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Development of a subcutaneous ear implant to deliver an anaplasmosis vaccine to dairy steers

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Abstract

Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production. Use of chlortetracycline-medicated feed to control active anaplasmosis infections during the vector season has raised concerns about the potential emergence of antimicrobial resistance in bacteria that may pose a risk to human health. Furthermore, the absence of effectiveness data for a commercially available, conditionally licensed anaplasmosis vaccine is a major impediment to implementing anaplasmosis control programs. The primary objective of this study was to develop a single-dose vaccine delivery platform to produce long-lasting protective immunity against anaplasmosis infections. Twelve Holstein steers, aged 11-12 weeks, were administered a novel 3-stage, single-dose vaccine against Anaplasma marginale (Am) major surface protein 1a. The vaccine consisted of a soluble vaccine administered subcutaneously (s.c.) for immune priming, a vaccine depot of a biodegradable polyanhydride rod with intermediate slow release of the vaccine for boosting immune response, and an immune-isolated vaccine platform for extended antigen release (VPEAR implant) deposited s.c. in the ear. Six calves were randomly assigned to two vaccine constructs (n=3) that featured rods and implants containing a combination of two different adjuvants, diethylaminoethyl (DEAE)-Dextran and Quil-A (Group A). The remaining 6 calves were randomly assigned to two vaccine constructs (n=3) that featured rods and implants containing the same adjuvant (either DEAE-Dextran or Quil A) (Group B). Twenty one months post-implantation, calves were challenged intravenously with Am stabilate and were monitored weekly for signs of fever, decreased packed cell volume (PCV) and bacteremia. Data were analyzed using a mixed effects model and chi-squared tests (SAS v9.04.01, SAS Institute, Cary, NC). Calves in Group A had higher PCV than calves in Group B (P = 0.006) at day 35 post-infection. Calves in Group A were less likely to require antibiotic intervention compared with calves in Group B (P = 0.014). Results indicate that calves exhibited diminished clinical signs of anaplasmosis when antigen was delivered with a combination of adjuvants as opposed to a single adjuvant. This demonstrates the feasibility of providing long lasting protection against clinical bovine anaplasmosis infections using a subcutaneous ear implant vaccine construct.

Keywords

Anaplasma marginale, anaplasmosis, Bos taurus, cattle, implant, vaccine

Disciplines

Large or Food Animal and Equine Medicine | Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology

Comments

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NOTE: THIS IS A RAPID COMMUNICATION SUBMISSION

Rapid Communication: Development of a subcutaneous ear implant to deliver an anaplasmosis vaccine to dairy steers

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Conflict of Interest

The authors declare no conflict of interest.

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ABSTRACT: Bovine anaplasmosis is the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production. Use of chlortetracycline-medicated feed to control active anaplasmosis infections during the vector season has raised concerns about the potential emergence of antimicrobial resistance in bacteria that may pose a risk to human health. Furthermore, the absence of effectiveness data for a commercially available, conditionally licensed anaplasmosis vaccine is a major impediment to implementing anaplasmosis control programs. The primary objective of this study was to develop a single-dose vaccine delivery platform to produce long-lasting protective immunity against anaplasmosis infections. Twelve Holstein steers, aged 11-12 weeks, were administered a novel 3-stage, single-dose vaccine against *Anaplasma marginale (Am)* major surface protein 1a. The vaccine consisted of a soluble vaccine administered subcutaneously (s.c.) for immune priming, a vaccine depot of a biodegradable polyanhydride rod with intermediate slow release of the vaccine for boosting immune response, and an immune-isolated vaccine platform for extended antigen release (VPEAR implant) deposited s.c. in the ear. Six calves were randomly assigned to two vaccine constructs (n=3) that featured rods and implants containing a combination of two different adjuvants, diethylaminoethyl (DEAE)-Dextran and Quil-A (Group A). The remaining 6 calves were randomly assigned to two vaccine constructs (n=3) that featured rods and implants containing the same adjuvant (either DEAE-Dextran or Quil A) (Group B). Twenty one months post-implantation, calves were challenged intravenously with *Am* stabilate and were monitored weekly for signs of fever, decreased packed cell volume (PCV) and bacteremia. Data were analyzed using a mixed effects model and chi-squared tests **(**SAS v9.04.01, SAS Institute, Cary, NC). Calves in Group A had higher PCV than calves in Group B (P = 0.006) at day 35 post-infection. Calves in Group A were less likely to require antibiotic intervention compared with calves in Group B (P = 0.014). Results indicate that calves exhibited diminished clinical signs of

anaplasmosis when antigen was delivered with a combination of adjuvants as opposed to a single adjuvant. This demonstrates the feasibility of providing long lasting protection against clinical bovine anaplasmosis infections using a subcutaneous ear implant vaccine construct.

Key Words: *Anaplasma marginale*, anaplasmosis, *Bos taurus*, cattle, implant, vaccine

INTRODUCTION

Anaplasmosis, caused by the rickettsial hemoparasite, *Anaplasma marginale (Am)*, is the most prevalent tick-transmitted disease of cattle worldwide and causes significant disease loss to beef producers in the United States (Uilenberg, 1995; Kocan et al. 2003). In the absence of an effective vaccine, control of anaplasmosis infection is predicated on the administration of low doses of in-feed chlortetracycline for several months (Reinbold et al. 2010b). As of January 2017, control of active anaplasmosis using in-feed chlortetracycline requires veterinary oversight in the form of a veterinary feed directive (VFD). As a result, federal law restricts this medicated feed to use by or on the order of a licensed veterinarian (FDA, 2019). The VFD places an additional regulatory burden on livestock producers and makes anaplasmosis control in extensive and smaller livestock operations especially challenging.

Vaccination strategies to control anaplasmosis are urgently needed to assist livestock producers in combating this disease. Major surface protein (MSP) 1a (MSP1a) is one of six MSP previously described on *Am* derived from bovine erythrocytes (Palmer et al. 2001) and is involved in immunity to *Am* infection in cattle (Palmer et al. 1987). Previous work has shown that cattle vaccinated with erythrocyte-derived *Am* antigens demonstrated preferential recognition for MSP1a (Brown et al. 2001). The present study was conducted to determine the optimal delivery and adjuvant combination of *Am* MSP1a using a 3-stage vaccine administered as a single injection and long-term subcutaneous (s.c.) ear implant. The unique vaccine implant design allows for a sustained release of the target antigen with an immunoregulatory design to minimize tolerance and achieve long-term

immunization in a single dose. We hypothesize that this device will mimic the life-long concomitant immunity associated with persistent *Am* infection after field exposure.

MATERIALS AND METHODS

All animal studies were conducted under an approved Institutional Animal Care and Use Committee (IACUC) protocol (IACUC #: 3959) on file at Kansas State University, Manhattan, Kansas.

Antigen

The peptide antigen R1OK was designed from an *Am* strain originally isolated from a cow in Oklahoma as previously described by Blouin et al. (2000) and characterized by de la Fuente et al. (2003a). The MSP1a genotype of this *Am* Oklahoma strain is K;S-C-H. The multiple antigenic peptide (MAP) for this vaccine was the R1OK peptide, NH2-ADGSSAGGQQQESSVSSQSDQASTSSQLG-COOH, derived from MSP1a tandem repeat K;S (de la Fuente et al. 2003a), which was synthesized as an 8-subunit MAP (Biosynthesis, Lewisville, TX) and shipped as a powder.

Priming Solubilization

To yield an immune-priming dose, 2 mg of R1OK-MAP was solubilized in 2 mL of MES buffer [0.1 M 2-(N-morpholino) ethanesulfonic acid, 0.9% sodium chloride, pH 4.7] and linked via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride to 2 mg Imject Blue carrier protein (BP) (Thermo Fisher Scientific, Waltham, MA) in 1 mL of MES buffer following manufacturer's recommendations. The conjugate was washed through a polyethersulfone 3K molecular weight cutoff protein concentrator (Pierce, Thermo Fisher Scientific) three times in pH 7.42 phosphate-only buffer prepared according to manufacturer's instructions (Thermo Fisher Scientific). The soluble vaccine consisted of 300 µg R1OK-MAP in 1.0 mL pH 7.42 phosphate-only buffer mixed with 1.0 mL Montanide ISA 61 VG adjuvant (Seppic, Paris, France) for a final volume of 2 mL per injection.

Rod and implant design

The boosting dose relied on a bioerodible polyanhydride (PA) rod 15mm long and 4mm in diameter composed of 20% 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 80% 1,6-bis(*p*-

carboxyphenoxy) hexane (CPH) (20:80 CPTEG:CPH) (Schaut et al. 2018). The molecular weight and copolymer composition of the 20:80 CPTEG:CPH copolymer were 6.618 kDa and 23:77, respectively. The boosting dose rod consisted of 208 mg of PA, 100 µg of R1OK-MAP, and 100 mg of diethylaminoethyl-dextran (DEAE-Dextran) or 500 µg Quil-A (Sigma-Aldrich, Saint Louis, MO) as indicated. The VPEAR implant (Jones et al., 2016) for long-term release (up to 3 years) consisted of 140 mg of PA, 100 µg of R1OK-MAP and 100 mg of DEAE-Dextran or 500 µg Quil-A as indicated. Dry mixtures of all components were pressed in a custom-made mold at 0.5 tons-on-ram for 5 s, using a hydraulic press (International Crystal Laboratories Inc., Garfield, NJ). The implant was designed and formulated as previously described (Schaut et al. 2018). All but three calves required implant replacement after initial rejection. Rejections were preceded by local inflammation and formation of an abscess around the implant sites. Implant replacements were of identical design to the original implant, except the amount of adjuvant was reduced to either 10 mg of DEAE-Dextran or 50 µg of Quil-A.

Study Animals

A cohort of 12 Holstein steers ranging from 11-12 weeks of age, weighing 102.1 ± 2.3 kg (mean ± SEM) was enrolled in the project. Calves were randomly assigned to vaccine treatment groups using the RAND function in a spreadsheet program (Microsoft Excel, Richmond, WA). Six calves were assigned to two vaccine constructs that featured rods and implants containing a combination of different adjuvants (DEAE-Dextran and Quil-A) (Group A). The remaining 6 calves were randomly assigned to two vaccine constructs that featured rods and implants containing the same adjuvant (either DEAE-Dextran or Quil A) (Group B). All calves received an initial soluble vaccine priming dose s.c. before implantation (See Table 1). As this was a proof-of-concept study conducted over 2 years and animal numbers were limited, we did not enroll a negative control group to evaluate the differences in composition of the vaccine constructs.

Animal vaccinations

The soluble vaccine was administered into the s.c. tissue on the right side of the neck. Immediately afterward, the rod was inserted into the s.c. tissue at the base of the right ear pinna through a six-gauge needle; the implant was then manually inserted through the same incision. The incision was closed with a single suture. Reimplantation, when required, was done into the left (contralateral) ear, five weeks after the initial implant. All but three calves required reimplantation within the first 5 weeks of the study with devices containing a reduced adjuvant load. All implants were subsequently maintained for the duration of the study, suggesting that rejection may have been due to excessive activation of an immune response to the initial implant device. Reimplantation was not believed to impact total antigen delivery as the rate of release from the implants was engineered to remain consistent for the duration of the study regardless of whether the device was reimplanted.

***Anaplasma marginale* infection challenge**

A cryopreserved field isolate of *Am*, with the MSP1a genotype M-F-F, was administered IV at 21 months after vaccination to infection challenge immunized animals. This represented day zero of the infection challenge phase of the study. Calves weighed a mean ± SEM of 632.7 kg ± 16.3 kg and were approximately 24 months of age at the time of challenge. The *Am* challenge isolate was obtained from a persistently-infected cow in Oklahoma in 2017. Briefly, to prepare the cryopreserved *Am* isolate, 60 mL of blood from the donor cow was collected into a blood bag containing heparin and sub-inoculated into a splenectomized calf to amplify *Am*. The resulting infected red blood cells were washed three times in phosphate buffered saline (PBS), resuspended 1:1 in a stabilate buffer (31.2% dimethylsulfoxide in 1X PBS) after the final wash, and stored in liquid nitrogen as described by Love (1972). Two milliliters of cryopreserved *Am* (M-F-F genotype) stabilate were intravenously inoculated into the jugular vein of each vaccinated steer. The target *Am* challenge dose was approximately 2 x 10^9 bacteria per inoculation.

Sampling

Beginning at day zero of the infection challenge component of the study, approximately 20 mL of whole blood was drawn from the jugular or coccygeal vein into evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing EDTA (1.8 mg/mL whole blood) or no anticoagulant once weekly post-infection to monitor development of anemia, bacteremia, and antibody response to *Am*. Anemia was evaluated by quantifying packed cell volume (PCV) from whole blood that was collected into EDTA tubes and centrifuged (Micro-Hematocrit Centrifuge CMH30, UNICO, Dayton, NJ). Development of bacteremia was monitored by PCR and microscopic examination of Wright-Giemsa-stained blood smears (HEMA-3, Fisher HealthCare, Pittsburgh, PA). Total genomic DNA was extracted from 100 µL of whole blood collected into EDTA tubes using the *Quick*-DNA Miniprep Kit (Zymo Research, Irvine, CA) according to manufacturer instructions, and DNA was eluted in 35 µL of DNA Elution Buffer. A quantitative, real-time PCR (qPCR) assay targeting a portion of the single-copy *Am* gene MSP5 was used to quantify *Am* infection levels in blood as previously described (Hammac et al. 2013). Quantitative specificity for this qPCR assay is 100 copies per template and qualitative sensitivity is 10 copies per template. Serum was centrifuged at 2,000 x g for 10 min at 20°C from whole blood collected into evacuated tubes containing no anticoagulant. Serum samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for *Am* serological screening using a commercial cELISA that detects host antibodies produced against *Am* MSP5 (Catalog No: 283-2, VMRD, Pullman, WA).

Animal Health

Anaplasmosis is potentially fatal, and animal health was monitored closely to determine need for antibiotic intervention. Animal PCV and rectal temperature were measured twice per week. Veterinary exams were performed if: temperatures measured greater than 39.2°C or less than 36.7°C, PCV was measured at less than 22%, respiration rate was measured at greater than 60 breaths per minute, inappetence was noted for more than 24 hours, or if severe depression was observed for more than 24 hours. Veterinary physical exams included verification of symptoms in

addition to assessment of depressed mentation and icteric mucus membranes. Cattle were treated with a single label dose of 200 mg/mL oxytetracycline (Bio-Mycin 200, Boehringer Ingelheim Vetmedica Inc, Duluth, GA) at 20 mg/kg body weight if two or more of the preceding symptoms were displayed as determined by the attending veterinarian (EJR). If an animal required antibiotic intervention, then vaccination was deemed a failure.

Statistical Analysis

Outcome variables PCV, cELISA percent inhibition, bacteremia, and body temperature were analyzed using a generalized linear mixed model incorporating both fixed effects and random effects (PROC GLMMIX; SAS university edition v9.04.01, SAS Institute, Cary, NC). All of these responses best fit to log-normal models. Day post-infection, vaccine treatment group (each of four vaccine treatments tested), vaccine construct (Group A or B; depending on combination or single adjuvants), and their interactions were analyzed as fixed effects in the model with cattle nested in vaccine construct designated as a random effect. Where there was evidence for a vaccine construct by day post-infection interaction, simple effect comparisons of least squares means were conducted using Tukey-Kramer adjustment for multiple comparison. For all outcomes, statistical significance was set *a priori* at $P < 0.05$. Two-tailed chi-squared tests, with and without Yate's corrections, were used to compare disease outcome between vaccine constructs (combination adjuvants vs. single adjuvant). A Fisher's exact test was also used for comparison between vaccine constructs.

RESULTS AND DISCUSSION

The effect of vaccine treatment group alone was examined but no differences were found when comparing bacteremia ($P = 0.136$) or body temperature ($P = 0.068$). Animals administered the combination adjuvant vaccine construct had higher PCV than those receiving the single adjuvant vaccine construct at day 35 post-infection ($P = 0.006$) (Figure 1). Bacteremia peak coincided with

PCV nadir (Figure 1). It is noteworthy that a chi-squared test without Yate's correction revealed that animals vaccinated with combination adjuvants (vaccine construct A) were less likely to require antibiotic intervention compared with calves vaccinated with single adjuvants (vaccine construct B) (P = 0.014). A Fisher's exact test revealed a similar trend (P = 0.061), as did a chi-squared test with Yate's correction (P = 0.066). These results indicate that calves exhibited diminished clinical signs of anaplasmosis when vaccine antigen was delivered with a combination of adjuvants as opposed to a single adjuvant.

These results are in agreement with previous studies that indicated that immunization with native MSP1 (a heterodimer containing disulfide and noncovalently bonded polypeptides MSP1a and MSP1b) of the erythrocytic stage of *Am* conferred protection against pathogen challenge (Palmer et al. 1986). Likewise, Hope et al. (2004) demonstrated the value of using multiple adjuvants to confer immunity and decrease the need for antibiotic intervention in infected animals. This may be due to a broader repertoire of immune effector cells being stimulated by multiple adjuvants. Previous work has suggested that Quil-A (a heterogenous fraction of saponin) induces activation of dendritic cells and leads to strong antibody and T cell responses (Maraskovsky et al., 2009). Though mode of action hasn't been studied in detail, DEAE-Dextran has shown antibody enhancing properties in anti-fertility vaccines (Vizcarra et al., 2012) and appears to stimulate antigen-specific antibodies and eosinophilia when used in helminth vaccines (Piedrafita et al., 2013).

Assuming vaccine efficacy is based on a reduced need for antimicrobial therapy and increased survival, our data indicate that the use of multiple adjuvants in the vaccine construct could limit disease severity. Though caution is necessary considering the small sample size, it is noteworthy that 100% (6/6) of the animals vaccinated using a single adjuvant (DEAE-Dextran or QuilA) required antibiotic intervention during the study, compared to only 33% (2/6) of animals vaccinated using combination adjuvants (DEAE-Dextran and QuilA).

Despite the documented negative impact of anaplasmosis in cattle herds (Alderink et al. 1982), there remains no effective means of disease prevention. Controlling the disease once endemic is challenging, as transmission of *Am* can be mediated through biological vectors such as ticks (Dikmans, 1950), mechanical vectors such as horseflies (Baldacchino et al. 2014), blood-contaminated fomites such as needles (Reinbold et al. 2010a), or transplacentally from cow to calf during gestation (Zaugg, 1985). Average weight loss associated with disease progression is reported to be 190 lbs. (Alderink et al. 1982) with adult (>2yrs of age) cattle being more susceptible to severe clinical disease and death (Kocan et al. 2003). Symptoms diminish in surviving animals, but recovered cattle maintain low, sometimes undetectable, levels of infection (Coetzee et al. 2005). These carrier animals subsequently serve as local reservoirs for disease transmission (Swift and Thomas, 1983). Previous work has estimated that, when introduced to a naïve herd, anaplasmosis can result in a 3.6% reduction in calf crop, a 30% increase in cull rate, and a 3% mortality rate in clinically infected adult cattle (Alderink et al. 1982). In spite of these challenges, strategies to control anaplasmosis have not changed markedly in the last several decades (Kocan et al. 2003).

Though not available in the U.S., use of live vaccines containing attenuated or less pathogenic strains of *Am* or *A. centrale* for the control of clinical anaplasmosis is widespread in many parts of the world (Rogers et al. 1988). These vaccines are predicated on the principle of concomitant immunity, the paradoxical immune status in which resistance to reinfection coincides with the persistence of the original infection. Live vaccine-vaccinated cattle develop persistent infections which induce lifelong protective immunity in cattle such that revaccination is usually not required (Shkap et al. 2008). Although generally effective, use of live *Am* vaccines is not legal in the U.S. There are currently no USDA-approved vaccines for protection against *Am* infection or lowering disease severity. An experimental killed vaccine is available in 14 U.S. states, but no efficacy data for this vaccine are available (Aubry and Geale, 2011). Killed vaccines that contain purified *Am* organisms from erythrocytes are expensive to manufacture, may have the potential to induce isoimmune erthrolysis following repeated administration, have unknown efficacy against

heterologous strains, and usually require annual revaccination (Kocan et al. 2003). Thus, there remains no vaccine universally accepted as safe and effective against bovine anaplasmosis (Hammac et al. 2013).

This study tested the efficacy of a set of s.c. vaccine implants to protect against the development of antibiotic-intervention-requiring clinical anaplasmosis. Work presented here agrees with previous studies using cell culture-derived *Am* antigens (Kocan et al. 2001). Six MSPs of *Am* have been identified on erythrocyte-derived organisms (Kocan et al. 2003). The MSP1a is an *Am* adhesin for both bovine erythrocytes and tick cells (de La Fuente et al. 2003b). MSP1a has been explored as a vaccine target for *Am* because the individual tandem repeats contain B and T cell epitopes (Cabezas-Cruz et al. 2015). Cattle immunized with erythrocyte-derived *Am* have been shown to have a preferential antibody response to MSP1a (Brown et al. 2001). Immunization of cattle with MSP1a has also been shown to reduce infection of *Am* for the tick *Dermacentor variabilis* (de La Fuente et al. 2003c). The tandem repeats of the MSP1a subunit-based vaccines with *Am* MSP1a functional motifs have also been shown to induce a balanced humoral and cellular immune response in mice (Santos et al. 2013). It is possible that we would have observed a more robust protective response if we had used an *Am* strain that contained the K;S tandem repeat sequence, as the B and T cell epitopes differ between K;S, M and F tandem repeats (Catanese et al. 2016).

In the present study, our group demonstrated the feasibility of a subunit-based vaccine delivered in a single, subcutaneous ear implant 21 months prior to disease challenge. Future work may expand upon this observation by incorporating other conserved antigens, such as those previously identified (Riding et al. 2003) utilizing a similar vaccine delivery platform. Future work may also establish an optimal adjuvant concentration in order to achieve high immunogenicity without implant rejection.

LITERATURE CITED

Alderink, F. J., and R. A. Dietrick. 1982. Economic and epidemiological implications of anaplasmosis in Texas cattle herds. Proc Annu Meet US Anim Health Assoc 66–75.

Aubry, P., and D. W. Geale. 2011. A Review of Bovine Anaplasmosis. Transbound Emerg Dis 58:1–30. doi:10.1111/j.1865-1682.2010.01173.x

Baldacchino, F., M. Desquesnes, S. Mihok, L. D. Foil, G. Duvallet, and S. Jittapalapong. 2014. Tabanids: Neglected subjects of research, but important vectors of disease agents! Infect. Genet. Evol. 28:596–615. doi:10.1016/j.meegid.2014.03.029

Blouin, E., A. Barbet, J. Yi, K. Kocan, and J. Saliki. 2000. Establishment and characterization of an Oklahoma isolate of Anaplasma marginale in cultured Ixodes scapularis cells. Vet. Parasitol. 87:301–313. doi:10.1016/s0304-4017(99)00183-1

Brown, W. C., G. H. Palmer, H. A. Lewin, and T. C. Mcguire. 2001. CD4 T Lymphocytes from Calves Immunized with Anaplasma marginale Major Surface Protein 1 (MSP1), a Heteromeric Complex of MSP1a and MSP1b, Preferentially Recognize the MSP1a Carboxyl Terminus That Is Conserved among Strains. Infect. Immun. 69:6853–6862. doi:10.1128/iai.69.11.6853-6862.2001

Cabezas-Cruz, A., and J. D. L. Fuente. 2015. Anaplasma marginale major surface protein 1a: A marker of strain diversity with implications for control of bovine anaplasmosis. Ticks Tick Borne Dis 6:205–210. doi:10.1016/j.ttbdis.2015.03.007

Catanese, H. N., K. A. Brayton, and A. H. Gebremedhin. 2016. RepeatAnalyzer: a tool for analyzing and managing short-sequence repeat data. BMC Genomics 17. doi:10.1186/s12864-016-2686-2

Coetzee, J. F., M. D. Apley, K. M. Kocan, F. R. Rurangirwa, and J. V. Donkersgoed. 2005. Comparison of three oxytetracycline regimens for the treatment of persistent Anaplasma marginale infections in beef cattle. Vet. Parasitol. 127:61–73. doi:10.1016/j.vetpar.2004.08.017

de la Fuente, J., J. C. Garcia-Garcia, E. F. Blouin, and K. M. Kocan. 2003a. Characterization of the functional domain of major surface protein 1a involved in adhesion of the rickettsia Anaplasma marginale to host cells. Vet. Microbiol. 91:265–283. doi: 10.1016/s0378-1135(02)00309-7

de la Fuente, J., R. A. V. D. Bussche, T. M. Prado, and K. M. Kocan. 2003b. Anaplasma marginale msp1 Genotypes Evolved under Positive Selection Pressure but Are Not Markers for Geographic Isolates. J. Clin. Microbiol. 41:1609–1616. doi: 10.1128/jcm.41.4.1609-1616.2003

de la Fuente, J., K. M. Kocan, J. C. Garcia-Garcia, E. F. Blouin, T. Halbur, and V. Onet. 2003c. Immunization against Anaplasma marginale major surface protein 1a reduces infectivity for ticks. Int J Appl Res Vet Med 1:285-292. doi:10.1016/S0304-4017(01)00519-2

Dikmans, G. 1950. The transmission of anaplasmosis. Am. J. Vet. Res. 11:38.

FDA. 2019. Electronic Code of Federal Regulations (e-CFR). https://www.ecfr.gov/cgi-bin/text-idx?SID=000de4b0dcc4e12d05649c2a95^{0c}7476&mc=true&node=se21.6.558_1128&rgn=div8

(Accessed 13 August 2019)

Hammac, G. K., P.-S. Ku, M. F. Galletti, S. M. Noh, G. A. Scoles, G. H. Palmer, and K. A. Brayton. 2013. Protective immunity induced by immunization with a live, cultured Anaplasma marginale strain. Vaccine 31:3617–3622. doi:10.1016/j.vaccine.2013.04.069

Hope, M., G. Riding, M. Menzies, and P. Willadsen. 2004. A novel antigen from Anaplasma marginale: characterization, expression and preliminary evaluation of the recombinant protein. Vaccine 22:407–415. doi:10.1016/j.vaccine.2003.07.006

Jones, D., M. Brewer, B. Narasimhan, and J. Jackman. 2016. Vaccine Delivery Devices. U.S. Patent 10,130,454, filed July 30 2015, and issued February 4, 2016.

Kocan, K. M., T. Halbur, E. F. Blouin, V. Onet, J. D. L. Fuente, J. C. Garcia-Garcia, and J. T. Saliki. 2001. Immunization of cattle with Anaplasma marginale derived from tick cell culture. Vet. Parasitol. 102:151–161. doi:10.1016/s0304-4017(01)00519-2

Kocan, K. M., J. D. L. Fuente, A. A. Guglielmone, and R. D. Melendez. 2003. Antigens and Alternatives for Control of Anaplasma marginale Infection in Cattle. Clin. Microbiol. Rev. 16:698–712. doi:10.1128/cmr.16.4.698-712.2003

Love, J. N. 1972. Cryogenic preservation of Anaplasma marginale with Dimethyl sulfoxide. Am. J. Vet. Res. 33:2557-2560

Maraskovsky, E., M. Schnurr, N. S. Wilson, N. C. Robson, J. Boyle, and D. Drane. 2009. Development of prophylactic and therapeutic vaccines using the ISCOMATRIX adjuvant. Immunol. Cell Biol. 87:371–376. 10.1038/icb.2009.21

Palmer, G., A. Barbet, W. Davis, and T. Mcguire. 1986. Immunization with an isolate-common surface protein protects cattle against anaplasmosis. Science 231:1299–1302. doi:10.1126/science.3945825

Palmer, G. H., S. D. Waghela, A. F. Barbet, W. C. Davis, and T. C. Mcguire. 1987. Characterization of a neutralization-sensitive epitope on the Am 105 surface protein of Anaplasma marginale. Int. J. Parasitol. 17:1279–1285.

Palmer, G. H., F. R. Rurangirwa, and T. F. Mcelwain. 2001. Strain Composition of the Ehrlichia Anaplasma marginale within Persistently Infected Cattle, a Mammalian Reservoir for Tick Transmission. J. Clin. Microbiol. 39:631–635. doi:10.1128/jcm.39.2.631-635.2001

Piedrafita, D., S. Preston, J. Kemp, M. D. Veer, J. Sherrard, T. Kraska, M. Elhay, and E. Meeusen. 2013. The Effect of Different Adjuvants on Immune Parameters and Protection following Vaccination of Sheep with a Larval-Specific Antigen of the Gastrointestinal Nematode, Haemonchus contortus. PLoS ONE 8. 10.1371/journal.pone.0078357

Reinbold, J. B., J. F. Coetzee, L. C. Hollis, J. S. Nickell, C. M. Riegel, J. A. Christopher, and R. R. Ganta. 2010a. Comparison of iatrogenic transmission ofAnaplasma marginalein Holstein steers via needle and needle-free injection techniques. Am. J. Vet. Res. 71:1178–1188. doi:10.2460/ajvr.71.10.1178

Reinbold, J. B., J. F. Coetzee, L. C. Hollis, J. S. Nickell, C. Riegel, K. Olson, and R. R. Ganta. 2010b. The efficacy of three chlortetracycline regimens in the treatment of persistent Anaplasma marginale infection. Vet. Microbiol. 145:69–75. doi:10.1016/j.vetmic.2010.02.031

Riding, G., M. Hope, D. Waltisbuhl, and P. Willadsen. 2003. Identification of novel protective antigens from Anaplasma marginale. Vaccine 21:1874-1883. doi:10.1016/s0264-410x(03)00004-5

Rogers, R., C. Dimmock, A. D. Vos, and B. Rodwell. 1988. Bovine leucosis virus contamination of a vaccine produced in vivo against bovine babesiosis and anaplasmosis. Aust. Vet. J. 65:285–287. doi:10.1111/j.1751-0813.1988.tb16144.x

Santos, P. S., A. A. S. Sena, R. Nascimento, T. G. Araújo, M. M. Mendes, J. R. S. Martins, T. W. P. Mineo, J. R. Mineo, and L. R. Goulart. 2013. Epitope-Based Vaccines with the Anaplasma marginale MSP1a Functional Motif Induce a Balanced Humoral and Cellular Immune Response in Mice. PloS ONE 8. doi:10.1371/journal.pone.0060311

Schaut, R. G., M. T. Brewer, J. M. Hostetter, K. Mendoza, J. E. Vela-Ramirez, S. M. Kelly, J. K. Jackman, G. Dellanna, J. M. Howard, B. Narasimhan, W. Zhou, and D. E. Jones. 2018. A single dose polyanhydride-based vaccine platform promotes and maintains anti-GnRH antibody titers. Vaccine 36:1016–1023. doi:10.1016/j.vaccine.2017.12.050

Shkap, V., B. Leibovitz, Y. Krigel, T. Molad, L. Fish, M. Mazuz, L. Fleiderovitz, and I. Savitsky. 2008. Concomitant infection of cattle with the vaccine strain Anaplasma marginale ss centrale and field strains of A. marginale. Vet. Microbiol. 130:277–284. doi:10.1016/j.vetmic.2008.02.013

Swift, B. L. and G. M. Thomas. 1983. Bovine anaplasmosis: elimination of the carrier state with injectable long-acting oxytetracycline. J. Am. Vet. Med. Assoc. 183:63–65.

Uilenberg, G. 1995. International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Vet. Parasitol. 57:19–41. doi:10.1016/0304-4017(94)03107-8

Vizcarra, J. A., S. L. Karges, and R. P. Wettemann. 2012. Immunization of beef heifers against gonadotropin-releasing hormone prevents luteal activity and pregnancy: Effect of conjugation to different proteins and effectiveness of adjuvants1. J. Anim. Sci. 90:1479–1488. 10.2527/jas.2011-4554

Zaugg, J. L. 1985. Bovine anaplasmosis: transplacental transmission as it relates to stage of gestation. Am. J. Vet. Res. 46:570-572.

Table 1. Random assignment of calves to vaccine treatment groups.

Vaccine Treatment Group[a] (n = 3)	Vaccine Construct[b] (n = 6)	Priming Dose (300 ug R1OK-MAP-BP/ Montanide)	Boosting dose (rod) adjuvant	Implant adjuvant	Vaccine Outcome (Failure/ Treatment)
1	B	1 dose s.c.	DEAE-dextran	DEAE-dextran	3/3
2	B	1 dose s.c.	Quil A	Quil A	3/3
3	A	1 dose s.c.	Quil A	DEAE-dextran	1/3
4	A	1 dose s.c.	DEAE-dextran	Quil A	1/3

[a] Calves were randomly assigned to one of four vaccine treatment groups

[b] Calves were divided between two vaccine constructs (A or B) denoting single or combination adjuvants - diethylaminoethyl-Dextran (DEAE-Dextran) and Quil A

Figure 1. Mean (± SEM) bacteremia and packed cell volume (PCV) of vaccinated animals challenged with *Anaplasma marginale.* (A) PCV is shown over time and separated by vaccine construct. (B) Bacteremia is shown over time and separated by vaccine construct. PCV was significantly higher among animals within the combination adjuvant construct than those within the same adjuvant construct at day 35. * $P = 0.006$.

Fig1-2 SEM 300dpi