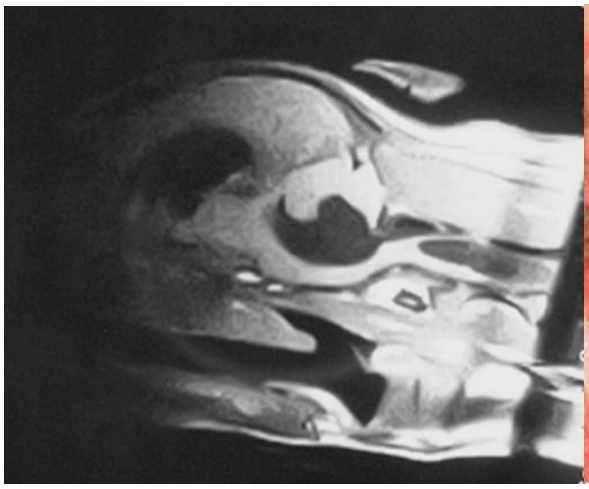
# Update Veterinary Science from Scotland and Hokkaido

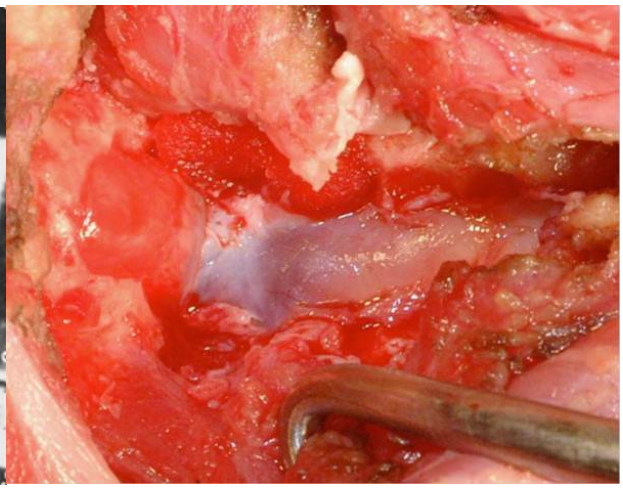
**Date : 13:30-17:00, November 27 (Tue), 2012**

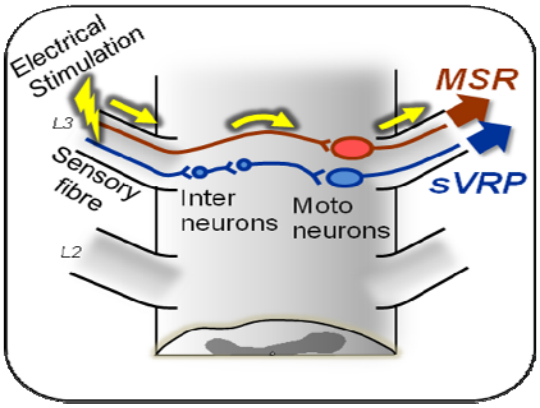
**Venue : Lecture Hall, Graduate School of Veterinary Medicine, Hokkaido University**

VIA VERITAS VITA

Hokkaido University,
Program for Leading Graduates Schools
Fostering Global Leaders in Veterinary
Science toward Contributing to "One Health"






Electrical Stimulation
MSR
L3
Sensory fibre
Inter neurons
Moto neurons
sVRP
L2

The 4rd International Symposium

Program for Leading Graduate Schools

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13:30 - 13:40 *Welcoming address*
Professor Shigeo Ito
Dean, Graduate School of Veterinary Medicine, Hokkaido University

13:40 - 14:25 ***Adenosine in the spinal cord: Contribution to CO2-evoked analgesia***
Professor Kenichi Otsuguro
Department of Biomedical Science,
Graduate School of Veterinary Medicine, Hokkaido University

14:25 - 15:10 ***Recent Topics of Veterinary Neurology***
Professor Thomas James Anderson
School of Veterinary Medicine, College of Medical,
Veterinary and Life Sciences, University of Glasgow

15:10 - 15:25 Coffee break

15:25 - 16:10 ***Role of Inhibitory Molecules in Bovine Leukemia Virus Pathogenesis and as Target for Therapy***
Professor Satoru Konnai
Department of Disease Control, Graduate School of Veterinary Medicine,
Hokkaido University

16:10 - 16:55 ***Development and application of non-invasive techniques for the assessment of airway disease in the horse***
Professor Sandy Love
Equine Clinical Studies**,** School of Veterinary Medicine, University of Glasgow

16:55 -17: 00 *Closing remarks*
Professor Kazuhiro Kimura
Vice Dean, Graduate School of Veterinary Medicine, Hokkaido University

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Student of Graduate Course, Department of Biomedical Science, Graduate School of Veterinary Medicine, Hokkaido University

・*April 1997 – March 1999*
Research Fellow of the Japan Society for the Promotion of Science.

・*April 1999 – May 2002*
Researcher of Pharmacology and Molecular Biology Research Laboratories, Sankyo CO. LTD.

・*June 2002 – March 2007*
Instructor at Laboratory of Pharmacology, Graduate School of Veterinary Medicine, Hokkaido University

・*April 2007 – May 2009*
Assistant Professor at Laboratory of Pharmacology, Graduate School of Veterinary Medicine, Hokkaido University

・*June 2009 – present*
Associate Professor at Laboratory of Pharmacology, Graduate School of Veterinary Medicine, Hokkaido University

# Adenosine in the spinal cord: Contribution to $CO_2$-evoked analgesia

**Ken-ichi Otsuguro**

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Adenosine is now recognized as a neuromodulator in the CNS including the spinal cord. Adenosine modulates several cellular functions via the activation of adenosine receptors, which are divided into four subtypes ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$). The amount of extracellular adenosine is increased by various stimuli or conditions such as hypoxia or tissue damages. In this study, we investigated the influences of carbon dioxide ($CO_2$) on spinal neuronal activities, and found that adenosine is involved in the depression of synaptic transmission during hypercapnia in the spinal cord. Exposure of rats to 20% $CO_2$ (hypercapnia) but not 5% $CO_2$ decreased the pain-related behavior evoked by capsaicin (pain-producing substance), implying the inhibition of nociceptive transmission in the spinal cord. To examine the effects of hypercapnia on spinal neuronal activities, we measured the reflex potentials in the isolated spinal cord preparation of neonatal rats. The isolated spinal cords were exposed to hypercapnia by superfusing with artificial cerebrospinal fluid (ACSF) gassed with 20% $CO_2$ + 80% $O_2$ (pH 6.7). Hypercapnic acidosis (20% $CO_2$, pH 6.7) caused rapid and reversible depression of spinal reflex potentials. On the other hand, isocapnic acidosis (5% $CO_2$, pH 6.7) showed only a small depression. Hypercapnic acidosis did not inhibit the action potentials of the primary afferents and the depolarization evoked by glutamate application, suggesting that hypercapnia depressed the synaptic transmission in the spinal cord. The hypercapnic depression was partially recovered by 8-cyclopentyl-1,3-dimethylxanthine (CPT), a selective $A_1$ receptor antagonist. The application of adenosine also depressed the spinal reflex potentials, which was completely recovered by CPT. The amount of extracellular adenosine was increased during hypercapnic acidosis. This hypercapnic increase in adenosine was suppressed by homocystein thiolacton, used to trap intracellular adenosine, but not by ARL67156, an ecto-ATPase inhibitor, indicating that the adenosine accumulation was caused by the release of adenosine but not by the degradation of ATP in the extracellular space. Hypercapnic acidosis inhibited the activity of adenosine kinase in the spinal cord. These results indicate that hypercapnia causes the accumulation of extracellular adenosine and depresses synaptic transmission via the activation of adenosine $A_1$ receptors. It is suggested that hypercapnia inhibits adenosine kinase activity and thus increases the intracellular adenosine concentration, which is released into the extracellular spaces. The spinal synaptic depression during hypercapnia can partly explain the analgesic effects by $CO_2$.

## Thomas James Anderson

T. James (Jim) Anderson graduated from the Royal (Dick) School of Veterinary Studies at Edinburgh University, Scotland in 1984. He spent four years in general y veterinary practice before coming to the University of Glasgow Veterinary School, Scotland to train in small animal surgery in 1998. In 1992 he graduated Master of Veterinary Medicine for his studies on canine osteosarcoma and in 1993 was awarded the Royal College of Veterinary Surgeons Diploma in Small Animal Orthopaedics. He undertook research training in basic neuroscience which led to the award of a PhD in 1997, which was followed by a period in neuroscience research. In 1999 he returned to the clinical teaching environment to lead the small animal neurology service. In 2002 he was awarded the European Diploma in Veterinary Neurology. In 2004 he took on leadership of the small animal clinical group and in 2007 he became the Associate Head of School for Learning and Teaching. His clinical interests include Great Dane myopathy; the impact on owners of caring for a dog with epilepsy; the use of acute phase proteins in the diagnosis of inflammatory central nervous system disease; and the development of biomarkers of neurological disease.

# Recent advances in small animal neurology

**Thomas James Anderson**

**School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences,**

**University of Glasgow**

The biggest strides in small animal veterinary neurology in recent years have been through the application of new technologies, developed in other fields. The recent pace and breadth of investigation and development has been driven by the increasing sophistication of the technologies available, generally reducing costs, a culture of collaboration and a concomitant development in the rigor of peer review, both for grant applications and publications.

Generic molecular biology techniques have expanded from the focus of confirming specific candidate properties found in other species (e.g. man) to small animals through to "-omic" studies, where the full spectrum of properties can be interrogated in relation to specific circumstances. In a veterinary context geneomics is the most developed field, followed by proteomics.

The candidate gene approach has been the basis of the investigation of genetic background to diseases for many years (L-2-hydroxyglutaric aciduria, Staffordshire Bull Terrier). However, this approach is most suitable for single gene disorders and requires a priori knowledge to select the candidate. Technological developments, including the canine genome project, have facilitated prospective genome wide approaches. These approaches have led to discoveries which would have been not possible or delayed if pursued using the candidate gene approach. Such studies have revealed the genetics of a number of well recognised diseases where the expected gene has been confirmed (e.g. Lafora's disease in Wire haired dachshunds), new mutations underlying familiar diseases described (narcolepsy in Doberman's and degenerative myelopathy – DM - in multiple breeds). Breeds, a significant feature of domestic species, have been a powerful tool in genetic research. The identification of mutations associated with specific diseases has been important in developing diagnostic techniques.

However, identification of an abnormal gene associated with a disease is in some cases insufficient to confirm disease (degenerative myelopathy) and in other cases the disease may be present without the common mutation due to another mechanism (narcolepsy in Chihuahua).

A complimentary approach is to examine the proteins in biological samples as indicators of the abnormal state. The response of specific (e.g. hypocretin in narcolepsy) and non-specific (e.g. for inflammatory disease - steroid responsive meningitis-arteritis) have been of value in diagnosis and disease management of neurological conditions respectively. Prospective analysis of the proteins

in biological samples, “proteomics”, is a potentially useful tool is developing both diagnostic techniques and disease markers.

Cerebrospinal fluid (CSF) represents a biological sample with the potential as a source of proteins reflecting disease in the CNS. Studies at the University of Glasgow, School of Veterinary Medicine have confirmed that the analysis of CSF represents specific challenges in relation to its characteristics of high salt and low protein content, though these can be overcome by selective preparation. An analysis of CSF using both a candidate protein approach (based on protein in expression in amyotrophic lateral sclerosis – ALS – an orthlogue of DM; transthyretin (TTR) and cystatin C) and proteome analysis (clusterin and haptoglobin) have revealed proteins that warrant further analysis as disease markers of DM. One of these proteins (clusterin) is a novel marker that appears to be related to neurodegenerative diseases. In considering these proteins as potential clinical markers stability experiments have demonstrated that sample handling can significantly influence concentrations in the serum.

Neurosurgery has also benefited through the application of techniques developed in other areas e.g. orthopaedics and implant technology. The development of fixator and novel plate systems for complex fractures has had applications in small animal spinal surgery. The adoption of new artificial disc technology for canine “wobblers” has the potential to overcome the issues associated with the previous techniques used for vertebral distraction. A project at the University of Glasgow, Veterinary School on the surgical management of thoracic hemi-vertebrae in the pug (Professor Jacques Penderis) has been possible because of the developments in plate technology, the advances in the management of post operative thoracotomy patients (specifically pain)and the acceptance that airway surgery may be required as a prerequisite to definitive management. This approach has required the development of multidisciplinary teams to manage these patients.

In conclusion, many of the developments in the field of small animal neurology are occurring because neurologists are looking out with their speciality and adopting and adapting techniques to the requirements of the understanding of the diseases of their small animal patients.


## Satoru KONNAI

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PhD. 09/2003 (Hokkaido University)

**Research and training:**

| | |
|---|---|
| 04/1999 - 03/2003 | Graduate student at the Laboratory of Infectious Diseases, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University and RIKEN Institute, investigating on virology and immunology of bovine leukemia virus. |
| 04/2003 -11/2003 | Research associate at the Graduate School of Veterinary Medicine, Hokkaido University, working on virology and immunology of bovine leukemia virus |
| 12/2003 – 11/2004 | Postdoctoral researcher at the Graduate School of Veterinary Medicine, Hokkaido University, working under Professor M. Onuma on anti-tick vaccine development. |
| 12/2004 – 11/2008 | Assistant Professor at the Graduate School of Veterinary Medicine, Hokkaido University, working under Professor K. Ohashi on bovine infection immunity |
| 12/2008 – present | Associate Professor at the Graduate School of Veterinary Medicine, Hokkaido University, working under Professor K. Ohashi on bovine infection immunity |

**Current research :**

Since enormous economic losses of livestock are caused by those infections on a global scale, establishment of effective preventive measures to control these infections is the most important subject to maintain maximum food production in the restricted spaces we have on the earth. For these reasons, my research activities are concentrated on systematic analysis of the pathogenesis of viral, protozoan, rickettsial and vector-borne infections of domestic animals. In addition, I am currently developing the diagnostic methods of the intractable diseases, and novel vaccine (anti-tick vaccine) or therapeutic strategies (using antibody or recombinant protein) against the diseases.

Publication lists


1. Konnai S., Suzuki S., Shirai T., Ikebuchi R., Okagawa T., Sunden., Y, Mingala CN., Onuma M., Murata S., Ohashi K. 2012. Enhanced expression of LAG-3 on lymphocyte subpopulations from persistently lymphocytotic cattle infected with bovine leukemia virus. *Comp. Immunol. Microbiol. Infect. Dis*., (*in press*).

2. Konnai S, Yamada S, Imamura S, Nishikado H, Githaka NW, Ito T, Takano A, Kawabata H, Murata S, Ohashi K. 2012. Identification of TROSPA homologue in *Ixodes persulcatus*, Schulze, the specific vector for human Lyme borreliosis in Japan. *Ticks Tick borne dis.,* (*in press*).

3. Mekata H, Konnai S, Mingala CN, Abes NS, Gutierrez A, Dargantes AP, Witola WH, Inoue N, Onuma M, Murata S, Ohash K. 2012. Kinetics of regulatory dendritic cells in inflammatory responses during *Trypanosoma evansi* infection. *Parasite Immunol*., 34: 318-329.

4. Okagawa T, Konnai S, Ikebuchi R, Suzuki S, Sunden Y, Onuma M, Murata S, Ohashi K. 2012. Increased bovine Tim-3 and its ligand expressions during bovine leukemia virus infection. *Vet Res*. 43:45.

5. Konnai S, Nishikado H, Yamada S, Imamura S, Ito T, Onuma M, Murata S, Ohashi K. 2011. Molecular identification and expression analysis of lipocalins from blood feeding taiga tick, *Ixodes persulcatus* Schulze. *Exp. Parasitol.*, 127: 467-474.

6. Ikebuchi R, Konnai S, Shirai T, Sunden Y, Murata S, Onuma M, Ohashi K. 2011. Increase of cells expressing PD-L1 in bovine leukemia virus infection and enhancement of anti-viral immune responses *in vitro* via PD-L1 blockade. *Vet. Res*., 42: 103.

7. Shirai T, Konnai S, Ikebuchi R, Okagawa T, Suzuki S, Sunden Y, Onuma M, Murata S, Ohashi K. 2011. Molecular cloning of bovine lymphocyte activation gene-3 and its expression characteristics in bovine leukemia virus-infected cattle. *Vet. Immunol. Immunopathol*., 144: 462-467.

8. Mingala C.N, Konnai S, Ikebuchi R, Onuma M, Ohashi K. 2011. Characterization of CTLA-4, PD-1 and PDL-1 of swamp and riverine type water buffaloes. *Comp. Immunol. Microbiol. Infect. Dis*., 34: 55-63.

9. Ikebuchi R, Konnai S, Sunden Y, Onuma M, Ohashi K. 2010. Molecular cloning and expression analysis of bovine programmed death-1. *Microbiol. Immunol*., 5: 291-298.

# Role of Inhibitory Molecules in Bovine Leukemia Virus Pathogenesis and as Target for Therapy

**Satoru KONNAI, Shiro MURATA, Kazuhiko OHASHI**

**Laboratory of Infectious Diseases, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, 060-0818, Japan**

Bovine leukemia virus (BLV), a retrovirus related to human T-cell leukemia virus types 1 (HTLV-1), causes enzootic bovine leucosis (EBL). Recently, the cases of BLV-induced EBL and infected cattle have been increasing in Japan, a worrisome trend given that there is no effective treatment and vaccination. The latter could be attributed to the lack of sufficient understanding of immunological mechanisms leading to immune evasion. During BLV-infection especially at the persistent lymphocytosis and lymphoma stages, T-cell dysfunction characterized by impaired cell proliferation and downregulation of Th1 cytokines accelerates disease progression through mechanisms yet to be elucidated.

Recent work in our laboratory has revealed that inhibitory receptor molecules (Programmed death-1, Programmed death-ligand-1, T cell immunoglobulin domain and mucin domain-3, Lymphocyte activation gene-3, Cytotoxic T-lymphocyte antigen-4) plays a critical role in immune exhaustion and disease progression in BLV-infection, and blocking the inhibitory pathways *in vitro* increases cytokine responses and enhances function leading to a decrease in the viral load. Therefore, such an evaluation of inhibitory receptor expression kinetics is essential to improve the design of an effective immunotherapy that can induce cell-mediated immune responses. Presently, we focus on develop a novel vaccine or therapeutic method against BLV infection, perhaps using anti-inhibitory molecule chimeric antibodies or a recombinant Fc fusion proteins.

In domestic animals including cattle, there are still many intractable diseases with poor-prognosis because of lack of effective treatment and vaccination. A deeper understanding of the inhibitory molecule pathway in domestic animals will facilitate the elucidation of events leading to immune dysfunction during the progression of incurable diseases including BLV-infection. Studies are underway to evaluate the possible clinical application of the Programmed death-1/ Programmed death-ligand 1 blockade as a novel strategy to control bovine diseases using the BLV infection model.

**Sandy Love**

- **Professor of Equine Clinical Studies** (Large Animal Clinical Sciences and Public Health)
- **Associate Academic** (Institute of Biodiversity Animal Health and Comparative Medicine)

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Following graduation from The University of Glasgow Veterinary in 1982, Sandy Love worked in a mixed veterinary practice in Yorkshire prior to undertaking a Fellowship in Veterinary Surgery at The University of Bristol. Since 1985 he has been at The University of Glasgow, initially as a Horserace Betting Levy Board Research Student and then as an equine medicine clinician. In 1995, he was appointed to the Chair of Equine Clinical Studies. He is clinical director of the Weipers Centre Equine Hospital and Post Graduate Convener at The School of Veterinary Medicine. He has over 120 peer-reviewed publications as well as over 20 contributions to veterinary textbooks. He is a former President of the British Equine Veterinary Association.

# Development of non-invasive techniques for assessment of respiratory disease in the horse.

**Sandy Love**

**A. G. Whittaker, T. D. H. Parkin, C. Wyse, M. Duz, M. P. Cathcart and K. J. Hughes**

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Background

Over the last decade, interest has developed in the analysis of volatile gases in exhaled breath (EB) and non-volatile molecules within exhaled breath condensate (EBC) of man and animals. Examples of equine research using exhaled breath analysis include investigations into respiratory physiology and the diagnosis of respiratory disease. In humans, increased concentrations of several EB/EBC biomarkers have been found in asthma, chronic obstructive pulmonary disease and cystic fibrosis. These studies have established the value of non-invasive, reliable and repeatable approaches to investigate and monitor lower airway inflammation (LAI). Collection and analysis of EB/EBC is simple, although the application and interpretation in human research has been confounded by methodological differences between studies. Additionally, EB/EBC may be influenced by physiological variables, respiratory dynamics and environmental factors which may result in large intra- and inter-subject variability for many biomarkers.

The gaseous phase of EB represents a mixture of approximately 3000 volatiles within the airways. Most studies of EB have investigated the detection of nitric oxide (NO), carbon monoxide (CO), ethane and pentane.

The epithelial lining fluid of the lungs contains numerous non-volatile substances which can be transported as aerosols in EB. Aerosols can be collected by cooling EB to form a liquid or frozen exhaled breath condensate (EBC). In veterinary species, the majority of studies on EBC have focused on hydrogen peroxide ($H_2O_2$), pH and leukotriene B4.

Compared with conventional methods (endoscopy, bronchoalveolar fluid cytology) of assessing respiratory disease there are advantages and limitations of collection and analysis of exhaled breath and exhaled breath condensate:

***Advantages***

- Non-invasive
- Simple to collect samples
- Samples can be collected outside of the clinic/laboratory, e.g. in the horse's normal stabling
- Does not influence the local environment of the respiratory tract
- Applicable for longitudinal sampling
- Useful for monitoring disease progression and response to treatment

***Limitations***

- Lack of standardisation of methodology
- Sophisticated analytical equipment required for many compounds in EB
- Biomarker concentrations are often centred around the lower detection limit of the analytical equipment
- Anatomical origin and contribution of biomarkers is unknown
- Poorly defined reference ranges for many biomarkers
- Variable dilution of solutes in EBC
- Commercially available collection devices are not available for veterinary use
- Concentrations of some biomarkers are dependent on respiratory flow rates, collection temperature and humidity
- Environmental contamination (particularly relating to the hydrocarbon gases) must be taken into account
- Many biomarkers in EBC are highly labile, requiring storage at -80 °C

**Reasons for the development of non-invasive techniques for use in the horse**

Lower airway inflammation is common in the horse as a feature of various diseases including recurrent airway (RAO), summer-pasture associated RAO, inflammatory airway disease and exercise induced pulmonary haemorrhage. Diagnosis and monitoring of these conditions often require tracheal endoscopy and bronchoalveolar lavage (BAL), which are invasive and frequently require animal sedation incompatible with regulatory issues in competition horses. Moreover, BAL *per se* may induce airway inflammation and so confound interpretation of sequential samples. A series of studies were performed in groups of Welsh Mountain ponies and Thoroughbred horses with a view to developing novel non-invasive techniques for assessment of respiratory disease.

**Summary of findings**

From the series of studies on exhaled breath and EBC in the horse it was shown that:

- Pentane, ethane and carbon monoxide are detectable biomarkers in equine EB and their exhalation was correlated with respiratory inflammation
- pH of equine EBC had minimal intra- and inter subject and intra- and inter day variability
- collection temperature and breath sampler device design had significant influence on pH of equine EBC
- pH of equine EBC was lower in horses with lower airway inflammation
- there was a large intra- and inter- day variability of $H_2O_2$ concentration in equine EBC
- exhaled nitric oxide could not be consistently measured in equine EB
- exhaled carbon monoxide (CO) could be consistently measured and the concentration of CO in EB was associated with environmental temperature
- pH of equine EBC decreased after ridden exercise and was not affected by environment temperature.

**Conclusions**

Identification and measurement of biomarkers in EB and/or EBC may provide opportunities for both physiological and pathological investigation of the lower airways in the veterinary species. The simple and non-invasive nature of breath sample collection holds attraction for the contribution of these techniques as a monitoring tool to assess progression and/or response to treatments of various disorders of the lower respiratory tract in the horse. At the current time, it seems unlikely that EB/EBC analysis will be appropriate for clinical diagnostics of individual diseases, ie they have insufficient sensitivity/specificity. Before exhaled biomarkers can be applied as clinical research tools, the many influences of collection and analytical methodologies, environmental factors, different pulmonary disease states and animal physiological parameters on biomarker concentrations require further robust validation.

To date, pH of equine EBC appears to represent the most appropriate bio marker for assessment of lower airway disease in the horse.