BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME BERGHMAN, Luc R.	POSITION TITLE Associate Professor
eRA COMMONS USER NAME berghman	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Leuven, Belgium	MSc	1982	Zoology
University of Leuven, Belgium	PhD	1988	Zoology
University of Leuven, Belgium	Postdoc	1988-1994	Zoology

A. Personal statement

The goal of the proposed research is to identify the role of the bufodienolides in disorders of vascular integrity, injury and inflammation. The most widely studied of the pathologies in which bufodienolides are implicated is the pregnancy-specific syndrome preeclampsia (PE), but a broad array of other disorders share dysfunctional vasculature as one of their symptoms, potentially mediated by one or several bufodienolides. Those diseases include, but are not limited to, multiple sclerosis, stroke, traumatic brain injury, and hemorrhagy; other pathologies are likely to be added to this list as our research goes on. The bufodienolides that we have focused on until now are marinobufagenin (MBG), as one of the major culprits in preeclampsia and likely one of the very earliest biomarkers in the development of the disease, and resibufogenin (RBG), a congener of MBG that appears to be capable of curing and preventing preeclampsia in a rodent model.
In collaboration with the PI of this proposal, Dr. J.B. Puschett, MD, we have recently developed a chemifluorescent enzyme-mediated immunoassay for the assay of marinobufagenin (MBG), and documented an increase in the urinary levels of MBG, which precedes the other symptoms of the PE syndrome (hypertension, proteinuria and intrauterine growth restriction). Urinary levels of MBG were demonstrated to increase in a rat model of PE as well as in human patients. My specific role in the current proposal will consist of developing antibodies (polyclonal and monoclonal) against RBG (potentially the first drug against preeclampsia), cinobufotalin (CINO) and telocinobufagin (TCINO) for use in the design of immunoassays similar to the one recently developed for MBG. Since it is as yet unclear which other bufodienolides may be implicated in the pathogenesis of the array of disorders studied in the scope of the current proposal, additional bufodienolides may be added to our list of targets. My team has the expertise, leadership and motivation necessary to successfully carry out the proposed work. Production of antibodies – monoclonal, polyclonal and recombinant in mammals as well as birds – is a hallmark of my career of 25 plus years. I have developed antibodies, and immunochemical applications based on those antibodies, for a huge variety of antigens, including steroids, neuropeptides, protein hormones, bacterial and viral antigens, *etc*. The production of my first monoclonal antibodies goes back 25 years, and in the meantime I have developed antibody production and characterization protocols that allow us to produce virtually any antibody with a success rate of close to 100%. The current proposal is a logical extension of previous research projects that include an ongoing project focusing on the role of MBG in PE and in an array of other pathologies. My lab has been fruitfully collaborating with Drs. Puschett and Romo for five years now.

B. Positions and Honors

1988: Postdoctoral Research Fellow, National Fund for Scientific Research, Belgium
1994: Senior Research Associate, National Fund for Scientific Research, Belgium

1994: Assistant Professor, Department of Zoology, University of Leuven (partim)
06/1998 – 08/2005: Assistant Professor, Department of Poultry Science, TAMU
09/1998 – 08/2005: Assistant Professor, Department of Veterinary Pathobiology, TAMU
09/2005 – present: Associate Professor, Depts. of Poultry Science and Vet. Pathobiology
Poultry Science, Associate Editor.

C. Selected peer-reviewed publications (10 selected from 105 peer-reviewed publications)

1. A chemifluorescent immunoassay for the determination of marinobufagenin in body fluids. Abi-Ghanem D, Lai X, **Berghman** LR, Horvat D, Li J, Romo D, Uddin MN, Kamano Y, Nogawa T, Xu JP, Pettit GR, Puschett JB. J Immunoassay Immunochem. 2011 Jan;32(1):31-46.

   My lab developed a chemifluorescent competitive ELISA that quantifies marinobufagenin (MBG) levels in biological fluids. Based on a polyclonal antibody raised against a novel MBG-bovine serum albumin conjugate, this assay achieved an MBG detection limit of less than 9 pg/mL. It represents a powerful tool for the diagnosis and prevention of human hypertensive states, particularly preeclampsia.

2. Marinobufagenin Levels in Preeclamptic Patients: A Preliminary Report. (2011) Agunanne E, Horvat D, Harrison R, Uddin MN, Jones R, Kuehl TJ, Ghanem DA, **Berghman** LR, Lai X, Li J, Romo D, Puschett JB. Am J Perinatol. 2011 Mar 4. PMID:21380994

   The first application of the above chemifluorescent enzyme-linked immunosorbent assay. Marinobufagenin levels were higher in preeclamptics than in the controls in both serum and urine at various gestational age periods. These data are consistent with a role for marinobufagenin in the etiology of preeclampsia.

3. Production and characterization of agonistic monoclonal antibodies against chicken CD40. Chen CH, Abi-Ghanem D, Njongmeta L, Bray J, Mwangi W, Waghela SD, McReynolds JL, Ing NH, **Berghman** LR. Dev Comp Immunol. 2010 Nov;34(11):1139-43. Epub 2010 Jul 3.

   These are the first academically available monoclonal antibodies against chicken CD40 and have allowed us to demonstrate the CD40-CD154 interaction in birds. More specifically we have gathered data that show that these antibodies can become the foundation of a new vaccination platform based on in vivo targeting of dendritic and other antigen- presenting cells resulting in dramatically enhanced immune responses.

4. Carboxypeptidase E, an essential element of the regulated secretory pathway, is expressed and partially co-localized with chromogranin A in chicken thymus. Zhang* X, Zhu J, Loh YP, **Berghman** LR. Cell Tissue Res. 2009 Sep;337(3):371-9. Epub 2009 Jul 15. PMID:19603184

   Our findings, based on novel monoclonal antibodies against chromogranin A, suggest for the first time that the thymic diffuse neuroendocrine system serves as a relay for nervous stimuli delivered by the autonomic nervous system. Thus, these newly defined neuroendocrine cells might play an important role in the immuno-neuro-endocrine cross-talk in the thymus.

5. Phage display selection and characterization of single-chain recombinant antibodies against Eimeria tenella sporozoites. Abi-Ghanem* D, Waghela SD, Caldwell DJ, Danforth HD, **Berghman** LR. Vet Immunol Immunopathol. 2008 Jan 15;121(1-2):58-67. Epub 2007 Aug 11. PMID:17897723
   These were the first reported recombinant antibodies against the sporozoites of Eimeria tenella, a causative agent of coccidiosis in chickens. The development of these reagents required practical knowledge of the phage display technology needed for the selection of high affinity recombinant antibodies, and development of an innovative way to produce antigenically intact sporozoites.

6. Validation of a new antiserum directed towards the synthetic c-terminus of the fos protein in avian species: immunological, physiological and behavioral evidence. D'Hondt, E., Vermeiren, J., Peeters K., Balthazart J., Tlemçani O., Ball G. F., Duffy D. L., Vandesande F. and **Berghman** L. (1999). J

Neurosci Methods 91: 31-45.

This paper reports the development and validation of the first antiserum for the immunocytochemical localization of the c-fos protein in the nervous system of a wide variety of birds. This antiserum has become the gold standard for this purpose and was/is used in numerous publications aiming at demonstrating the activation of certain populations of neurons in the avian brain.

7. The molecular characterization of chicken pituitary N-terminal pro-opiomelanocortin (POMC): affinity isolation of the isoforms and cloning of the POMC cDNA. **Berghman**, L.R., Devreese, B., Verhaert, P., Gerets, H., Arckens, L., Vanden Broeck, J., van Beeumen, J., Vaudry, H. and Vandesande, F., 1998. *Mol Cell Endocrinol* 25: 119-130.

   Monoclonal antibodies (Mabs) against the chicken pituitary corticotropes were used to isolate and physicochemically characterize a population of closely related N-terminal POMC peptides from crude chicken pituitary extracts. The nucleotide sequence obtained by reversed transcription PCR (RT-PCR) completely confirmed the new amino acid sequence data including pro-gamma-MSH, the joining peptide and ACTH for the first time in birds.

8. Immunocytochemical demonstration of chicken hypophyseal thyrotropes and development of a radioimmunological indicator for chicken TSH using monoclonal and polyclonal homologous antibodies in a subtractive strategy. **Berghman**, L.R., Darras, V.M., Chiasson, R.B., Decuypere, E., Kühn, E.R., Buyse, J. and Vandesande, F. (1993). General and Comparative Endocrinology 92, 189-200.

   These new monoclonal antibodies against the alpha- and beta- subunit of chicken gonadotropins were used for the first immunocytochemical and radioimmunological characterization of an avian thyrotropin. The resulting RIA indicator was used extensively for the physiological assessment of the thyroidal axis in birds.

9. One step purification of chicken growth hormone from a crude pituitary extract by use of a monoclonal immunoadsorbent. Berghman, L.R., Van Beeumen, J., Decuypere, E., Kühn, E.R. and Vandesande, F. (1988). Journal of Endocrinology 118, 381-387.

   This paper reported the first application of homologous monoclonal antibodies for the purification of an avian hypophyseal hormone and the development of a homologous immunoassay, in this case for chicken growth hormone. The purification was a one-hour, one-step protocol that yielded > 99% pure growth hormone in a pure mixture representing the multiple molecular forms of pituitary chicken GH.

10. Glycosylated chicken growth hormone. **Berghman**, L.R., Lens, P., Decuypere, E., Kühn, E.R. and Vandesande, F., 1987. General and Comparative Endocrinology 68, 408-414.

    Based on the above described new monoclonal antibodies against chicken growth hormone, this paper revealed for the first time the existence of a glycosylated counterpart of the well-established protein form of chicken growth hormone, similar to what had been established for human growth hormone and prolactin shortly before.

D. Research support

<u>Ongoing Research Support</u>

- USDA-NRI 2008-35204-04554 – L.R. Berghman (PI) *et al.* 2008-2011

"Enhancing chicken mucosal IgA response against Clostridium perfringens toxins"

C. perfringens alpha-toxin plays a critical role in the pathogenesis of avian necrotic enteritis (NE). We propose to use in ovo administration of a non-replicating adenovirus-vectored alpha-toxin fragment that is targeted to chicken antigen-presenting cells. We hypothesize that in ovo immunization with an adenovirus-vectored truncated C. perfringens alpha-toxin fused in-frame to an agonistic CD40-targeting single chain antibody (scFv) and co-delivery of an IgA switch factor, BAFF, will induce robust C. perfringens alpha-toxin-specific IgA response and protection in a model system of poultry necrotic enteritis.

- Department of Homeland Security. HSHQDC-11-C-0016 – Mwangi, W. (PI), L.R. Berghman (Co-PI) *et al.* 2011-2013

"*Development of multi-component vaccines for African Swine Fever"*

African Swine Fever (ASF) is caused by a large enveloped double-stranded DNA arbovirus. The African Swine Fever virus (ASFV) is a highly contagious foreign animal disease pathogen that causes devastating hemorrhagic fever in pigs with ~100% mortality rates. It causes major economic losses, threatens food security,

and limits pig production in affected countries. There is no vaccine against ASFV and outbreaks are mainly controlled by culling animals exposed to the disease. The contribution of my lab is the production of monoclonal and polyclonal antibodies against two candidate ASF vaccine proteins.

Completed Research Support

- USDA-NRI Y. Li (PI), L.R. Berghman et al. (Co-PI) 2007-2009

"*Biosensor for rapid, specific detection of avian influenza virus H5N1"*

The overall objective of this proposal was to develop an antibody-based biosensor for the rapid and specific detection of avian influenza virus H5N1. The contribution of the Berghman lab will be the production of monoclonal antibodies and monospecific polyclonal anti-peptide antibodies against hemagglutinin (HA) 5 and neuraminidase (NA) 1.