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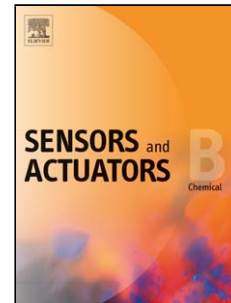
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ELECTRONIC NOSE RESPONSES AND ACUTE PHASE PROTEINS CORRELATE IN BLOOD USING A BOVINE RESPIRATORY INFECTION

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Abstract

This study aimed (i) to assess the ability of electronic nose (e-nose) technology to differentiate between blood samples of experimentally infected and non-infected subjects, and (ii) to evaluate e-nose responses given by volatile organic compounds in relation to the acute phase reaction generated in the host. In an animal model of gram-negative bacterial infection (20 calves; intratracheal inoculation of *Mannheimia haemolytica* A1), the concentrations of the acute phase proteins (APPs; i.e. lipopolysaccharide binding protein and haptoglobin) were measured in serum samples before and after challenge, and headspaces of pre- and post-inoculation serum samples were analysed using a conducting polymer based e-nose. Significant changes of certain e-nose sensor responses allowed discrimination between samples before and after challenge. The maximal changes in responses of sensitive e-nose sensors corresponded to the peak of clinical signs. Significant correlations linked decreasing responses of multiple e-nose sensors to increasing concentrations of APPs in the peripheral blood.

Keywords: electronic nose, acute phase proteins (APP), animal model, gram-negative bacterial infection, *Mannheimia haemolytica* A1, host response

Some results were partially shown at ISOEN 2009 (Italy, Brescia, 15-17.04.2009).

1. Introduction

From the past, it is well known that a number of infectious or metabolic processes could liberate specific odours characteristic of the disease indicating that there are significant relationships between diseases and alterations of the airborne chemicals emitted from the body. Based on this ancient knowledge, the diagnosis of infectious diseases on the basis of an altered profile of volatile organic compounds (VOCs) is a new area of clinical biochemistry and can therefore be used for the early detection of infectious diseases in both, veterinary and human medicine analysing readily collectable samples (i.e. blood, urine, exhaled breath, swabs).

In human medicine, ‘artificial’ or ‘electronic’ nose (e-nose) technology has been employed in several areas of medical diagnosis, including rapid detection of tuberculosis [1], *Helicobacter pylori* [2], bacterial sinusitis [3] and urinary tract infections [4, 5]. In veterinary medicine, the usefulness of e-nose technology was principally demonstrated for the first time in 2005 by discriminating serum samples obtained from badgers and cattle infected with *Mycobacterium bovis* from those obtained in non-infected controls [6]. The e-nose technology itself consists of an array of non-specific gas sensors. It functions in a manner similar to the olfactory system by ‘smelling’ various classes of volatile compound. To distinguish VOC mixtures, complex data analysis is then used in order to classify a sample by pattern recognition. Despite these encouraging results, there is still a lack of knowledge about the source of VOCs detected. In the mammalian body, the composition of volatile gases may change significantly because of (i) the various speeds at which different metabolic products are removed from the blood, or (ii) the production of metabolic products arising from certain diseases or infection, either from the host response or the metabolising infective organism.

The determination of acute phase proteins (APPs) is another diagnostic option that has been widely used in the last decades. The term ‘acute phase proteins’ describes a group of plasma proteins that are mainly synthesised in the liver and change in their concentrations substantially during the so called ‘acute phase reaction’, i.e. an early reaction of the host in response to many

different stimuli (such as infections, inflammation, tissue damage, immunological disorders or stress) [7]. The fact that circulating concentrations of APPs are measurable in the peripheral blood enables their use as quantifiable indicators of inflammation or infection. Over the last few decades, APPs have become biomarkers of choice in human medicine for monitoring inflammation and infection. In veterinary medicine, APPs are accepted biomarkers for infection and inflammation in experimental studies to reflect the acute phase response as an integral part of the innate immune system [8]. There is evidence that concentrations of serum APPs are related to the severity of underlying disease and therefore may act as markers of both the presence and the extent of disease [9].

Mannheimia haemolytica (formerly *Pasteurella haemolytica*) is one of the most important gram-negative bacteria isolated from the respiratory tract in cattle. Gaining access to the lungs when host defences are compromised by stress or other infections, it is known to become a significant component of enzootic broncho-pneumonia in calves [10]. Especially in fatal bovine respiratory diseases of feedlot calves, *M. haemolytica* was the most common agent identified in peracute, acute, and subacute pathologic processes [11]. Experimental models of infection have been used for decades and for many reasons; for example to understand the pathogenesis of this particular bacterium in the bovine respiratory system [12-16], to determine the effect of treatments [17-19], or to develop different vaccination protocols [20-23]. In this experimental study, calves with *Mannheimia haemolytica* infection were used as a model to assess host response under the defined condition of a gram-negative bacterial infection in a natural host. Blood samples were collected before and after experimental challenge and were analysed for both (i) the concentration of APPs and (ii) the response characteristics of e-nose sensors due to VOCs present in the headspace of blood. First, we hypothesised that an e-nose can discriminate blood samples of animals obtained after infection from those obtained before infections. Secondly, we tested whether the measurable e-nose response could be correlated with the acute phase reaction generated in the host, because nothing is currently known about the relationship

between the acute phase reaction of the host and the change in its metabolic profile of VOCs. To the best of our knowledge this is the first study demonstrating that a changed VOC profile of serum headspace correlates with acute phase reaction in the host, following a pathogenic infection.

2. Animals, material and methods

2.1 Animals

A cohort of 20 conventional cross-bred calves was used. Animals were introduced at the age of 3 to 15 days and were housed in 4 groups (each group consisted of 5 calves) under controlled conditions according to the guidelines for animal welfare in the European Union. The calves were bedded on barley/wheat straw and fed with commercially available milk substitutes and coarse meal. Water and hay were supplied *ad libitum*. Daily clinical observations (including monitoring of respiratory rate, nasal secretions, ocular secretions, rectal temperature, appetite, and body weight) were used to confirm the clinical status of calves before they were included in the experiment. All phases of the study were performed at a specialised veterinary institute (Federal Research Institute for Animal Health, Germany) under supervision of a veterinarian, and had ethical approval from the Commission for the Protection of Animals of the state of Thuringia (registration number: 23-51/99).

2.2 Experimental challenge and study design

Aged 52 ± 5 days (body weight: 64 ± 6 kg, mean \pm SD), all calves were inoculated with *Mannheimia haemolytica* biotype A serotype 1 (*M. haem.* A1). As described by Schimmel in 1987 [24], an 18-hour broth culture of *M. haem.* A1, strain 2353 (P588) was used for the experimental challenge resulting in an acute respiratory infection. Ten ml of the culture containing $1.5 - 2.0 \times 10^9$ cfu per ml were administered intratracheally per calf and on two

consecutive days (the interval between the two inoculations was 30 hours). Surviving calves were euthanized for necropsy 5 days after the first inoculation of *M. haem.* A1.

This study was designed as an intra-individually controlled study in order to decrease the number of animals used for experimental purposes for ethical reasons. Accordingly, the animals were their own controls considering the pre-infection data as a baseline. Thus, each animal was carefully and consecutively characterised before challenge (non-infected status or baseline data) as well as after challenge (infected status) by identical examinations and techniques. Daily clinical examinations were performed on each animal throughout the full study as explained above.

2.3 Collection of blood and serum preparation

Two blood samples were collected from each animal 7 days and one hour before experimental challenge. Eight blood samples were obtained post-inoculation (*p.i.*): 3 h, 6 h, 12 h, 24 h, 48 h, 3 d, 4 d, and 5 d after the first bacterial exposure. Jugular venous blood was collected using plastic syringes for serum production (S-Monovette®-Serum, Sarstedt AG & Co, Nuembrecht, Germany). Serum was harvested by centrifugation (20 minutes at 1500 g) and stored at -20°C until analysed.

2.4 E-nose headspace analyses

In this study, a conducting polymer-based electronic nose containing an array of 13 sensors was used (e-nose, ST214, Scensive Tech. Ltd., Leeds, UK). Serum headspace was sampled using the static sampling method described previously [25]. Briefly, serum was defrosted and pipetted into Nalophan bags. Polypropylene tubes were inserted into the bags which were sealed at both ends. The bag volume was 0.75 litre and the sample volume was 900 μ l. All bags were then filled with zero grade air and incubated for 15 minutes at 25°C. Time for adsorption and desorption during analyses was 20 seconds. The total time per replicate was 55 seconds. Seven replicates per sample were taken, and divergence (i.e. maximum amplitude of sensor signal in sample analysis) of replicates 3 to 5 were used for further analysis since they proved to be stable in flow [25]. All samples were blinded and analysed randomly. Ambient air temperature was kept constant at 25°C. Three replicates were used for analysis.

2.5. Analyses of acute phase proteins (APP)

Lipopolysaccharide binding protein (LBP) — Concentrations of LBP in sera were measured using an enzyme-linked immunosorbent assay (ELISA) described elsewhere [26]. Intra- and inter-assay coefficients of variance were below 10%. The detection limit of the LBP assay was 0.45 ng/mL.

Haptoglobin (Hp) — Concentrations of Hp in sera were measured by means of an ELISA as described previously [26, 27]. The coating antibody was IgG (rabbit) anti-human haptoglobin (DAKO, Hamburg, Germany). The same antibody, but conjugated with horseradish peroxidase, was used as the detection reagent. Hp-standard was a calibrated bovine blood plasma. Calibration was done with a colorimetric assay (Tridelta Development Ltds., Greystones, Co., Wicklow, Ireland) and also against purified bovine Hp isolated by affinity chromatography. The detection limit of the bovine haptoglobin ELISA was 0.8 μ g/ml and the intra- and interassay coefficients of variance were less than 11%.

2.6 Statistical analyses

Before applying any statistical test, all data were checked for normal distribution. Normally distributed data are expressed as mean \pm standard deviation (SD) while data without normal distribution are given as median and range (difference between minimum and maximum). Biological variation such as the individual calf, the infectious status (non-infected versus infected), and the time point of blood collection within the study, was also assessed for e-nose responses and APPs using mANOVA. Significant differences between datasets were identified using the multiple range test. Analysis of linear regression (Pearson) was used to identify linear correlations between two variables. Spearman rank correlation was applied when dealing with non-normally distributed data. The level of significance for all statistical methods applied was $P \leq 0.05$. Results were displayed as 'Box and Whisker' plots. Outlier values are 1.5 to 3 times of the length of a box away from the median and extreme values are further away than 3 times of the length of the box. In addition, principle component analysis (PCA) was performed based on data averaged over all individuals in order to eliminate biological variability (Matlab 2006b including the PLS toolbox version 3.5, Eigenvector Inc.)). Consequently, three data points were available per time point of the experiment. One data point represented one replicate averaged over all subjects. Raw e-nose divergences and auto-scaled data were analysed using PCA. Auto-scaling is a normalisation technique which scales all values of a column (e.g. for each sensor) by the standard deviation of each column of the matrix in order to eliminate different ranges in magnitudes between the columns.

3. Results

3.1 Clinical course of the study

After experimental inoculation, 6 of 20 calves (30%) died spontaneously before the end of the study. Four calves died between 24-48 hours, and two died between 48-72 hours after the first challenge with *M. haem.* A1.

In surviving calves, the following clinical signs were dominant: within the first 6 hours after challenge the appetite dropped markedly and the majority of animals became quiet, dull or depressed; significant increases in respiratory rates were already observed within the first 3 hours and in rectal temperature within the first 6 hours after challenge. The maximum temperature was measured 2 days after challenge (Figure 1). Both rectal temperature and breathing frequency (data not shown) remained significantly increased in the group until the end of the study. Surviving calves (n = 14) were euthanized for necropsy at 5 d *p.i.*

[INSERT figure 1 about HERE]

3.2 Acute phase proteins (APP)

Analysis of serum samples before infection (n = 40) showed concentrations of LBP of 2.4 ± 2.2 $\mu\text{g/ml}$ (mean \pm SD) and 0.02 ± 0.04 mg/mL (mean \pm SD) of Hp. After challenge with *M. haem.* A1, APPs increased significantly with the following time kinetics (Figure 2): for both LBP and Hp, the first significant increase was observed 12 hours *p.i.* Maximal concentrations were seen 24 and 48 hours *p.i.* for LBP (about 600% of baseline data) and 48 hours *p.i.* for Hp. Significantly elevated concentrations of both APPs were measurable until the end of the observation period (5 d *p.i.*). A significant relationship was found between LBP and Hp as expressed by the coefficient of Spearman's rank correlation of $r_{\text{sp}} = 0.73$ ($P < 0.0001$, n = 178).

Rank correlations between rectal temperature and APPs were $r_{sp} = 0.51$ ($P < 0.0001$, $n = 178$) for LBP, and $r_{sp} = 0.49$ ($P < 0.0001$, $n = 178$) for Hp, both indicating significant relationships between the rise in body temperature and increased concentrations of APPs in the blood.

[INSERT figure 2 about HERE]

3.3 Response of e-nose sensors

E-nose sensor responses of the complete array were analysed both in terms of changes due to the *infection status* (i.e. responses before and after experimental challenge) and over the *time course* of the study. Regarding the *infection status*, responses of sensors 3, 6 and 7 showed differences between all pre- and all post- inoculation samples (mANOVA, $P < 0.05$). Divergences of sensitive sensors became more negative after challenge.

In terms of the *time course*, responses of sensors 2 to 8, 10 and 11 showed kinetic profiles as illustrated in Figure 3 as a typical example. In general, divergences of sensitive e-nose sensors were approximately the same before and shortly after challenge, then declined and reached a minimum at days 2 and 3 *p.i.*, and increased again until the end of the study. However, responses of sensors 10 and 11 continued to decrease. This implies that there were a number of volatiles changing their concentrations in headspace in different ways during the course of infection, detected by the e-nose.

[INSERT figure 3 about HERE]

Performing PCA, no discrimination between time points were found due to the different ranges in magnitude of the sensors. After auto-scaling, a clear discrimination of time points 2 and 3 days after infection and the remaining data points were found. Principle component 1 and 2 covered in

total 92.62% of all variance. This discrimination was found on a group level when averaging over subjects and confirmed significant differences found using mANOVA and multiple range test for individual sensors without averaging.

[INSERT figure 4 about HERE]

3.4 Correlations between e-nose results, rectal temperature and acute phase proteins

As shown in Table 1, significant correlations were found between responses of e-nose sensors and the severity of the infection expressed by either APP concentrations in the blood or rectal temperature. Strongest correlations were found between e-nose responses and the concentration of both APPs. With increasing concentrations of LBP, sensors 2-4 and 6-8 became more negative and sensors 10 and 11 became more positive. As the concentration of haptoglobin increased, responses of sensors 2-8 and sensor 13 became more negative. According to most of the sensor responses, the general temporal profile after infection was inversely proportional to increasing concentrations of APPs.

The most negative responses of e-nose sensors were found 48 hours after challenge which corresponded to the highest peaks of Hp concentrations. In contrast, peaks in LBP concentrations were observed earlier, i.e. 24 hours after infection (see 3.2.). Therefore, coefficients of correlation between e-nose sensors and LBP were lower compared to those between e-nose sensors and haptoglobin.

With respect to body temperature, only the responses of sensors 8 and 9 were significantly linked to fever; showing an increase in divergence with increasing rectal temperature. Coefficients of correlation were very low; ranging between 0.09 and 0.14.

[INSERT table 1 about HERE]

4. Discussion

The purpose of this study was (i) to assess the potential of electronic nose (e-nose) technology to detect differences in the patterns of volatile organic compounds (VOCs) released from the peripheral blood in an acute respiratory infection of calves, and (ii) to evaluate e-nose signals in comparison with the acute host response and rectal temperature; both known non-specific biomarkers of infectious or inflammatory diseases.

The experimentally induced *M. haemolytica* challenge used in this study represents a bovine model of a severe bacterial infection with acute clinical illness and spontaneous deaths. Originally the study was performed to study the course of the disease and to develop potential medication but could also be used for assessing clinical symptoms and changes in headspace composition. There is evidence that bacterial lipopolysaccharide (LPS) was most likely heavily involved in the pathogenesis of acute host response because *Mannheimia* species belong to the group of gram-negative bacteria producing LPS, and because LPS binding protein (i.e. LBP) increased significantly after challenge (Fig. 2A). The fact that one third of the calves died within 3 days after exposure reveals the well known phenomenon of limited ability of calves to tolerate LPS or even a hyperresponsiveness to endotoxin that has been described by others [28].

The systemic host response to infection includes the acute-phase response (APR) which is a highly conserved, complex, and non-specific series of immunological reactions that is mediated by pro-inflammatory cytokines and results in an entire array of metabolic and physiologic changes. One characteristic feature of APR after bacterial infection is a remarkable change in concentration of APPs [29]. Many of these APPs originate from the liver and represent alterations in the intermediary metabolism during the host defence to inflammation, injury and infection [7]. Being called ‘chemicals thermometers’, APPs respond in a close relationship to disease progression or recovery [8]. In this study, LBP and Hp, both accepted as major APPs in bovines [7, 26, 30], were used in addition to clinical symptoms (i.e. rectal temperature) in assessing the severity of the disease. While LBP has been assessed in the acute phase of

experimental infection models in bovine and porcine species so far [7, 31], Hp has also been identified as an useful diagnostic tool for detecting infectious respiratory diseases in calves even under field conditions [32].

Discrimination ability of the e-nose

With the e-nose used in this experiment, clinically healthy cattle could be discriminated from challenged individuals with severe clinical symptoms. Significant differences in divergences in e-nose sensor array responses were found at the group level considering the median of groups per day. These differences obtained with sensors 3, 6 and 7 allowed discrimination between infected and non-infected individuals. Discrimination based on individual samples was not possible because of large variation across all sensors due to methodological factors and inconsistencies of the sensor surface associated with them [25]. Discrimination was also made difficult by the temporal changes in divergences over the course of the infection. Many sensors (e.g. 2 to 8, 10 and 11) showed a significant decline at days 2 and 3 after challenge while responses obtained from other time points after infection were similar to pre-infection divergences. This minimised the total differences between all pre- and post challenge samples but on the other hand, this temporal profile reflected the progression of the infection.

The fact that e-nose sensor signals were reduced (i.e. decreasing reactions in e-nose response) after challenge is in good agreement with data reported from human medicine showing the ability of an electronic nose to detect changes in the human body odour as a result of renal dysfunction by reducing multivariate sensor signals [5, 33]. However, the biochemical background for both (i) the discrimination between infection and non-infection and (ii) the temporal profile after infection still remains unclear since it could not be elucidated in this study which molecules were responsible for the changes. To clarify this point, further analytical options and techniques are required (e.g. GC-MS).

Comparison between e-nose responses and the severity of the disease

In this study, the severity of the experimentally induced bacterial infection was confirmed and characterised by clinical illness and spontaneous deaths. Body temperature and concentrations of APPs measurable in the peripheral blood provided additional quantitative assessment of the host response. Different APPs may show either an increase or a decrease in concentration following infection [7]. The two APPs included in this study, LBP and Hp, increased significantly after exposure to *M. haem. A1*. These findings confirm results from previous studies reporting an increased concentration of many APPs, including Hp and LBP, following an infection of the respiratory tract of calves with gram-negative bacteria (*Pasteurella multocida* or *Mannheimia haemolytica*) [34, 35].

LBP and Hp were positively correlated with one another in this study and expressed similar temporal profiles over the experimental period. Interestingly, the correlation between LBP and Hp was stronger ($r_{SP} > 0.7$) compared to the correlations between body temperature and each of the APPs ($r_{SP} \sim 0.5$). Nevertheless all three parameters reflected the APR in calves after bacterial infection with *M. haem. A1*, thus providing a good basis for the evaluation of responses of e-nose sensors.

The divergence of multiple sensors as a part of the e-nose array changed significantly over time comparing pre- and post challenge samples. Comparing the temporal profile of the e-nose responses with rectal temperature and APP concentrations revealed general correlations of several sensors and the surrogate markers for infection. Correlations between e-nose responses and LBP or Hp were more prominent compared to correlations between e-nose sensors and rectal temperature. However, correlation coefficients were low ($r_{SP} \leq 0.20$). This might be due to the fact that the temporal profile observed for each of e-nose signals, rectal temperature and APP concentrations were sometimes out of phase with one another, even though they were comparable in shape. Nevertheless, the correlation between the concentration of APPs as surrogate markers of

infection and e-nose sensor responses demonstrate the potential for VOC analysis by e-nose or other methods for monitoring and diagnosing infection and/or host response.

Conclusions

To the best of our knowledge, this is the first study evaluating e-nose technology by comparing responses with APPs in the peripheral blood.

E-nose analysis of the headspace of blood samples was shown to have potential for detecting animal infection. E-nose sensors allowed discrimination at the group level between pre-inoculation and post-inoculation samples in an animal model exposed to the gram-negative bacteria *M. haem. A1*. These findings warrant validation of e-noses in diagnosing different infections caused by different micro-organisms.

There are significant correlations between divergences of e-nose sensors and acute phase markers present in the blood of the infected host. The results are encouraging and provide the basis for the non-invasive detection of host responses based on the detection of VOCs. Further studies are required to identify VOCs that could be used as specific biomarkers for certain infections or diseases.

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Table 1: Summary of Spearman's rank correlation evaluating the relationship between responses of e-nose sensors and rectal temperature or blood concentrations of acute phase proteins, respectively

Responses of e-nose sensors	Rectal temperature in °C		c [LBP] in µg/ml		c [Haptoglobin] in µg/ml	
	r_{sp}	P	r_{sp}	P	r_{sp}	P
Divergence sensor 01	0.0488	0.2737	0.0220	0.6211	-0.0091	0.8391
Divergence sensor 02	0.0386	0.3871	-0.1255	0.0049	-0.1628	0.0003
Divergence sensor 03	0.0755	0.0904	-0.1304	0.0034	-0.1264	0.0046
Divergence sensor 04	0.0411	0.3564	-0.1101	0.0136	-0.1660	0.0002
Divergence sensor 05	0.0515	0.2477	-0.0723	0.1050	-0.1257	0.0048
Divergence sensor 06	0.0262	0.5563	-0.1057	0.0177	-0.1288	0.0039
Divergence sensor 07	0.0331	0.4576	-0.1069	0.0165	-0.1335	0.0028
Divergence sensor 08	0.0888	0.0465	-0.1059	0.0176	-0.1006	0.0241
Divergence sensor 09	0.1398	0.0017	-0.0093	0.8348	-0.0046	0.9185
Divergence sensor 10	0.0749	0.0930	0.1119	0.0121	0.0628	0.1590
Divergence sensor 11	0.0839	0.0597	0.1223	0.0061	0.0776	0.0817
Divergence sensor 12	0.0448	0.3148	0.0265	0.5525	-0.0427	0.3385
Divergence sensor 13	0.0047	0.9153	-0.0239	0.5915	-0.1003	0.0244

Legend to Table 1:

r_{sp} : coefficient of Spearman rank correlation.

$P \leq 0.05$ indicates significant correlations (highlighted in **bold**).

Biographies

Henri Knobloch (MPhil), studied biotechnology at the University of Applied Sciences, Jena, (Germany) and did his first degree in non-invasive analysis, method development and standardisation of H₂O₂ concentrations in exhaled breath condensate and blood. He is currently finishing his PhD at Cranfield Health/ Cranfield University (UK). His research interests are trace gas analysis and the application and evaluation of electronic nose technology as a diagnostic tool.

Wieland Schroedl (DVM) received his Doctor of Veterinary Medicine from the Leipzig University (Germany) in 1994. His professional activities in the Institute of Bacteriology and Mycology (University of Leipzig) focus on validation of new methods for the quantification of acute phase proteins in animals, the detection of specific antibodies against various microbial antigens (LPS, botulinum toxins, candida, etc.) and especially the investigation of interactions between microbes and host immune defence.

Claire Turner (BSc. Hons., Ph.D, Dip. Comp., MIBiol) completed a PhD in the Department of Chemical and Biochemical Engineering at University College, London in 1993, and since then, has had an interest in the monitoring of biological systems. She is currently a Lecturer and Head of the Volatiles Research Group, Cranfield Health, Cranfield University and has a particular interest in the potential for VOCs from body fluids to be used to diagnose disease.

Mark Chambers (BSc. Hons, PhD) obtained his Ph.D at Cambridge University and has held post-doctoral positions at Imperial College of Science, Technology & Medicine. In 1996 he joined the Veterinary Laboratories Agency, an Executive Agency of the UK Government Department for Environment, Food and Rural Affairs where he leads a team of eight scientists

working on the development of vaccines, immunological reagents, and novel diagnostic assays for tuberculosis.

Petra Reinhold (DVM, PhD) received her Doctor of Veterinary Medicine from the Leipzig University (Germany) in 1984, followed by a PhD in Veterinary Science from the University of Liege (Belgium) in 1996. Her professional activities at the ‘Friedrich-Loeffler-Institut’ (Federal Research Institute for Animal Health, Germany) focus on validation of new techniques for assessing the pulmonary function, the gas exchange, and pulmonary inflammatory mediators in healthy and infected animals and in domestic animal models of respiratory diseases. In addition, she currently teaches respiratory physiology in the veterinary professional curriculum at the Free University of Berlin, Germany.

Figure captions and legends

Figure 1: Rectal temperature measured in calves before and after an experimentally induced infection with *Mannheimia haemolytica* A1. Rectal temperature was highest 48 hours after infection.

Legend:

Box-and-Whisker Plot represents median value, 25% and 75% percentiles (box), range, outlier values (o), and extreme values (*). Different letters indicate significant differences between time points (mANOVA).

Figure 2: Concentrations of lipopolysaccharide binding protein (LBP, (A)) and haptoglobin (Hp, (B)) in serum samples collected before and after an experimentally induced infection with *Mannheimia haemolytica* A1 in calves. After infection, highest concentrations of acute phase proteins were measured 24 hours *p.i.* for LBP, and 48 hours *p.i.* for Hp.

Legend:

Box-and-Whisker Plot represents median value, 25% and 75% percentiles (box), range, outlier values (o), and extreme values (*). Different letters indicate significant differences between time points (mANOVA).

Figure 3: Changes of e-nose sensor 7 over time evaluating the headspace of serum samples obtained from calves before and after an experimentally induced infection with *Mannheimia haemolytica* A1. After infection, responses were significantly decreased and reached a minimum at days 2 and 3 *p.i.*.

Legend:

Box-and-Whisker Plot represents median value, 25% and 75% percentiles (box) and range. Different letters indicate significant differences between time points (mANOVA).

Figure 4: Principle component analysis of headspace serum samples. A clear discrimination was found between samples obtained at time points 2 and 3 (left) compared to remaining samples (right).

Legend:

Data was auto-scaled. The numbers next to data points indicate the sampling time.

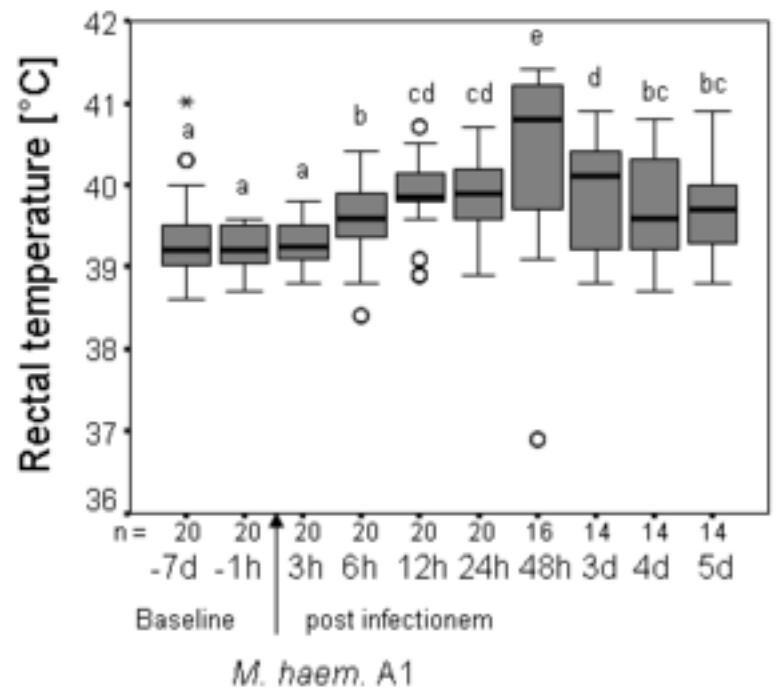
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