Evaluation of novel vaccines for Enzootic Abortion of Ewes (EAE)

Key external stakeholders:
Veterinarians, Sheep farmers, Animal health pharmaceutical companies.

Practical implications for stakeholders:
- Two proteins from Chlamydia abortus, the causative agent of EAE were purified.
- The proteins were tested as novel vaccines both singly and in combination.
- Neither protein alone protected ewes against EAE.
- The combination of proteins provided partial protection against EAE. Ewes vaccinated with both proteins and challenged with Chlamydia abortus had fewer abortions, longer gestation lengths and lambs of higher birth weight than unvaccinated ewes.
- The proteins could contribute to a novel subunit vaccine for EAE.

Main results:
- Two proteins of Chlamydia abortus, the causative agent of enzootic abortion of ewes (EAE), that were predicted to be immunogenic were selected and expressed.
- The proteins were used both singly and in combination to vaccinate ewes 6 months prior to mating. Both proteins were safe and well tolerated and induced an antibody response by 14 days post-inoculation. This antibody response increased over time.
- The protective qualities of the proteins were evaluated in pregnant ewes challenged on day 90 of gestation with Chlamydia abortus. Neither protein was effective alone; however, the combination of both proteins offered 50% protection against abortion and resulted in longer gestation lengths and higher lamb birth weights than unvaccinated ewes.
- These proteins could contribute to a novel safe EAE vaccine.

Opportunity / Benefit:
The recombinant proteins MIP and CPAF from Chlamydia abortus were formulated as adjuvanted vaccines and administered to ewes as a subcutaneous injection over the flank. They were found to be safe and well tolerated. The proteins induced an immune response in the ewes with antibodies against the proteins observed by 14 days post-inoculation which increased by 35 days post-inoculation. The ability of the proteins to protect against Chlamydia abortus challenge was determined; neither protein alone protected the ewes. However, a combination of both proteins was 50% effective in preventing abortions and resulted in longer gestation lengths and lambs of higher birth weights. Therefore these proteins could contribute to a new, safer, subunit vaccine for the protection of ewes from enzootic abortion.

Collaborating Institutions:
UCD, DAFM, USDA, UTSA

Contact
Orla Keane
Email: orla.keane@teagasc.ie
http://www.teagasc.ie/publications/
1. Project background:

*Chlamydia abortus*, the causative agent of enzootic abortion of ewes (EAE), is the second most common diagnosable form of infectious ovine abortion in Ireland. It is also a serious risk to pregnant women. EAE results in necrotizing placentitis leading to abortion in late pregnancy, premature lambing or birth of weak lambs. The disease can spread easily, especially at lambing time when a large number of bacteria can be excreted with the placenta and in vaginal discharge. In Ireland EAE has been estimated to cost upwards of €5 million per annum. The rate of abortion in infected flocks is typically 2-3% per flock per year but the introduction of infection into a naïve flock can result in abortion storms. After abortion infected ewes develop immunity and rarely abort again but these ewes can continue to shed bacteria at subsequent births resulting in transmission of the infection to other ewes in the flock. There is one commercial vaccine available in Ireland for EAE, which is a live attenuated vaccine. This vaccine reduces, but does not stop, the shedding of infective bacteria. This vaccine has also recently been found to be a cause of abortion in some flocks, indicating the need for safer vaccines.

2. Questions addressed by the project:

- Can recombinant proteins be purified from *Chlamydia abortus*, the causative agent of enzootic abortion of ewes?
- Do these proteins induce an immune response in naïve ewes?
- Do the proteins induce protective immunity in ewes and prevent abortion after challenge with *Chlamydia abortus*?

3. The experimental studies:

Three separate studies were carried out:

1. Recombinant maltose binding protein-tagged MIP and CPAF from *Chlamydia abortus* were produced in *Escherichia coli* and purified via amylose affinity chromatography.
2. A vaccine trial was conducted with 50 EAE-seronegative ewes. The ewes were randomly allocated to one of 5 treatment groups which received i) MIP ii) CPAF iii) MIP and CPAF iv) buffer (negative control) or v) Enzovax (positive control). The treatment was administered as a subcutaneous injection over the flank and the immune response was determined. All vaccines were well tolerated and the recombinant proteins induced an antibody response by 14 days post-vaccination which increased by 35 days post-vaccination.
3. The ability of the vaccines to protect against challenge was investigated by challenging 47 vaccinated pregnant ewes on day 90 of gestation with *Chlamydia abortus*. Neither MIP nor CPAF protected against challenge; however the MIP/CPAF combination provided 50% protection against abortion and resulted in longer gestation lengths and lambs of higher birth weight. The commercially available Enzovax (MSD) vaccine provided 100% protection.

- **Main results:**
  - Two proteins from *Chlamydia abortus* that were potential vaccine candidates were selected and recombinant maltose binding protein-tagged versions of the proteins were produced and purified.
  - Vaccination of EAE-seronegative ewes with the recombinant proteins MIP, CPAF and MIP/CPAF in combination as a subcutaneous injection over the flank was safe and well tolerated.
  - Antibodies to the recombinant proteins could be detected by 14 days post-vaccination and increased by 35 days post-vaccination indicating that the ewes mounted an immune response to the proteins.
  - To determine if the immune response was protective, vaccinated pregnant ewes were challenged on
day 90 of gestation with *Chlamydia abortus*; neither protein was protective when administered singly, however in combination the proteins offered 50% protection against abortion.

- The combination of MIP/CPAF also resulted in longer gestation lengths and higher lamb birth weights compared to negative control ewes.
- MIP and CPAF in combination could contribute to a novel subunit vaccine for EAE.

4. **Opportunity/Benefit:**

The recombinant proteins MIP and CPAF from *Chlamydia abortus*, the causative agent of enzootic abortion of ewes were purified. These recombinant proteins were administered to ewes as a subcutaneous injection over the flank and were found to be safe and well tolerated. The proteins induced an immune response in the ewes with antibodies against the proteins observed by 14 days post-inoculation which increased by 35 days post-inoculation. The ability of the proteins to protect against *Chlamydia abortus* challenge was established; neither protein alone protected the ewes. However, a combination of both proteins was 50% effective in preventing abortions and resulted in longer gestation lengths and lambs of higher birth weights. Therefore these proteins could contribute to a new, safer, subunit vaccine for the protection of ewes from enzootic abortion.

5. **Dissemination:**


6. **Compiled by:** Dr Orla Keane