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Expression of xenobiotic metabolising enzymes in lungs of horses with or without histological evidence of lower airway inflammation

Maria Löfgren | Pia Larsson | Ronny Lindberg | Katarina Hörnaeus | Eva Tydén

Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence
Maria Löfgren, Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, PO Box 7028, SE-750 07 Uppsala, Sweden.
Email: maria.lofgren@slu.se

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Abstract

Mild, moderate and severe equine asthma is a problem for equine welfare. The aetiology of the disease is not known in detail but is likely multi-factorial. One important factor may be inhaled dust which carries harmful substances which may be bioactivated and thus can lead to local inflammation in the airways. The aim of this study was to investigate gene expression and protein localisation of cytochrome P450 (CYP) enzymes, superoxide dismutase and glutathione-S-transferases (GST) involved in bioactivation and detoxification of harmful substances in lungs of horses with or without histological evidence of lower airway inflammation. Significantly lower gene expression of CYP2A13 and GSTM1 was observed in lungs from horses with histological evidence of lower airway inflammation compared with horses without. A higher expression, although not significant, was found for CYP1A1 in horses with histological evidence of lower airway inflammation. There were no differences in gene expression of GSTP1 and SOD3. The proteins were localised in the respiratory epithelium which is of relevance as a defence to local exposure of inhaled harmful substances. In conclusion, our study reports differential gene expression of enzymes involved in bioactivation and detoxification of foreign substances in the lungs of horses with histological evidence of lower airway inflammation compared with horses without.

KEYWORDS
CYP, equine asthma, gene expression, GST, immunohistochemistry, SOD

1 | INTRODUCTION

Respiratory disorders represent a major problem for equine welfare and are one of the main causes of poor performance in athletic as well as pleasure horses (van Erck-Westergren, Franklin, & Bayly, 2013). The latest consensus term "equine asthma" includes horses with mild to moderate equine asthma previously referred to as inflammatory airway disease (IAD) and horses with severe equine asthma previously referred to as recurrent airway obstruction (also called heaves). Clinical signs of mild to moderate equine asthma include occasional coughing and poor performance but no increased respiratory efforts at rest. Clinical signs of severe equine asthma are regular to frequent coughing, exercise intolerance and increased respiratory efforts at rest (Couëtil et al., 2016). Remission of disease may occur in affected horses if there is a reduction in organic dusts, moulds and allergens in the stable environment. However, in severe

cases, horses are often further hyperresponsive to environmental stimuli and improvement fails (Ivester, Couëtil, & Zimmerman, 2014; Leclere, Lavoie-Lamoureux, & Lavoie, 2011; Pirie, 2014).

Several enzymatic and non-enzymatic systems cooperate in the metabolism and elimination of foreign compounds (xenobiotics; Figure 1). Phase I xenobiotic-metabolising enzymes catalyse the first step, referred to as the functionalisation, in which the substrate is modified by oxidation, reduction or hydrolysis reactions. However, compounds may also be bioactivated by phase I enzymes such as the cytochrome P450 (CYP) enzymes (Casarett & Klaassen, 2008). Bioactivation may lead to formation of reactive intermediates that can bind to DNA and in turn generate reactive oxygen species (ROS) via redox cycling (Cohen & d'Arcy Doherty, 1987; Ding & Kaminsky, 2003). ROS induce oxidative stress which amplifies the inflammation and causes tissue damage (Soffler, 2007; Figure 1).

Phase II enzymes conjugate the phase I metabolite with, for example, glutathione, sulphate or glucuronide to enable elimination. These reactions are generally beneficial as they enable detoxification and elimination of the xenobiotics from the tissues (Casarett & Klaassen, 2008).

Enzymatic defence mechanisms against oxidative stress include antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione-S-transferases (GST) and glutathione peroxidase (GPx) (Sahiner, Birben, Erzurum, Sackesen, & Kalayci, 2011; Soffler, 2007). The metabolic balance between production of reactive intermediates and detoxification reactions depends on the profile of xenobiotic enzymes (Phase I and II) in a given tissue. An imbalance between production of ROS and antioxidant activity may lead to oxidative stress and further on to inflammatory processes in the airways (Art, Kirschvink, Smith, & Lekeux, 1999; Deaton, 2006; Kirschvink, de Moffarts, & Lekeux, 2008; Nadeem, Chhabra, Masood, & Raj, 2003; Sahiner et al., 2011). Thus, inhalation of harmful substances leads to a local exposure of the cells lining the airway, resulting in a high concentration which may lead to an imbalance of the metabolic pathways.

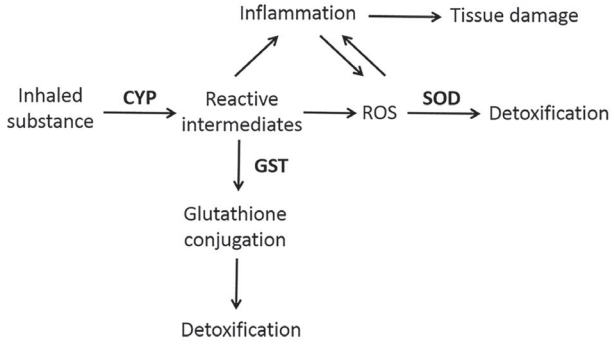

FIGURE 1 Schematic drawing of xenobiotic metabolism. Cytochrome P450 (CYP) enzymes metabolise inhaled substances (xenobiotics) into reactive intermediates which cause inflammation leading to tissue damage. Detoxification of reactive intermediates can be done through conjugation with glutathione which is mediated by glutathione-S-transferases (GST). Accumulation of reactive intermediates leads to inflammation and generation of reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$) which cause oxidative stress. Defence mechanisms include superoxide dismutase (SOD) which catalyse conversion of superoxide anions into hydrogen peroxide which can be further detoxified into water

The aetiology of mild, moderate and severe equine asthma is not known in detail and is likely multi-factorial (Couëtil et al., 2016). A correlation between equine asthma and inhalation of organic dusts is evident (Ivester et al., 2014). Studies of enzymes involved in bioactivation and defence against xenobiotics are of importance to further elucidate disease mechanisms of equine asthma. CYP enzymes belonging to families 1–3 are especially important for the bioactivation of xenobiotics and are expressed in tissues regularly exposed to foreign compounds (Ding & Kaminsky, 2003). Other enzymes such as GSTP1, GSTM1 and SOD3 participate in detoxification of reactive metabolites and have a central role in the defence against oxidative stress in the airways (Sahiner et al., 2011). Several studies in humans indicate that genetic variation and induction of the isoenzymes in the CYP family as well as GSTP1, GSTM1 and SOD3 may play a role in the onset of inflammatory processes that might lead to asthma and bronchitis (Bowatte, Lodge, Perret, Matheson, & Dharmage, 2016; Dahl et al., 2008; Gaurav et al., 2017; Korytina, Yanbaeva, Babenkova, Etkina, & Victorova, 2005; Sahiner et al., 2011).

CYP enzymes have been detected in equine airways (Larsson, Persson, Tydén, & Tjälve, 2003; Tydén, Löfgren, Hakhverdyan, Tjälve, & Larsson, 2013; Tydén, Olsén, Tallkvist, Tjälve, & Larsson, 2008). We have previously reported CYP3A-related metabolism in tissues of the upper airways (Tydén et al., 2008) and a capacity of cells in the respiratory tissues to bioactivate the mycotoxin aflatoxin B_1 in the horse (Larsson et al., 2003). However, only little is known about expression and localisation of other CYP enzymes and detoxifying enzymes in equine airways.

The overall aim of the study was to extend the knowledge about expression and localisation of phase I and detoxifying enzymes in equine airways. The study was performed on respiratory tissues from abattoir material from horses. Histological examination of the collected samples revealed that a share of the horses displayed histological evidence of lower airway inflammation. Therefore, the specific aim was to examine the gene expression and the cellular localisation of CYP1A1 and CYP2A13 and the detoxifying enzymes GSTP1, GSTM1 and SOD3 in the lungs of horses with or without histological evidence of lower airway inflammation. Our hypothesis was that there is a difference in expression and localisation between horses with or without histological evidence of lower airway inflammation. To the best of the authors' knowledge, these enzymes have not been previously investigated in horses.

2 | MATERIALS AND METHODS

2.1 | Animals

Tissue samples from the respiratory tract of 19 standardbred trotters were collected at a local abattoir. Demographic information

about the horses is presented in Table 1. The horses were examined by an official veterinarian before slaughter in accordance with European Union legislation. Controls were also carried out to ensure the horses were free of medications during the stipulated period prior to slaughter, as documented in the horse passports. The horses were stunned using a captive bolt stunner followed by immediate exsanguination to cause death. Samples were collected within 30 min of death.

2.2 | Tissue collection

Samples from the central area of the left diaphragmatic lung lobe were collected for RNA isolation, assessment of pulmonary histology and immunohistochemistry.

2.3 | Histological examination

For assessment of pulmonary histology, lung samples were fixed in 10% neutral buffered formalin. A minimum of three specimens of the piece were embedded in low-melting paraffin (56°C), cut 4 μm thick and stained with haematoxylin and eosin. The tissue sections were examined by a Diplomate of the European College of Veterinary Pathologists (RL) blinded to other results of the study.

2.4 | Preparation of total RNA

Tissue samples were collected in RNase-free plastic tubes and snap frozen in liquid nitrogen. Total RNA was prepared using NucleoSpin RNA II columns with DNAse I (Macherey-Nagel GmbH & Co.). Washing buffers and rDNAse buffer were used to remove contaminating DNA. The integrity of the RNA was verified visually using 28S rRNA and 18S rRNA on a 1% agarose gel containing 18% formaldehyde at 60 V. RNA quantification was performed according to the Quant-iT RiboGreen protocol (Molecular Probes Inc.) using a microplate reader (Wallac 1420 VICTOR2™ software version 2.0).

2.5 | Primer design

Gene-specific intron spanning primers for equine CYP1A1, CYP2A13, GSTM1, GSTP1 and SOD3 and the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed using Primer3 (Whitehead Institute) and were custom synthesised by Cybergene (Huddinge, Sweden). Nucleotide primers were designed to amplify the particular equine isoenzyme and to avoid cross-reactivity within the family. Primer sequences and accession numbers for the sequences in the horse genome are given in Table 2. The specificity of primers was verified by sequencing of

TABLE 1 Demographic information and histological examination of the horses

Horse no.	Age (years)	Gender	Histological examination		
			Bronchitis	Bronchiolitis	Classification
1	16	♀	–	–	Histo-
2	18	♀	–	–	Histo-
3	20	♀	–	–	Histo-
4	21	(♂)	–	–	Histo-
5	5	♀	–	–	Histo-
6	16	♀	–	–	Histo-
7	9	(♂)	–	–	Histo-
8	17	♀	–	–	Histo-
9	24	♂	–	–	Histo-
10	7	♀	–	–	Histo-
11	14	♀	–	–	Histo-
12	21	(♂)	++	++	Histo+
13	18	♀	+	+	Histo+
14	11	♀	+++	++	Histo+
15	18	♀	–	+	Histo+
16	20	♀	++	++	Histo+
17	12	♀	–	+	Histo+
18	23	♀	–	+	Histo+
19	23	♀	++	++	Histo+

Note: ♀, mare; ♂, stallion; (♂), gelding; -, not detected; +, mild; ++, moderate; +++, severe; Histo-, horses without histological evidence of lower airway inflammation; Histo+, horses with histological evidence of lower airway inflammation.

PCR products at the Centre for Genomic Research at the Karolinska Institute (KI-seq, Stockholm, Sweden).

2.6 | Gene expression

Analysis of the gene expression was performed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). PCR reactions were performed using the OneStep QuantiTect™SYBR® Green RT-PCR Kit (Qiagen Inc.) according to the manufacturer's protocol using 300 ng of RNA as a template in total reaction volumes of 25 μl with 12.5 μl 2x Master Mix, 0.25 μl Quantitect RT enzyme, 0.5 μM each of forward and reverse primer. Real-time PCR samples were run in a Rotor-Gene 3000 (Corbett Research) and the data were analysed using Rotor-Gene 6.1.9 software. All amplification runs were performed under the following conditions: 50°C for 30 min, 95°C for 15 min, followed by 45 cycles of 94°C for 15 s, optimal annealing temperature for 30 s and 72°C for 30 s. Melt curve analyses were performed at the end of each PCR reaction to ensure specificity of the primers. Three technical replicates were run in the PCR. To calculate the real-time PCR efficiency (*E*), a fourfold dilution series was used for each gene investigated, according to the equation = 10[−1/slope] (Schmittgen & Livak, 2008), where quantification cycle (Cq) number is plotted against logarithm RNA input. The comparative quantification of mRNA was determined from the quantification cycle (Cq) for the gene of interest and expressed in relation to the reference gene GAPDH (according to Schmittgen and Livak 2008). Expression levels of GAPDH were constant between the tissues investigated.

2.7 | Immunohistochemistry

Sections from tissue blocks of the lung used for histology were also used for immunohistochemistry. The sections were deparaffinised and rehydrated, endogenous peroxidase was quenched with 1.6% H_2O_2 in PBS, and non-specific protein binding was minimised with 10% normal goat serum in PBS. Endogenous avidin and biotin were blocked with Avidin/Biotin Blocking kit (Vector Laboratories Inc.). The tissue sections were incubated overnight at 4°C with the polyclonal primary antibodies shown in Table 3. The secondary goat anti-rabbit antibody was applied for 2 hr at room temperature. The ABC Vectastain kit (Vector Laboratories Inc.) was used to demonstrate immune reactivity. Tissue sections were developed with 3,3'-Diaminobenzidine (DAB) and counterstained with haematoxylin. For specificity controls, sections were treated as above, but the primary antibodies were replaced with non-immune rabbit serum with dilutions equivalent to those for the primary antibodies.

2.8 | Statistical methods

Statistical significance of differences in the level of gene expression in lungs from horses with or without histological evidence of lower airway inflammation was calculated using the Mann-Whitney *U*-test for non-paired data. A $p < .05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism, version 7.04, for Windows (GraphPad Software).

3 | RESULTS

3.1 | Histological examination of the lung

Demographic information and histology of the bronchi and bronchioles in the horses are presented in Table 1. Eleven of 19 horses had normal histology in all specimens (Figure 2a,c) and were classified as without histological evidence of lower airway inflammation. Eight of 19 horses had lesions consistent with chronic airway disease, with inflammatory changes in bronchi and bronchioles and classified as with histological evidence of lower airway inflammation with bronchiolitis demonstrable in all the horses (Figure 2d,e) and bronchitis in five of eight horses (Figure 2b). The extent of inflammation ranged between horses from mild to severe (Table 1). In seven of eight horses, inflammatory cell infiltrates were predominantly mixed neutrophilic to lymphocytic and plasmacytic, whereas in one of eight horses, the inflammation showed large components of eosinophils. Inflamed

TABLE 2 Accession numbers and primer sequences for CYP1A1, CYP2A13, GSTM1, GSTP1, SOD3 and GAPDH respectively

Enzyme	Accession no.	Forward primer 5′–3′	Reverse primer 5′–3′
CYP1A1	XM_023653657.1 XM_014734095.2	CCGTTATCTGCCCAACTCTG	CTTCTCGTCTGACAGCTGGA
CYP2A13	NM_001111337.1	GGGAACCGCTTTGACTATGA	GCTCCACCTTCTTGGCTATG
GSTM1	XM_001495375.6 XM_005610361.3 XM_014740196.2	TCGCAAGCACAACCTGTGT	ACCAAGGCCCTCTTCCCTAGA
GSTP1	XM_001498106.6	GCTCCCTCGGGCTGTATGGGA	AGTCCTCCTTGCCGGCCTCA
SOD3	XM_005615084.3	TCTCAGGAGCCAGCCTGCGT	TTGGCGTGCATGTCGCGGAT
GAPDH	NM_001163856.1	CAATGCCTCCTGCACCAC	GGCAGCACCAGTAGAAGCA

airways showed epithelial hyperplasia in three of eight horses (Figure 2b) and bronchiolar hyperplasia with goblet cell metaplasia in 1 of eight horses (Figure 2e).

3.2 | Evaluation of gene expression in lungs from horses with and without histological evidence of lower airway inflammation

Gene expression of CYP1A1, CYP2A13, GSTP1, GSTM1 and SOD3 in the lung was compared between horses with and without histological evidence of lower airway inflammation.

TABLE 3 Polyclonal antibodies used in the immunohistochemistry

Antibody	Company	Dilution
Primary CYP1A1 rabbit anti-human	Millipore, USA	1:2,000
Primary GSTP1 rabbit anti-human	Abcam, UK	1:400
Primary GSTM1 rabbit anti-human	Biosite, USA	1:500
Primary SOD3 rabbit anti-human/mouse/rat	Biosite, USA	1:3,000
Secondary goat anti-rabbit	Vector laboratories, USA	1:200

The gene expression of CYP1A1 in horses with histological evidence of lower airway inflammation was double that in horses without histological evidence of lower airway inflammation, although no significance was observed between the groups, $p = .08$ (Figure 3a). Horses with histological evidence of lower airway inflammation did also show larger inter-individual variation in gene expression level of CYP1A1 compared with horses without histological evidence of lower airway inflammation. Both CYP2A13 (Figure 3b) and GSTM1 (Figure 3c) showed an opposite pattern to CYP1A1 with significantly lower expression in the lung of horses with histological evidence of lower airway inflammation (CYP2A13 $p = .02$, GSTM1 $p = .04$). The largest inter-individual variation in gene expression for these two enzymes was observed in horses without histological evidence of lower airway inflammation (Figure 3b,c). There was no difference in gene expression levels in the lung of horses with and without histological evidence of lower airway inflammation for GSTP1 ($p = .9$) (Figure 3d) and SOD3 ($p = .4$) (Figure 3e).

3.3 | Immunohistochemical examination of lungs from horses with and without histological evidence of lower airway inflammation

The immunohistochemistry revealed presence of proteins reacting with anti -CYP1A1, -GSTP1, -GSTM1 and -SOD3 in horses with and without histological evidence of lower airway inflammation (Figure 4). The enzymes were preferentially expressed in surface epithelial cells in bronchi and bronchioles but with a varying intensity.

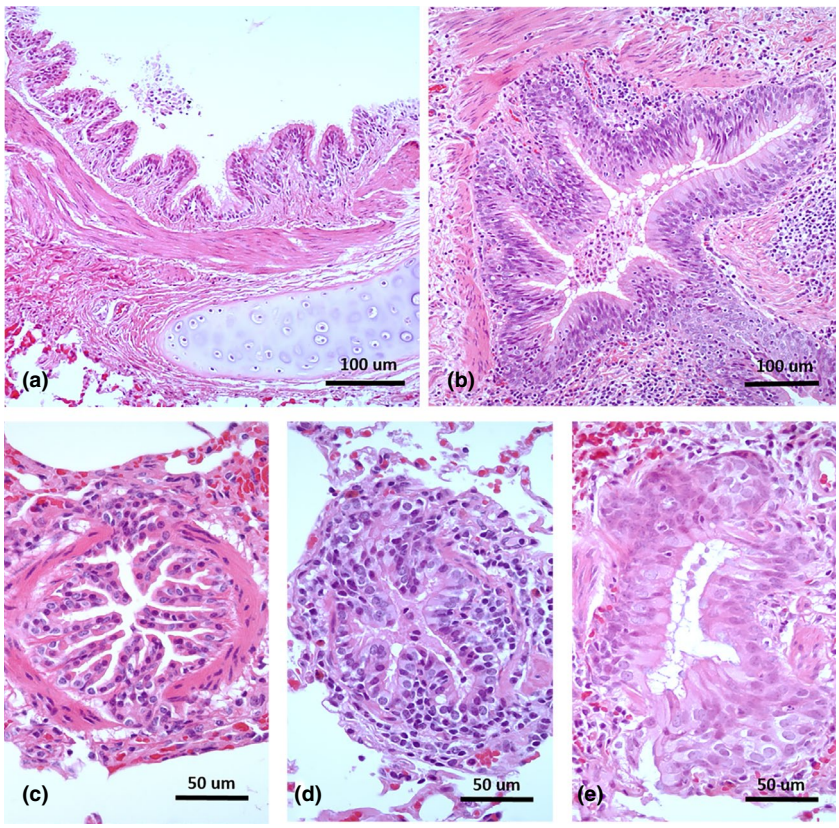

FIGURE 2 Pulmonary histology. (a) Normal bronchus, (b) chronic neutrophilic to lymphocytic bronchitis with epithelial hyperplasia, (c) normal bronchiole, (d) chronic lymphoplasmacytic bronchiolitis with normal epithelium, (e) bronchiolar hyperplasia with goblet cell metaplasia. A–E: Haematoxylin and eosin. A, B: bars = 100 μm, C–E: bars = 50 μm

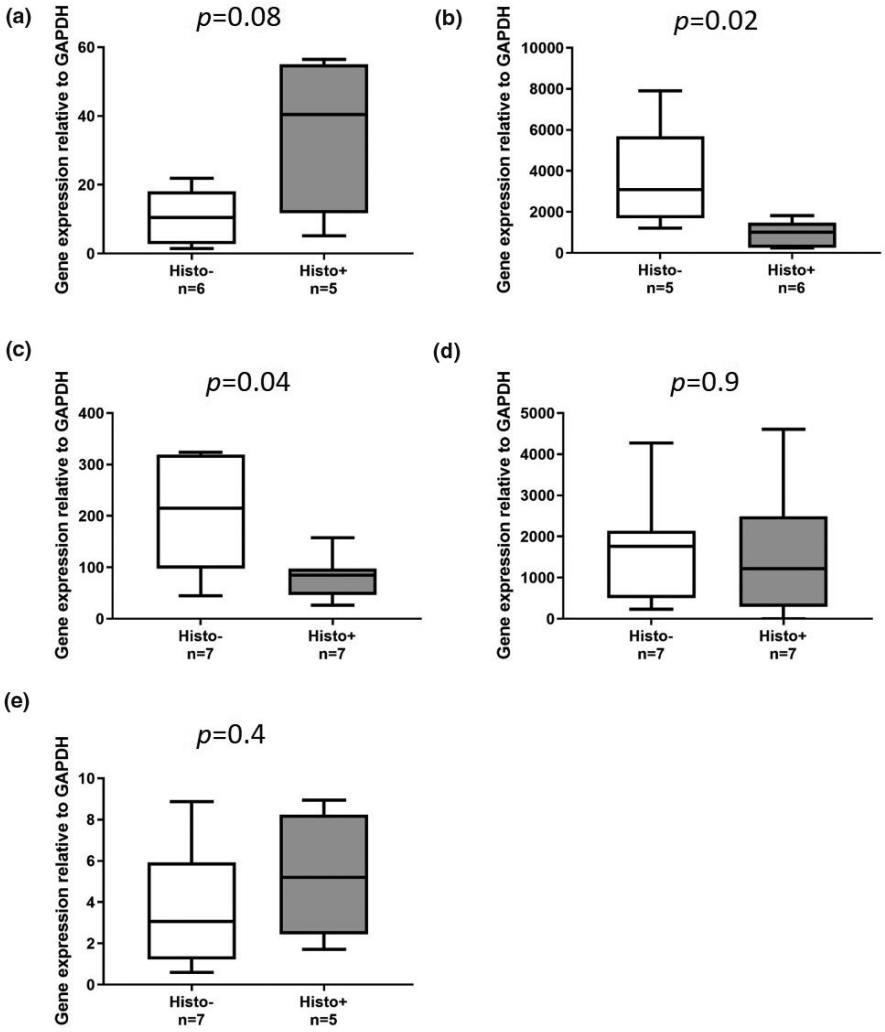

FIGURE 3 Box plots showing the level of gene expression of cytochrome P450 (CYP) enzymes (a) CYP1A1 and (b) CYP2A13, and enzymes involved in defence against toxic metabolites glutathione-S-transferases, (c) GSTM1, (d) GSTP1 and superoxide dismutase, (e) SOD3 in the lungs of horses without (Histo−) or with (Histo+) histological evidence of lower airway inflammation respectively. Gene expression is related to expression of the reference gene GAPDH (according to Schmittgen & Livak, 2008). The central horizontal line in each box represents the median, whereas the top and bottom lines represent the 25th and 75th percentiles. Whiskers indicate the min and max. *p*-values are shown above each enzyme, a $p < .05$ indicate a significant difference between horses without or with histological evidence of lower airway inflammation

In bronchiolar epithelium an intense staining was seen with CYP1A1, SOD3 and GSTM1 in some bronchiolar cells, probably representing club cells (Figure 4a–c). A similar picture was observed with GSTP1, although the staining was weaker (Figure 4d). No staining was observed in the negative controls with the primary antibodies replaced with non-immune rabbit serum. Immunohistochemistry has earlier been published for CYP 2A (Larsson et al., 2003) and therefore not included here.

4 | DISCUSSION

Our study reports expression of CYP1A1, CYP2A13, GSTM1, GSTP1 and SOD3 possibly involved in metabolism and detoxification of inhaled compounds in equine lung tissue. Protein localisation of these enzymes was observed in epithelial cells which is of relevance for metabolism of inhaled xenobiotics after local exposure.

All samples were collected at an abattoir and the clinical history of the horses was therefore not known. Further studies of horses with well-defined clinical history are needed to confirm clinical relevance of the findings in our study. The histology of 8 of 19 horses with airway lesions showed predominant chronic bronchiolitis of varying severity, some with bronchiolar hyperplasia, and with inflammatory infiltrates of neutrophils, lymphocytes and plasma cells or, in one horse, eosinophils. These morphological findings are consistent with a chronic clinical airway condition, most likely mild to moderate equine asthma formerly known as IAD (Caswell & Williams, 2007; Couëtil et al., 2016). However, there were several horses with age above 20 years, so age-related immune system changes cannot be ruled out and may have played a role in some of the histological lung changes. Differential gene expression with significantly lower expression of CYP2A13 and GSTM1 was seen

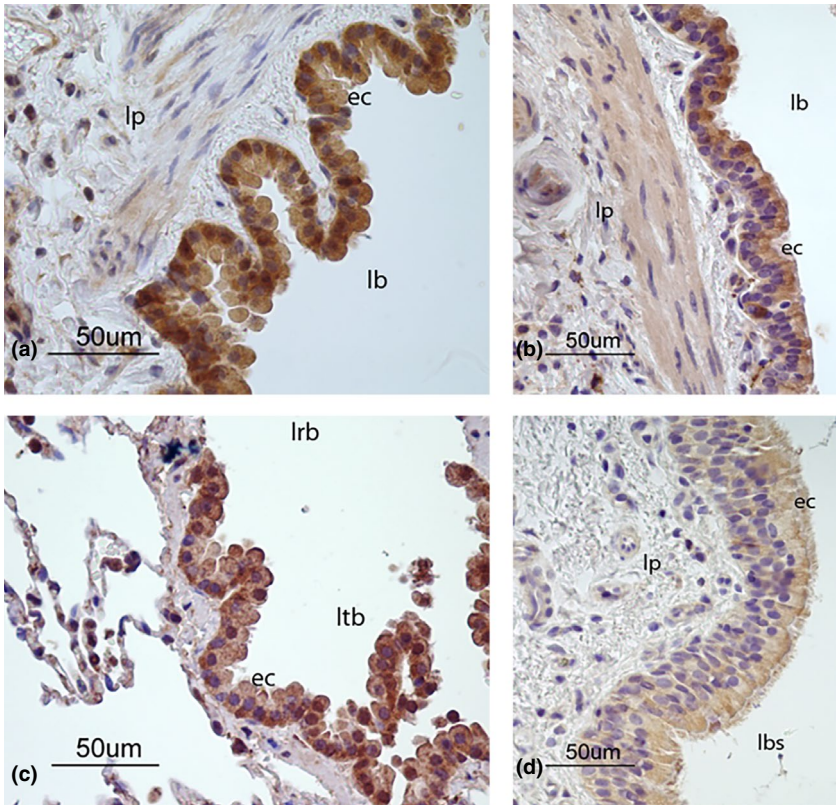

FIGURE 4 Immunohistochemical staining (brown) of (a) CYP1A, (b) SOD3, (c) GSTM1 and (d) GSTP1 in paraffin sections of horse lung tissue. ec, epithelial cells; lbs, lumen of bronchus; lb, lumen of bronchioles; lp, lamina propria; lrb, lumen of respiratory bronchioles; ltb, lumen of terminal bronchioles. Bars = 50 μm

in lungs of horses with histological evidence of lower airway inflammation compared with horses without histological evidence. Higher expression of CYP1A1, although not significantly higher, was observed in horses with histological evidence of lower airway inflammation.

Enzymes in the respiratory tract have an important role in activating or detoxifying the inhaled compounds. Local exposure may induce CYP enzymes leading to bioactivation and generation of reactive metabolites causing inflammatory responses due to tissue damage (Ding & Kaminsky, 2003). As reviewed by Aitken, Richardson and Morgan (2006) and Morgan (1997, (2001, 2009) inflammation commonly suppresses CYP enzymes in the liver but little is known about the effect of inflammation on specific CYP isoforms and tissue-specific effects. Different inflammatory stimuli such as individual cytokines are suggested to regulate specific CYP isoforms differentially and further research is needed to clarify the regulatory mechanism (Aitken et al., 2006; Morgan, 1997, 2001, 2009). Our results showing a trend towards higher expression of CYP1A1 in horses with histological evidence of lower airway inflammation indicate possible involvement of this enzyme in equine airway disease, although this has to be further confirmed. CYP1A1 induction has previously been related to inhalation of tobacco smoke in humans (Hukkanen, Pelkonen, Hakkola, & Raunio, 2002), inhalation of diesel exhaust particles in mice (Takano et al., 2002) and aflatoxin B_1 exposure of human lymphocytes and monocytes (Bahari, Mehrzad, Mahmoudi, Bassami, & Dehghani, 2014). The horses classified with histological evidence of lower airway inflammation in our study showed various degrees of inflammation with mainly neutrophilic and mononuclear cell infiltration. CYP enzymes have been studied in human IADs such as chronic obstructive pulmonary disease (COPD). Gene expression of CYP1A1 was not detected in lungs of humans with COPD but was detected in low levels in lungs of healthy humans (Berg et al., 2014). This is in contrast to our results showing a trend towards higher expression in horses with histological evidence of lower airway inflammation that might be explained as induction of CYP1A1 by substances in the stable air. The high inter-individual differences in horses with histological evidence of lower airway inflammation in our study can also be explained by different exposure and inhalation of xenobiotics in their stable environment. CYP2A13 is an extrahepatic enzyme predominantly expressed in the respiratory tract of humans. In a human lung cell line, CYP2A13 gene expression was suppressed by lipopolysaccharide (LPS) and the cytokine interleukin-6. In addition, pneumonia induced by LPS in a mouse model demonstrated a suppressed gene expression of CYP2A13 in the lungs (Wu, Liu, Ling, Lawrence, & Ding, 2013). Gene expression was previously detected in low levels in the lungs of healthy humans but not detected in humans with COPD (Berg et al., 2014). Suppression by inflammatory mediators, such as LPS and interleukin-6, might explain the lower expression of CYP2A13 in horses with histological evidence of lower airway inflammation in our study.

Oxidative stress is known to be involved in airway inflammation (Auerbach & Hernandez, 2012; Rahman & Adcock, 2006; Sahiner et al., 2011; Soffler, 2007). Protection against oxidative stress includes conjugation of reactive intermediates with glutathione which is mediated by GSTs. Expression and genetic variants of GSTs affect the capacity of detoxification (Bowatte et al., 2016). Decreased gene expression of GSTM1 and GSTP1 was found in lungs of humans with COPD (Tomaki et al., 2007). In our study, horses with histological evidence of lower airway inflammation showed lower gene expression

of GSTM1 than horses without histological evidence but no difference was observed for GSTP1. A lower gene expression of GSTM1 could indicate decreased capacity to detoxification of xenobiotics and thus decreased protection against oxidative stress.

Accumulation of reactive intermediates can lead to generation of ROS such as superoxide which induce oxidative stress. SOD catalyses conversion of superoxide into hydrogen peroxide which can be further detoxified into water (Comhair & Erzurum, 2010). Expression of antioxidant enzymes such as SOD3 determines the capacity to scavenge ROS. Decreased activity of SOD has been measured in lungs of human asthmatics (Comhair, Bhathena, Dweik, Kavuru, & Erzurum, 2000; Smith, Shamsuddin, Sporn, Denenberg, & Anderson, 1997). Overexpression of SOD3 in transgenic animal models has been shown to protect against acute lung injury induced by endotoxin (Hassett et al., 2011) and inflammation induced by hypoxia (Folz, Abushamaa, & Suliman, 1999). In the present study, horses with histological evidence of lower airway inflammation did not differ from horses without histological evidence in gene expression of SOD3.

All studied enzymes were identified in epithelial cells by immunohistochemistry. This localisation is consistent with a role in metabolism of xenobiotics due to local exposure of the respiratory epithelium to inhaled compounds (Ding & Kaminsky, 2003). A previous study from our group identified protein expression of CYP2A6/2B6 in the same locations (Larsson et al., 2003). Furthermore, that study showed covalently bound metabolites of bioactivated aflatoxin B_1 in epithelial cells of the equine tracheal, bronchial and bronchiolar mucosa. CYP1A, SOD3, GSTP1 and GSTM1 have previously been detected in the human respiratory tract epithelium (Anttila et al., 1993; Oury, Chang, Marklund, Day, & Crapo, 1994; Saarikoski et al., 1998; Terrier, Townsend, Coindre, Triche, & Cowan, 1990).

In conclusion, our study reports differential gene expression of enzymes involved in metabolism and detoxification of xenobiotics in the lungs. We observed significantly lower gene expression of CYP2A13 and GSTM1 in lungs from horses with histological evidence of lower airway inflammation compared with horses without histological evidence. Further studies of enzymes in the respiratory tract will likely increase the understanding of disease mechanisms in equine asthma.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

Maria Löfgren: Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft. **Pia Larsson:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing-review & editing. **Ronny Lindberg:** Formal analysis; Investigation; Validation; Visualization; Writing-review & editing. **Katarina Hörnaeus:** Formal analysis; Investigation; Methodology; Validation; Writing-review & editing. **Eva Tydén:** Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing.

ORCID

Maria Löfgren https://orcid.org/0000-0002-8020-3267
Ronny Lindberg https://orcid.org/0000-0003-1118-5533
Eva Tydén https://orcid.org/0000-0001-6958-2692

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