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1 **Short Communication**

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3 **Effect of inclusion or exclusion of epithelial cells in equine respiratory cytology analysis**

4

5 **Abstract**

6 There is variation amongst published studies as to whether epithelial cells are included in
7 differential counts for tracheal wash (TW) and bronchoalveolar lavage (BAL) cytology in
8 horses. The aim of this study was to determine whether inclusion / exclusion of epithelial cells
9 affects interpretation of airway cytology. Using criteria of >20% TW neutrophils, >10% BAL
10 neutrophils and >5% BAL mast cells to indicate airway inflammation, 21%, 4% and 8% of
11 horses changed from being categorised as ‘normal’ to ‘abnormal’ when epithelial cells were
12 excluded from differential counts. It is recommended that future equine respiratory research
13 studies explicitly state whether epithelial cells are included or excluded in differential counts.
14 A consensus on epithelial cell inclusion during cytology reporting is required.

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17 Keywords: horse, tracheal wash, bronchoalveolar lavage, cytology, epithelial cell

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30 There is variation amongst published studies as to whether epithelial cells are included in
31 differential counts for tracheal wash (TW) and bronchoalveolar lavage (BAL) cytology. A
32 published review of respiratory cytology from poorly performing horses revealed 4 of 6
33 studies included epithelial cells in differential counts for TW and 7 of 13 for BAL (Richard et
34 al. 2010). Utilising this published data, the mean proportion of epithelial cells was 33% for the
35 TW studies and 5% for BAL studies. In many studies specific 'cut offs' for the proportion of
36 neutrophils, mast cells and eosinophils have been used to indicate abnormal airway
37 inflammation. However, these recommendations have been determined from studies and
38 subsequently applied to studies when epithelial cells are both included and excluded in
39 differential counts (Couetil et al. 2016, Christley et al. 2001, Hodgson 2002, Malikides et al.
40 2003, da Silva et al. 2017, Sweeney et al. 1992). In other studies, it is unclear whether
41 differential counts were obtained from total nucleated cells or total inflammatory cells.
42 Knowledge of epithelial cell proportions may be of clinical value. In the UK, where TW are
43 widely performed as part of routine clinical racehorse practice, differentials including
44 epithelial cells are used in some clinics to provide recommendations for suitability to race.
45 Further research is required to improve our understanding of the circumstances which may
46 affect epithelial cell proportions; an increase in BAL epithelial cells is reported in horses after
47 exercising in cold air (Davis et al 2002) and they have been used as markers of mucosal injury
48 in humans and laboratory animals (Davis et al 2002).

49 The aim of this study was to determine whether inclusion or exclusion of epithelial cells
50 affects the interpretation of airway cytology in TW and BAL. A convenience sample of TW
51 (n=100) and BAL (n=50) cytology findings from poorly performing horses over a two-year
52 period were reviewed retrospectively. The TW study population comprised 89 Thoroughbreds
53 and 11 mixed breeds; 22 mares, 88 geldings; with a mean age of 6 years (2-14years). The BAL
54 study population comprised 44 (88%) Thoroughbreds and 6 (12%) mixed breeds; 9 mares, 41
55 geldings; with a mean age of 6 years (2-15 years). Tracheal wash samples were obtained
56 transendoscopically using 20ml sterile saline. The BAL samples were obtained from one lung
57 using 300ml sterile saline in a single bolus. Differentials had been obtained by board certified
58 pathologists from Cytospin preparations stained with modified Wright's stain. Slides were
59 examined to assess cellularity, types and morphology before performing a manual differential
60 count of 200 cells. Differential counts were obtained with respiratory epithelial cells included

61 and were later recalculated to determine proportions with respiratory epithelial cells
62 excluded. If cells were present in sheets or large clumps, these were not included, because
63 the uneven distribution of cells would have the potential to skew the differential count. For
64 the tracheal wash >20% neutrophils were used to indicate airway inflammation (Hodgson
65 2002). Earlier guidelines suggested that >5% neutrophils, >2% mast cells and >1% eosinophils
66 in BAL indicated airway inflammation. However, the most recent consensus statement
67 advised that >10% neutrophils, >5% mast cells and >5% eosinophils, regardless of BAL
68 technique indicated airway inflammation (Couetil et al. 2016), with values in-between likely
69 equivocal and technique dependent. Therefore, for the purposes of this study BAL neutrophils
70 >10% neutrophils, mast cells >5% and eosinophils >5% eosinophils were used to indicate
71 airway inflammation.

72 The median proportion of epithelial cells in the TW was 37% (Interquartile range (IQR) 15-
73 74%). When epithelial cells were included in differential counts 41% of horses had greater
74 than 20% neutrophils. When epithelial cells were excluded from the differential count a
75 further 21% of horses had greater than 20% neutrophils, resulting in 62% of horses being
76 categorised as abnormal (table 1).

77 The median proportion of epithelial cells in BAL was 6% (IQR 2-10%). When epithelial cells
78 were included 24% of horses had >10% neutrophils. A further 4% of horses changed from
79 being categorised as normal to being categorised as abnormal when epithelial cells were
80 excluded. When epithelial cells were included 6% had \geq 5% mast cells; an additional 8% of
81 horses became categorised as abnormal when epithelial cells were excluded (table 1). No
82 horse had \geq 5% eosinophils.

83 The purpose of this study was to highlight the extent to which inclusion/exclusion of epithelial
84 cells might affect interpretation of airway cytology. There was a higher proportion of
85 epithelial cells in TW samples than BAL samples, which has the potential to more greatly
86 influence interpretation. Determination of epithelial cell proportions from TW may be
87 warranted as they represent a large proportion of the normal cells. The smaller epithelial cell
88 proportions in BAL, meant that exclusion of epithelial cells resulted in a change of category in
89 a smaller number of horses than the TW.

90 Variation is apparent in the reporting of cytological findings from sputum and BAL samples in
91 humans. In sputum cytological analysis, there is a lower proportion of respiratory epithelial
92 cells (~1.5%) (Spanevello et al 2000) than in equine TW samples. Whereas the proportion of
93 epithelial cells in human and equine BAL samples is similar (Meyer et al 2012). For sputum
94 analysis squamous epithelial cells are excluded but bronchial epithelial cells are included in
95 differential cell counts (Weiszhar and Horvath 2013). Whereas guidelines for human BAL
96 reporting advise the use of differential counts for immune/inflammatory cells only (Meyer et
97 al 2012). However, it is recommended that cytology reports do separately include the figure
98 for epithelial cells as a percentage of total nucleated cells (Meyer et al 2012).

99 For equine respiratory cytology further studies are required to better understand the clinical
100 value of reporting epithelial cells for both TW and BAL. Furthermore, the repeatability of
101 epithelial cell counts warrants study, along with a consensus on how groups or sheets of
102 epithelial cells or inflammatory cells should be considered. Although their presence can
103 provide interpretative information, they were not included in the count because the uneven
104 distribution was likely to skew the differential. It has been shown that the differential counts
105 of less common cell types such as mast cells may be unreliable even at counts of 400 cells and
106 that evaluation of 5 microscopic fields at 500x magnification had improved reliability
107 (Fernandez et al 2013). The cell counts of samples included in this study were lower and the
108 recalculation without epithelial cells means that the inflammatory cells counted were further
109 reduced.

110 It is recommended that future equine respiratory research studies explicitly state whether
111 epithelial cells are included or excluded in differential counts. A consensus on epithelial cell
112 inclusion during cytology reporting is required.

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	Differential of total nucleated cells (epithelial cells included)	Differential of inflammatory cells (epithelial cells excluded)
Tracheal wash – Neutrophil proportion > 20%	41%	62%
BAL – Neutrophil proportion > 10%	24%	28%
BAL – Mast cell proportion > 5%	6%	14%

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Table 1: Shows the proportion of horses categorised with airway inflammation when epithelial cells were included and excluded in differential counts.

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151 Conflict of interest statement: None of the authors of this paper haves a financial or
152 personal relationship with other people or organisations that could inappropriately
153 influence or bias the content of the paper.

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