Curriculum vitae

Dr. Mirinda van Kleef (Ph.D.)

Current position:

Specialist Researcher – Cellular Immune Responses, Agriculture Research Council - Onderstepoort Veterinary Institute (ARC-OVI).

1986: BSc (Biochemistry & Chemistry), University of Pretoria.
1987: BSc (Hons), Biochemistry, University of Pretoria.
1992: MSc, Biochemistry, University of Pretoria.
The identification, isolation and characterisation of antigenic proteins of *Cowdria ruminantium*.
2002: PhD, Microbiology, Rhodes University.
Identification of *Cowdria ruminantium* proteins that induce specific cellular immune responses.

Employment

1987-1992: Assistant veterinary researcher, Protozoology Division, OVI.
1992-1993: Veterinary researcher, Protozoology Division, OVI.
1994-1996: Researcher, Protozoology Division, ARC-OVI.
1996-1999: Researcher, Immunology Division, ARC-OVI.
1999-2003: Senior researcher, Immunology Division, ARC-OVI.
2003-2006: Assistant Director, Molecular Biology Division, ARC-OVI.
2006-date: Specialist Researcher, Project manager – Cellular Immune Responses, ARC-OVI.
2014-2017: Acting Research Team Manager: New Generation Vaccines Programme, ARC-OVI.
2019-2020: Acting Research Team Manager: Vaccine and Diagnostic Development Programme, ARC-OVI.

Research experience

1987-1992: Responsible officer. The identification, isolation and characterization of antigenic proteins of *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*). This led to the identification, isolation and characterisation of the major antigenic protein (MAP1) of *Ehrlichia ruminantium* which formed part of my MSc. This work contributed towards sequencing of the *map*1 gene and its further investigation as a vaccine and/or diagnostic candidate.

1992-1993: Responsible officer. Developed a positive selection immunosorbent chromatography technique for the purification of *E. ruminantium* organisms. This contributed to determining the genome size of the bacteria and construction of the first representative expression library.

1994-1999: Project leader. The mechanism of protective immunity to heartwater (OV19/3). USAID CDR grant number TA-MOU-95-C15-194). I was instrumental in establishing a veterinary cellular immunity lab at ARC-OVI that is equipped to perform several assays (proliferation assays, ELISpot assays, MACs cells sorting, flow cytometry, cytotoxic assays, MHC typing and quantitative cytokine determination using real time PCR).

1999-2002: Project leader. Identification of proteins which stimulate a protective cellular response against heartwater (OV9/20). (part of EU INCO-DEV grant number ICA4-CT-2000-30026. Integrated diagnostic and recombinant vaccine development for cowdriosis and anaplasmosis). This resulted in the successful identification of low molecular weight proteins of the Welgevonden strain that induce bovine CD4+-enriched T-cells to proliferate and produce interferon-gamma and formed part of my PhD research.

2003- present: Project Manager. Recombinant heartwater vaccine development. Contributed to the annotation of the *E. ruminantium* genome sequencing project that allowed us to identify additional genes from the available complete genome sequence that induce the appropriate protective immune responses for inclusion in a subunit vaccine. I was part of a research team that successfully completed expression of 209 *E. ruminantium* recombinant proteins. Of these 173 recombinant proteins were subsequently screened *in vitro* for their ability to induce immune bovine and sheep lymphocyte proliferation and IFN-γ expression. Twenty of the highest responders were chosen for vaccine trials in sheep. However, none induced protective immunity when administered as a cocktail DNA vaccine in sheep when needle challenged. Results suggest that T cell epitope mapping and thus removal of possible inhibitory epitopes may improve protective responses. Contributed to the evaluation of the ability of additional recombinant proteins and overlapping peptides of selected proteins to induce recall T cell responses *in vitro* (proliferation, ELISpot IFN-γ production, real time PCR cytokine profiles and flow cytometry (fluorescent antigen-transfected target cell (FATT)-CTL assay, intracellular cytokine staining and phenotype determination). Chose epitopes that induce the best and varied immune responses and tested them in vaccine trials in sheep as a multi-epitope DNA vaccine, that induced 60% protection against tick challenge. Work is continuing to improve this vaccine. Transcriptome sequencing data from vaccine trials are being analysed to determine in depth immune responses induced and correlates of protection.

Submitted a provisional IP disclosure specification "Multi-epitope DNA vaccine, adjuvant provide protection against tick transmitted heartwater in sheep. [SPOORSA-sa_cases.0158931.PM108651. FID8622725].

I initiated investigations to develop a single dose vaccine against heartwater by using biodegradable polymer microspheres as the vaccine delivery system. Biodegradable polymer microspheres can mimic priming and boosting because the release of their encapsulated material can be controlled. We successfully optimized the formulation of a set of polymers that mimic a 2xDNA / 1x protein boost, at three week intervals, *in vitro*. This micro particle cocktail now requires further optimisation *in vivo* regarding route of administration, dose and inclusion of additional boosting and also forms part of my current research activities.

<u>Research grant proposals awarded</u>

1) 2005. EU Funding (€112655; 4 years) was secured in August 2005. Epigenevac FP6-003713: Epidemiology and new generation vaccines for Ehrlichia and Anaplasma infections of ruminants. Partner.
2) 2005. NRF. Recombinant heartwater vaccine. Partner.
3) 2005. RMRDT. 0V9/20/C154. (R220 000; 2 years) Towards a recombinant vaccine for heartwater. PI.
4) 2006. DoA. 0V9/23/167. (R5 741 040; 3 years). Single dose recombinant vaccine against heartwater. PI.
5) 2006. NRF – Research Niche Area. Molecular studies of infectious and parasitic diseases of animals. Assistant leader.
6) 2007. RMRDT (R260 000; 3 years). Laboratory tick challenge for heartwater vaccine development. PI.
7) 2008 & 2009. Joy Liebenberg Trust (R200 000). Preliminary studies of cellular immune responses in horses to African horsesickness virus. Partner.
8) 2009. Joy Liebenberg Trust (R100 000; 1 year). Epitope mapping of a secreted protein of *Ehrlichia ruminantium* that induced recall cellular immune responses *in vitro.* Partner.
9) 2010. THRIP/RMRDT (R708 000; 3 years). Heartwater attenuated Welgevonden stock vaccine testing in Holstein cattle. PI.
10) 2010. THRIP/OBP/RMRDT. (R3 285 000; 3 years). A multi-epitope single dose biodegradable vaccine for heartwater. PI.
11) 2010. NRF rating = C1
12) 2011. NRF Incentive Funding. (R240 000; 6 years).
13) 2012. Vaccine Initiative ECSP project (R6 000 000; 3 years). New generation heartwater vaccine. PI.

14) 2015. NRF Research and technology fund. (R450 000; 3 years). Multi-epitope heartwater vaccine. PI.

Mentor of Students

2005 – 2011: Extraordinary Lecturer, without remuneration, in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria.

1) MSc. 2005 - 2007. Ms Nontobeko Thema. Cellular immune responses induced *in vitro* by secreted proteins of *Ehrlichia ruminantium.* Supervisor.
2) MSc. 2007- 2009. Mr Ndavhe Tshikhudo. Biodegradable microspheres as a single dose delivery system for *Ehrlichia ruminantium* vaccines. Supervisor.
3) MSc. 2007 - 2010. Ms Erika Faber. The identification of *Ehrlichia ruminantium* membrane-associated proteins that induce cellular immune responses *in vitro.* Co-supervisor.
4) MSc (distinction). 2013 – 2016. Ms Lauren Son. Identification and characterization of T cell epitopes of Erum2550, -2580 and -2590 proteins of *Ehrlichia ruminantium*. Supervisor.
5) MSc. 2013 – 2016. Ms Abigail Ngoepe. Identification and characterisation of Th1 and Th2 epitopes of the cowdria polymorphic gene 1 (Erum2510) of *Ehrlichia ruminantium* ORF Erum2510. Supervisor.
6) MSc. 2013 – 2016. Mr Rae Smith. Identification of *Ehrlichia ruminantium* epitopes of Erum5400, -8050 and -5270 proteins as possible vaccine candidates. Supervisor.
7) MSc. 2014 – 2017. Mr Iyani Calvin Raselabe. *In vitro* characterisation of the cellular immune response induced by African swine fever virus proteins p30, p54 and p72. Supervisor.
8) MSc. 2015 – 2019. Mr Freddy Mabetlela. Identification of African swine fever virus proteins that activate cellular immune responses. Supervisor.
9) PhD. 2005 - 2010. Dr Ivy Sebatjane. Developing an optimized recombinant vaccine for heartwater. Supervisor.
10) PhD. 2012 – 2018. Dr Nontobeko Thema. Identification and characterisation of *E. ruminantium* vaccine candidate epitopes. Co-supervisor.
11) PhD. 2013 – 2016. Dr Thsifiwa Nefefe. Determination of the sheep innate and adaptive immune transcriptome after infection with *Ehrlichia ruminantium*. Co-supervisor.
12) PhD. 2013 – 2017. Dr Mabotse Tjale. Transcriptome analysis of *Ehrlichia ruminantium* at the host tick bite site and during the developmental stages in cell culture. Co-supervisor.
13) PhD. 2017 – 2022. Ms Erika Faber. Transcriptome analysis of immune responses induced in horse PBMC by the attenuated AHSV4 (*in vivo*), virulent AHSV4 and its recombinant proteins (*in vitro*). Co-supervisor.

Scientific publications

1) Faber E, Tshilwane SI, **van Kleef M,** Pretorius A. Virulent African horse sickness virus serotype 4 interferes with the innate immune response in horse peripheral blood mononuclear cells in vitro. Infect Genet Evol. 2021;91:104836. doi: 10.1016/j.meegid.2021.104836.
2) Tjale, M.A., Liebenberg, J., Steyn, H, **Van Kleef, M**., and Pretorius, A. 2021. a Transcriptome analysis of *Ehrlichia ruminantium* in the ruminant host at the tick bite site and in the tick vector salivary glands. Ticks and Tick-borne Diseases 12 (2021) 101646.
3) Tshilwane, S.I., Thema, N., Steyn, H.C., **van Kleef, M**., and Pretorius, A. 2019. A multi-epitope DNA vaccine co-administered with monophosphoryl lipid A adjuvant provides protection against tick transmitted *Ehrlichia ruminantium* in sheep. Vaccine, 37:4354-4363.
4) Thema, N., Tshilwane, S.I., Pretorius, A., Son, L., Smith, R.M., Steyn, H.C., Liebenberg, J., and **Van Kleef M**. 2019. Identification and characterisation of conserved epitopes of *E. ruminantium* that activate Th1 CD4+ T cells: towards the development of a multi-epitope vaccine: Molecular Immunology, 107:106–114.
5) Thema, N., Tshilwane, S.I., Son, L., Smith, R.M., Faber, E., Steyn, H.C., Liebenberg, J., **Van Kleef, M.,** and Pretorius, A. 2019. *Ehrlichia ruminantium* antigens and peptides induce cytotoxic T cell responses *in vitro*. Veterinary Immunology and Immunopathology, 207:1–9.
6) Tjale, M., Pretorius, A., Josemans, A., **Van Kleef, M.,** Liebenberg, J. 2018. Transcriptome analysis of *Ehrlichia ruminantium* during the developmental stages in bovine and tick cell culture. Ticks and Tick-borne Diseases. 9:126-134.
7) Nefefe, T., Liebenberg, J., **Van Kleef, M.,** Steyn, H.C., Pretorius, A. 2017. Innate immune transcriptomic evaluation of PBMC isolated from sheep after infection with *E. ruminantium* Welgevonden strain. Molecular Immunology. 91:238–248.
8) Faber, F.E., **Van Kleef, M.,** Tshilwane, S.I., Pretorius, A. 2016. African horse sickness virus serotype 4 antigens, VP1-1, VP2-2, VP4, VP7 and NS3, induce cytotoxic T cell responses *in vitro*. Virus Research. 220:12–20.
9) Thema, N., Pretorius, A., Tshilwane, S.I., Liebenberg, J., Steyn, H., **Van Kleef, M**. 2016. Cellular immune responses induced in vitro by *Ehrlichia ruminantium* secreted proteins and identification of vaccine candidate peptides. Onderstepoort Journal of Veterinary Research. Vol 83, No 1 doi: 10.4102/ojvr.v83i1.1170
10) Pretorius, A., Faber, F.E. and **Van Kleef, M**. 2016. Immune gene expression profiling of PBMC isolated from horses vaccinated with attenuated African horsesickness virus serotype 4. Immunobiology. 221:236–244.
11) Liebenberg, J., Pretorius, A., Faber, F.E., Collins, N.E., Allsopp, B.A., **Van Kleef, M**. 2012. Identification of *Ehrlichia ruminantium* proteins that activate cellular immune responses

using a reverse vaccinology strategy Veterinary Immunology and Immunopathology. 145:340–349.

12) Pretorius, A., **Van Kleef, M.**, Van Wyngaardt, W., Heath, J. 2012. Virus-specific CD8+ T-cells detected in PBMC from horses vaccinated against African horse sickness virus. Veterinary Immunology and Immunopathology. 146:81– 86.

13) Sebatjane, S.I., Pretorius, A., Liebenberg, J., Steyn, H., **Van Kleef, M.** 2010. *In vitro* and *in vivo* evaluation of five low molecular weight proteins of *Ehrlichia ruminantium* as potential vaccine components. Veterinary Immunology and Immunopathology. 137:217–225.

14) Tshikhudo, N., Pretorius, A., Putterill, J., **Van Kleef, M.** 2010. Preparation and in vitro characterisation of *Ehrlichia ruminantium* plasmid DNA and proteins encapsulated into and DNA adsorbed onto biodegradable microparticles. Ticks and Tick-borne Diseases. 1:186–193.

15) Pretorius, A., **van Kleef, M.**, Collins, N.E., Tshikudo, N., Louw, E., Faber, F.E., van Strijp, M.F., Allsopp, B.A. 2008. A heterologous prime/boost immunisation strategy protects against virulent *Ehrlichia ruminantium* Welgevonden needle challenge but not against tick challenge. Vaccine. 26:4363-4371.

16) Steyn, H.C., Pretorius, A., McCrindle, C.M., Steinmann, C.M., **Van Kleef, M**. 2008. A quantitative real-time PCR assay for *Ehrlichia ruminantium* using pCS20. Veterinary Microbiology. 131:258-265.

17) Pretorius, A., Collins, N.E., Steyn, H.C., van Strijp, F., **van Kleef, M**. and Allsopp, B.A. 2007. Protection against heartwater with four *Ehrlichia ruminantium* open reading frames presented by DNA immunisation. Vaccine. 25:2316-2324.

18) Zweygarth, E., Josemans, A.J., Van Strijp, M.F., Lopez-Rebollar, L., **Van Kleef, M**. and Allsopp, B.A. 2005. An attenuated *Ehrlichia ruminantium* (Welgevonden stock) vaccine protects small ruminants against virulent heartwater challenge. Vaccine. 23:1695-1702.

19) Collins, N.E., Liebenberg, J., De Villiers, E.P., Brayton, K.A., Louw, E., Pretorius, A., Faber, E., Van Heerden, H., Josemans, A., **Van Kleef, M**., Steyn, H.C., Van Strijp, F., Zweygarth, E., Jongejan, F., Maillard, J-C., Berthier, D., Botha, M., Joubert, F., Thomson, N.R., Allsopp, M.T., and Allsopp, B.A. 2005. The genome of the heartwater agent, *Ehrlichia ruminantium*, contains multiple tandem repeats of actively variable copy number. Proceedings of the National Academy of Sciences of the United States of America. 102:838-843.

20) Collins, N.E., Pretorius, A., **Van Kleef, M**., Brayton, K.A., Allsopp, M.T., Zweygarth, E. and Allsopp, B.A. 2003. Development of improved attenuated and nucleic acid vaccines for heartwater. Dev. Biol. (Basel). 114:121-36

21)Collins, N.E., Pretorius, A., **Van Kleef, M.**, Brayton, K.A., Zweygarth, E. and Allsopp, B.A. 2003. Development of improved vaccines for heartwater. Annals of the New York Academy of Sciences. 990:474-484.

22)Gunter, N., Esteves, I., Kandassamy, Y., Martinez, D., Bensaid, A., **Van Kleef, M.**, Du Plessis, D. and Totte, P. 2002. *Cowdria ruminantium* antigens of around 15 kDa are potent inducers of IFN-gamma. Annals of the New York Academy of Sciences. 969:135-140.

23)**Van Kleef, M.**, Macmillan, H., Gunter, N.J., Zweygarth, E., Allsopp, B.A., Shkap, V., Du Plessis, D.H. & Brown, W.C. 2002. Low molecular weight proteins of *Cowdria ruminantium* (Welgevonden isolate) induce bovine CD4+-enriched T-cells to proliferate and produce interferon-gamma. Veterinary Microbiology. 85:259-273.

24)**Van Kleef, M.**, Gunter, N.J., Macmillan, H., Allsopp, B.A., Shkap, V. & Brown, W.C. 2000. Identification of *Cowdria ruminantium* antigens that stimulate proliferation of lymphocytes from cattle immunized by infection and treatment or with inactivated organisms. Infection and Immunity. 68:603-614.

25)Brayton, K.A., Fehrsen, J., De Villiers, E.P., **Van Kleef, M.** & Allsopp, B.A. 1997. Construction and initial analysis of a representative □ZAPII expression library of the intracellular rickettsia *Cowdria ruminantium*: cloning of *map*1 and three other *Cowdria* genes. Veterinary Parasitology. 72:185-199.

26)Jardine, J.E., Vogel, S.W., **Van Kleef, M.** & Van Der Lugt, J.J. 1995. Immunohistochemical identification of *Cowdria ruminantium* in formalin-fixed tissue sections from mice, sheep, cattle and goats. Onderstepoort Journal of veterinary Research. 62:277-280.

27)Van Vliet, A.H.M., Jongejan, F., Van Kleef, M. and Van Der Zeijst, B.A.M., 1994. Molecular cloning, sequence analysis, and expression of the gene encoding the immunodominant 32-Kilodalton protein of *Cowdria ruminantium*. Infection and Immunity. 62:1451-1456.

28)**Van Kleef, M.,** Neitz, A.W.H. and De Waal, D.T., 1993. Isolation and characterization of antigenic proteins of *Cowdria ruminantium*. Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux. 46:157-164.

29)Van Vliet, A.H.M., Jongejan, F., **Van Kleef, M.** and Van Der Zeijst, B.A.M., 1993. Cloning and partial characterization of the Cr32 gene of *Cowdria ruminantium.* Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux. 46:167-170.

30)**Van Kleef, M.**, Neitz, A.W.H. and De Waal, D.T., 1992. Characterization of the 27 kDa and 31 kDa proteins of *Cowdria ruminantium.* Journal of the South African Veterinary Association. 64:43.

31)**Rossouw, M.**, Neitz, A.W.H., De Waal, D.T., Du Plessis, J.L., Van Gas, L. and Brett, M.S., 1990. Identification of the antigenic proteins of *Cowdria ruminantium*. Onderstepoort Journal of veterinary Research. 57:215-221.

Book Chapter

Anipedia, www.anipedia.org: JAW Coetzer and P Oberem (Directors) In: Infectious Diseases of Livestock, JAW Coetzer, GR Thomson, NJ Maclachlan and M-L Penrith (Editors). BA Allsopp, M van Kleef and A Pretorius, Heartwater, 2018

Conferences attended

1) South African Biochemical Society, 10th Congress, Pietermaritzburg, Natal, South Africa, 1991. Presented poster: Identification of antigenic proteins of *Cowdria ruminanitum*.
2) Tick Host Pathogen Interface (THPI), Kruger National Park, South Africa, 1995. Presented poster: Purification of *Cowdria ruminantium* by positive selection immunoadsorbent chromatography.
3) Society for Tropical Veterinary Medicine (STVM), San Jose, Costa Rica, 1995. Presented poster: Unresponsiveness of T cells to *Cowdria ruminantium in vitro*.
4) STVM, CRIAD-EMVT, Montpellier, France, 1997. Presented poster: Identification of *Cowdria ruminantium* proteins that induce lymphocyte proliferation in an immune animal.
5) BIOY2K combined millennium meeting, Grahamstown, South Africa, 2000. Presented a talk: Identification of *Cowdria ruminantium* antigens that stimulate proliferation and IFN-gamma production by immunized cattle.
6) Vaccines and Immunotherapeutics in the 3rd millenium, Lorne, Australia, 2000. Presented poster: Identification of protective and diagnostic epitopes of *Cowdria ruminantium*.
7) 20th meeting of the American Society for Rickettsiology and the 5th International Conference on Bartonella as Emerging Pathogens from 2-7 September 2006 at Asilomar Conference grounds, Pacific Grove, California, USA. Presented a poster on 'Development of an *Ehrlichia ruminantium* recombinant vaccine: A DNA prime- recombinant protein boost immunisation strategy protects against virulent *E. ruminantium* Welgevonden needle challenge but not against tick challenge'.
8) Vaccine Technology II conference held in Albufeira, Portugal from 1 - 6 June, 2008. Presented a poster on 'Towards a recombinant vaccine for heartwater'.
9) 8th International TTP and 12th Biennial STVM conference 2014 held 24-29 August in Cape Town, South Africa.
10) Global conference on agricultural research for development (GCARD3) held 5-8 April 2016 at ARC-Roodeplaat. Presented: New Vaccines for African Animal Diseases.
11) DST-NRF Nanotechnology Symposium 2016. Realising the potential of nanotechnology in South Africa. Held 27-28 June 2016 at CSIR, Pretoria, South Africa

Meetings/workshops/courses attended

1) Invited to ILRI (by Dr. W. Jorgenson; all expenses paid by ACIAR) to learn bovine MHC typing, 1998, for the purpose of determining the influence of type II MHC phenotypes in cattle on vaccination.
2) PCR methodology course, OVI, Onderstepoort, 1998.
3) Flow cytometry workshops, Beckman Coulter, Johannesburg, 1999, 2000, 2001, 2002.
4) Invited participant at International Consortium on Ticks and Tick-borne Diseases (ICTTD) Concerted action meeting, Montpellier, France, 1997. (All expenses paid by ICTTD).
5) Invited participant at ICTTD Concerted action workshop on "Epidemiology and economics of ticks and tick-borne diseases" in Hammamet, Tunisia in September 1999. (All expenses paid by ICTTD).
6) Participated in *E. ruminantium* pilot meeting of the EU INCO-DEV grant number ICA4-CT-2000-30026, Montpellier, France, January 2001. (All expenses paid by EU).
7) How to write a convincing proposal, ISNAR workshop, ARC, Pretoria, SA, 20-24 October 2003.
8) Participated in *E. ruminantium* pilot meeting of the EU FP6-2002-INCO-DEV-1/ Specific Targeted Research Projects, Dakar, Senegal, 5-7 April 2005. (All expenses paid by EU).
9) Radiation Safety Training Course. 2005.
10) Interpersonal management Course. Execuprime Training. 2005.
11) Education for the humane use and care of laboratory animals and for promoting quality of experimental results - accreditation course. 2006.
12) Participated in a report back meeting of the EU contract Epigenevac FP6-003713: Epidemiology and new generation vaccines for Ehrlichia and Anaplasma infections of ruminants, CIRAD, Montpellier, France, June 2008.
13) Intellectual Property awareness and training workshop. D.M. Kisch Inc. 2008.
14) Radiation Safety Training Course. 2008.
15) Global African Swine Fever Research Alliance (GARA) workshop held from 10-14 November 2014 at the Farm Inn Conference Venue, Pretoria, South Africa.
16) Animal and Human Vaccine Development in South Africa: Past Achievements and Future Prospects. University of Pretoria. 30 January – 1 February 2014. Faculty of Natural and Agricultural Sciences, Plant Sciences Auditorium. Presented: New generation heartwater vaccine: Where are we?
17) Workshop: ARC and the law. Presented by ENS Attorneys, Sandton, 1-2 March 2016.
18) Course: Managing workplace discipline and incapacity. Presented by CCMA, 4-6 April 2017.

19) Course: Leadership Development Programme for Middle Managers. Enterprises University of Pretoria. 2017.
20) Course: Animal Research Ethics. 2018.
21) Participated in a Gap analysis workshop on Heartwater. Guadeloupe, October 9-11, 2018.