares aborted their foetuses.

![Figure 6: Aborted foetus from mare treated with whole PC slurry. Umbilical cord in cross-section on right showing oedema and discolouration.](image)

![Figure 7: Amnion from a control group pregnancy (left) and from a treatment group pregnancy (right). Note discolouration and prominent vascular pattern of the amnion from the treated pregnancy.](image)
Figure 8: Amniotic fluid from a control group pregnancy (8A) and treatment group pregnancy (8B). The fluid from the control group pregnancy is clear, whereas it is dark and haemorrhagic from the treatment group pregnancy. Stomach contents from control group (8C) and treatment group (8D) pregnancies.

The fluid from the control group pregnancy is clear and straw-coloured, whereas that from the treatment group pregnancy is cloudy and orange.

**Experiment 2**

**Objectives**

To determine if oral administration of macerated shed exoskeleton of the Processionary Caterpillar (PC) (*Ochrogaster lunifer*) induced abortion in mares in mid-gestation, and whether a dose relationship could be demonstrated.

**Methodology**

**Selection of mares**

The remaining eight mares of the original sixteen mares plus the four control mares from Experiment 1 were used for this experiment. The gestational age of the pregnancies ranged from 198 to 240 days on the first day of treatment. The mares were matched according to gestational age and divided into four groups of three mares.

**Collection and determination of dose of shed exoskeleton of caterpillars**

As previously described (see Experiment 1) shed caterpillar exoskeleton was collected at the same time as whole Processionary Caterpillars in April 2006. It was determined that each whole caterpillar weighed approximately one gram. The weight of one exoskeleton was approximately 0.01 g. It was decided that the lowest treatment dose of exoskeleton should equate to 100 g of whole caterpillar,
i.e. 100 exoskeletons which equated to 1 g of exoskeleton. Doses for mares in the other two treatment groups were 2 g and 5 g.

**Preparation of shed caterpillar exoskeleton for administration**

Exoskeletons were weighed into 1, 2 and 5 gram lots and each lot was placed in a laboratory blender with 500 mls of distilled water and blended into a slurry. The blender was washed between the blending of each lot. The slurry of each lot was stored in a separate container and used within one hour of preparation.

**Treatment of Mares**

Mares were brought into stables seven days prior to the commencement of the study and started on a diet of commercial concentrate and hay. On the day prior to the first day of treatment (Day -1) a full clinical examination including temperature, heart rate, respiratory rate, pulse, mucous membrane colour and refill, and gut sounds was performed on each mare between 8.00 am and 9.00 am. Appetite and faecal output was also recorded. At the same time blood samples were collected and immediately stored on ice. Blood samples were submitted for haematology and biochemistry. The remaining samples were then centrifuged and the plasma collected and frozen at -20°C. A rectal ultrasound was performed on each mare and the examination recorded. The clinical examinations were repeated between 5.00 pm and 6.00 pm. These examinations and blood collections were repeated daily for the duration of the trial.

Following the ultrasound examination of the mares on the first treatment day (Day 0), mares in the treatment groups received either 1, 2 or 5 g of macerated shed exoskeleton via nasogastric tube. Mares were treated daily for five days. Mares in the control group were intubated, but received only distilled water. All procedures were performed with the control group prior to handling the exoskeletons and performing treatments on the experimental group. Each mare was kept in the same stable throughout the trial to eliminate the risk of exposing the control mares to any caterpillar components.

**Autopsy procedure**

All aborted foetuses underwent an autopsy following a standard protocol (Todhunter et al., 2008).

**Results**

Only one mare (2 g group) showed any evidence of an urticarial reaction. This occurred for the first four treatments but not the fifth. One mare from the 5 g group did not eat her concentrate feed on the first day following treatment. However, she appeared to be unaffected by any of the later treatments. The remainder of the mares were clinically normal throughout the trial. Haematology results were inconsistent, with no significant difference between treated and control mares in blood cell parameters. Biochemistry results were unremarkable.

On Day 3, (48 hrs after the initial treatment) the foetus of one mare from the 5 g group was detected as dead on ultrasound examination. This mare aborted the following day. One mare from each of the 2 g and 5 g groups aborted on Day 6. In both cases the foetus was determined as dead on the day prior to abortion. One mare in the 1 g group aborted her foal at Day 67.

Autopsy of each foetus showed gross pathology consistent with that seen in the field cases of EAFL. The bacteria isolated from foetal organs, most predominantly stomach and lung, were normal gut flora that are normally non-pathogenic, and were of similar species to those isolated in Experiment 1. This was also consistent with those found in the field cases.
Of the remaining pregnancies, one mare from each of the treatment groups delivered live foals that showed some abnormality consistent with EAFL. The mare from the 1 g group delivered a clinically normal foal at 311 days gestation. The foal weighed 24 kgs (compared to the average birthweight of foals of 45-50 kg) but otherwise appeared to be healthy (Figure 9). There was a small lesion in the umbilical stump external to the abdomen. Bacterial culture of this lesion grew *Enterococcus faecium*, *E. casseliflavus* and *Streptococcus dysgalactiae*, which are organisms consistent with those grown from foetuses aborted during the two experimental trials, as well as field cases of EAFL. This foal had a marked elevation in white cell count at birth. One mare from the 2 g group delivered a fully developed foal at term that was born with acute diarrhoea. The blood culture taken from this foal at birth grew *Enterobacter cloacae* and *Esherichia coli*, bacteria consistent with those seen in the other experimental cases. The foal died at two days of age. Bacteria isolated from tissues were the same as those isolated from the blood culture. The one remaining mare in the 5 g group delivered an apparently normal foal at term; this foal had a moderately elevated white cell count at birth.

![Figure 9: Foals born on the same day, when this photograph was taken.](image)

The larger foal is from a control group mare, and the smaller from a mare treated with 1g PC exoskeleton daily for 5 days. The foal from the treated mare was born approximately 3 weeks before term but was strong and clinically normal. Haematology revealed an elevated white cell count in this foal.

The remaining mares, (three from the control group, and one each from the 1g and 2g treatment groups) delivered clinically normal foals.
Experiment 3

Objectives

To determine if oral administration of macerated shed exoskeleton of the Processionary Caterpillar (PC) (*Ochrogaster lunifer*) induced abortion in mares in the pre-placentation stage (<day 35) of pregnancy.

Methodology

Selection of mares

Twelve standardbred mares were purchased and housed in the Equine Unit at the University of Queensland, Gatton Campus, where they underwent a routine induction procedure as described previously (see Experiment 1). Following a 10 day quarantine period each mare was mated to the same stallion, and date of ovulation was recorded.

Collection and determination of dose of shed exoskeleton of caterpillars

Processionary caterpillar nests were collected in April 2007 and processed in the same manner as described for Experiment 1. The whole caterpillars and shed exoskeleton samples were stored at -20 °C.

Five grams of shed exoskeleton was used for this experiment, based on the outcomes of Experiment 2, where it was determined that this dose of shed exoskeleton caused abortion with minimal effect on the mare.

Preparation of exoskeleton material for administration

The exoskeleton samples were prepared as for Experiment 2.

Treatment of Mares

Mares were matched according to gestational age and divided into control and treatment groups (n=6).

All mares were brought into stables when they reached day 21 of gestation (day of ovulation = Day 0) and started on a diet of commercial concentrate and hay. Clinical examinations and blood sample acquisitions were performed on each morning of the trial, and pregnancies were monitored by ultrasound daily.

Each mare in the treatment group was treated with 5 g of shed exoskeleton preparation daily for five days, starting on Day 25 of gestation. Exoskeleton preparation was administered as described in Experiment 2; mares in the control group did not receive exoskeleton preparation.

All procedures were performed with the control group prior to handling the exoskeleton preparations and treating mares in the experimental group. Each mare was kept in the same stable throughout the trial to eliminate the risk of exposing the control mares to any caterpillar components.

Results

Pregnancies were maintained in all of the treatment and control group mares, except for one mare in the treatment group. In this mare the embryo had a detectible heart beat on Day 5 of treatment. Ultrasonography on the sixth day revealed uterine oedema consistent with endometrial inflammation. Fluid was present in the uterine lumen. The cervix remained closed. A sample of the fluid was
collected per vagina using a sterile artificial insemination pipette. The bacteria isolated from the uterine fluid were not consistent with those seen in either the experimental or clinical cases of EAFL.

**Experiment 4**

**Objectives**

To determine if oral administration of macerated shed exoskeleton of the Processionary Caterpillar (PC) (*Ochrogaster lunifer*) induced abortion in mares in the early placentation stage of pregnancy (Day 45-60).

**Methodology**

**Selection of mares**

Twelve standardbred mares were purchased and housed in the Equine Unit at the University of Queensland, Gatton Campus where they underwent a routine induction procedure as described previously (see Experiment 1). Following a 10 day quarantine period each mare was mated to the same stallion, and date of ovulation was recorded.

**Collection and determination of dose of shed exoskeleton of caterpillars**

Processionary caterpillar nests were collected in April 2007 and processed in the same manner as described for Experiment 1. The whole caterpillars and shed exoskeleton samples were stored at -20 °C.

Five grams of shed exoskeleton was used for this experiment, based on the outcomes of Experiment 2, where it was determined that this dose of shed exoskeleton caused abortion with minimal effect on the mare.

**Preparation of exoskeleton material for administration**

The exoskeleton samples were prepared as for Experiment 2.

**Treatment of Mares**

Twelve mares were matched according to gestational age and divided into two groups (n=6). The gestational age of the mares at the commencement of the trial ranged from Day 46 to Day 59. The mares were brought into stables five days prior to the commencement of the trial and started on a diet of commercial concentrate and hay. The mares were turned out into grass paddocks during the day after all examinations and treatments had been completed. Mares were kept in the same stables at night throughout the trial, and the treated mares were run in a paddock separate to, but adjoining the paddock in which the control mares were kept during the day. Clinical examinations and blood samples were performed on each morning of the trial, and pregnancies were monitored by ultrasound daily.

The mares in the treatment group were treated with 5 g of shed exoskeleton daily for five days. Exoskeleton preparations were administered as described in Experiment 2. The control group mares did not receive exoskeleton.

All procedures were performed with the control group prior to handling the exoskeleton preparations and treating mares in the experimental group.
Results

Four of the treated mares showed a very mild urticarial reaction following the administration of exoskeleton on the first three days of treatment. The mares were otherwise clinically normal.

On the sixth day of the trial the foetus of one treated mare was detected as being dead on ultrasound examination. The allantoic and amniotic fluids were both hyperechoic on scan (Figure 10). Corpora lutea were observed on both ovaries and the cervix was still tightly closed. The following day all of the fluid within the uterus was hyperechoic and uterine oedema was apparent in both uterine horns. This mare had a transient increase in temperature 24 hours after the foetus was detected as dead. Two days later a second treated mare was found to have a dead foetus on ultrasound scan, with hyperechoic amniotic fluid and a slight increase in echogenicity of the allantoic fluid.

The cervix of the first mare to show foetal death remained closed for 14 days, and in the second mare the cervix remained closed for eight days. As soon as a vaginal discharge became apparent a uterine swab was performed on these mares and cultured for bacteriology. The foetuses were both autolysed in utero and neither foetus was recovered. The bacteria grown from these mares were consistent with those seen in clinical cases of EAFL, however, these were the only two cases throughout all of the experiments that grew organisms that are normal environmental bacteria. All previous samples from the experimental cases had only grown normal gut flora.

Figure 10: Ultrasound of pregnancy prior to treatment (10A), on the fourth day of treatment (10B) and on the fifth day of treatment (10C). Note the presence of blood flow in 10A and 10B. There is no evidence of a heart beat in the foetus in 10C indicating foetal death. There is
Discussion

The term Equine Amnionitis and Foetal Loss (EAFL) has been used to describe abortions in mares that show an unusual and consistent pattern of clinical and pathological signs. Examination of reports for all equine abortion cases that involved submission of diagnostic material to the Scone Veterinary Diagnostic Laboratory between March and October 2004 indicated that EAFL was the most common identified cause, responsible for 28 (37%) of 76 cases subjected to a diagnostic investigation (Perkins, 2005). Although most affected farms had only one EAFL case diagnosed in 2004, there were a small number of farms where multiple cases were diagnosed and where pregnancy losses due to abortion were larger than expected. EAFL cases were also identified in southern Queensland and in mares bred in Victoria and residing in the region close to the border between NSW and Victoria.

The case definition for EAFL shows similarities to descriptions of abortions associated with a condition called Mare Reproductive Loss Syndrome (MRLS) reported in Kentucky USA in 2001 and 2002 (Perkins et al., 2007). The major causal factor that was shown experimentally to be associated with MRLS was exposure of pregnant mares to Eastern Tent Caterpillars (*Malacosoma americanum*; Sebastian et al., 2006; Riddle and LeBlanc, 2007). Several species of hairy caterpillars have been identified in eastern Australia. These include *Ochrogaster lunifer* (processionary caterpillar), *Euproctis edwardsi* (mistletoe brown tail moth caterpillar), and *Leptocneria reducta* (white cedar moth caterpillar). Processionary caterpillars were identified as present in large numbers on several broodmare farms in Qld and NSW where EAFL abortions were diagnosed. White cedar moth caterpillars have also been identified on some farms in the Hunter Valley, but mistletoe brown-tail moth caterpillars have not yet been identified on horse farms.

Experimental induction of EAFL

The initial experiments in this study (Experiments 1 and 2) were designed to simulate the situation in the field where most EAFL cases occur in mid to late gestation. The results showed that exposure to preparations of processionary caterpillars (PC) can induce pregnancy loss in mares, and that exposure to preparations of shed exoskeleton can also induce abortion. Gross pathology and bacteriology results from experimentally-induced abortions were similar to those seen in cases of EAFL.

It is well recognised that PC can cause an intense allergic reaction in humans. On one property where there were a number of horse abortions it was reported that horses grazing under trees populated with PC showed urticarial reactions (K.H. Todhunter, pers.com). In the current studies, some but not all mares displayed a transient urticarial reaction following experimental exposure to PC. In the studies using exoskeleton preparations, urticaria was not a predominant sign, although it did occur in some mares. The mares exposed to exoskeleton preparations showed no detectible clinical abnormalities. These findings were consistent with the descriptions of the field situation, where mares generally showed no signs of illness prior to abortion. This was also observed in cases of MRLS.

In the original outbreak in 2004, mares that were moved out of paddocks where a number of pregnancy losses had occurred continued to abort. In those mares the placental and foetal findings on autopsy were consistent with chronic infection, indicating that the initial insult occurred some time prior to abortion. In the exoskeleton studies reported here, mares aborted or delivered live but affected foals more than two months after treatment through to term.

The other distinctive feature of the field syndrome, and also observed in these studies, was the isolation of bacteria from aborted foetuses. The range of identities of these organisms in the experimental studies was consistent with those found in field-reported cases. The organisms were common environmental or intestinal bacteria that are not normally associated with illness in horses. Although there were changes in haematology in some mares that were suggestive of a mild infection,
those findings were not consistent. The isolation of bacteria and the pathology of the foetus and placenta were consistent with infection in the foeto-placental unit.

**Early embryonic loss and early foetal loss**

Two further trials (Experiments 3 and 4) were carried out as part of this project to determine if processionary caterpillar (PC) derived materials could also cause early embryonic loss, and if they could cause abortion in mares from 45 – 60 days of gestation, at which time the source of progesterone for maintenance of the pregnancy is the ovary of the mare, rather than the foeto-placental unit.

Early embryonic loss has not been reported in either MRLS or EAFL. While one treated mare did abort in the early embryonic loss trial reported here, the bacteria isolated from the uterus of the mare following foetal death were not consistent with those found in cases of EAFL. It is thus considered unlikely that exposure to PC during the first 35 days of gestation causes abortion in mares. This conclusion is supported by epidemiological studies for both EAFL and MRLS (Cohen et al., 2003a,b; Perkins, 2005).

It was shown that mares exposed to PC exoskeleton material during the early foetal stage (45 – 60 days gestation) could suffer from abortion. In neither of the two cases that occurred in this study, was a foetus retrieved from the mare, the time from foetal death to the time of vaginal discharge was eight days in one mare and 14 days in the other. In mares exposed to PC material during mid- to late-term pregnancy, abortion occurred within 24-48 hours of foetal death. It is possible that the presence of secondary corpora lutea in the early placentation stage prevents the expulsion of the foetus following foetal death.

Another point of interest was the isolation of normal pasture bacteria from the mares in the early foetal loss trial (Experiment 4). These bacteria were not isolated from aborted foetuses in the previous studies (Experiments 1 and 2) although they were a common finding in the field cases (K.H. Todhunter, pers.com). In the earlier studies (i.e. Experiments 1 and 2), mares were maintained in stables throughout the trial whereas in Experiment 4 mares were turned out into grass paddocks each morning after treatment and brought back into stables in the afternoon. This finding supported the conclusion that abortion occurs through passage of normal gut and environmental bacteria through the gut wall into the mare’s blood stream, and then through haematogenous spread to the foetus.

**Caterpillar exposure and possible mechanism of action**

Studies conducted in this project demonstrated that exposure of pregnant mares to processionary caterpillars (PC) can induce foetal loss. However, it is well known that horses are able to sift through feed to avoid eating material that that they do not wish to ingest. It is therefore reasonable to doubt that horses would eat whole caterpillars. Based on United States studies, where it was shown that abortion could be induced using exoskeleton dissected from Eastern Tent caterpillars (McDowell et al., 2004), the second study in this project investigated the possibility that exoskeleton material shed by the PC during its growth phases could also cause abortion. The shed exoskeleton accumulates in the caterpillar nests in trees; when the caterpillars leave the nest to migrate into the soil to pupate the nests are no longer rebuilt, and they lose their structural integrity and fall onto the pasture. The exoskeleton is extremely light (one exoskeleton weighs approximately 0.01g) and is easily blown around the pasture, where it would be difficult for horses to separate it out. This is likely to be especially important during periods of drought, or on unimproved pasture, where horses are more likely to graze close to the ground and also closer to the base of trees than they might in better seasons. It should be noted that the initial outbreak of abortions recorded in the Hunter Valley occurred from late March to May, which coincides with the time that the caterpillars move out of the trees, and occurred in mares grazing native pastures.
It would appear from the results of this study that elements of the caterpillars’ body, or a compound (toxin?) in the caterpillars, or a combination of the two, allows the transfer of bacteria across the mare’s gastrointestinal wall and the placenta, and that the resulting infection in the foetus leads to foetal death and then abortion. There was no indication from hormonal profiles that other factors had perturbed the endocrine profile of the mare in such a way as to result in abortion. The endocrine changes that were observed were subsequent to foetal death rather than a cause of foetal death. It is thus reasonable to assume that foetal death is caused by bacterial infection and that abortion then follows.

The mechanism of action by which PC and ETC cause abortion has been a subject of much discussion. It is recognised that these species of caterpillars have hairs or setae that are barbed. Tobin et al. (2004) proposed that ingested setae penetrate the gut wall through mechanical action and carry bacteria that are present in the mare’s gut with them, and that these bacteria then spread via the mare’s circulation to the uterus, where they cross the placenta and localise in the amniotic fluid. Bacteria in the amniotic fluid may then be ingested/inspired by the foetus and cause infection in foetal tissues. The question then arises as to how easy it might be for bacteria to cross the gut wall as well as the placenta. It has also been suggested that these caterpillars may carry toxin(s) that may have an effect on the gut and placenta, resulting in facilitation of transfer of bacteria from gut lumen to circulation and from circulation to uterine contents, with or without the mechanical assistance of the setae (Sebastian et al., 2004). It is possible that this mechanism may be initiated by the release of histamine as part of the allergic response to the caterpillar materials.

Further examinations are currently being conducted of histopathological changes in tissue samples from mares that have been treated with PC in an attempt to better understand the role of setae and/or toxins in the pathogenesis of EAFL. A laboratory animal model of EAFL is also being explored as an efficient alternative to allow more detailed ongoing studies of this condition.
Implications and Recommendations

Processionary caterpillars (PC’s) are found throughout all of the thoroughbred breeding areas in eastern Australia, and given the results of this study it is surprising that they have not previously been reported as being associated with abortions in mares. There is anecdotal evidence from case records and assessment of pathology reports from abortions prior to 2004 of abortions being reported with similar pathology to that described in field cases of EAFL in 2004. These findings suggest that EAFL was likely to have been present in Australia prior to 2004.

The diagnosed occurrence in 2004 may have potentially related to higher local concentrations of caterpillars, or a larger insect population in that period, or a combination of high caterpillar numbers in conjunction with drought conditions. It is likely that, as with other insect-associated diseases, occurrence of EAFL will be unpredictable and relate to the dynamics of population changes of the insects. The results of this project provide compelling evidence that processionary caterpillars cause EAFL, and justify efforts to control exposure of pregnant mares to the caterpillar.

Exposure to caterpillars and their shed exoskeletons may be minimised by ensuring that pregnant mares are not grazed on pastures where it is known that caterpillars are, or have been, present. Where this cannot be avoided, provision of supplementary feed during periods of drought to reduce grazing under and around trees may be beneficial. It is also advisable that supplementary feed be available from feeders elevated off the ground and away from trees. Replacement of trees that are the natural food source of the PC (i.e., eucalypt and acacia species) might be considered, but may not be practicable. Removal of caterpillar nests in February and March before the caterpillars leave the nests may also be helpful, but due to the height of the nests in most trees this may not be possible.

There is justification for further studies that seek a greater understanding of the life cycle and population dynamics of processionary caterpillars, and of the caterpillar-associated factor(s) that, when ingested, induce abortion in mares.
References


Appendix 1

Publications and Presentations Associated with this Research


Equine Amnionitis and Foetal Loss
— The role of caterpillars —

RIRDC Publication No. 09/155

By A.J. Cawdell-Smith and W.L. Bryden

Re productive loss is a major cause of wastage in the thoroughbred breeding industry and probably accounts for a loss of about 7 - 10% of pregnancies per annum.

In 2004 a series of abortions were described in the Hunter Valley region of NSW that showed an unusual and consistent pattern of clinical and pathological signs. The condition, which came to be known as equine amnionitis and foetal loss (EAFL), had not been previously reported. The case definition of EAFL showed similarities to descriptions of abortions associated with a condition called mare reproductive loss syndrome (MRLS) reported in Kentucky, USA in 2001 and 2002.

The aim of this project was to determine or elucidate the role of caterpillars in the pathogenesis of EAFL, specifically to determine if exposure of pregnant mares to processionary caterpillars (Ochrogaster lunifer) is associated with increased risk of abortion.

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