

# Tracheal Endoscopic and Cytological Findings and Blood Examination Results in Thoroughbred Racehorses Suspected to have Lower Respiratory Tract Disease

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*Cytology of tracheal aspirates, tracheal endoscopic and blood tests were carried out to 86 Thoroughbred racehorses presenting coughs or poor performance which were suspected to have lower respiratory tract disease (LRTD) to assess the conditions of the disorders. Racehorses were classified into coughing (66 horses) and non-coughing (20 horses) groups based on clinical symptoms. Nine Thoroughbred racehorses without respiratory abnormality were used as controls. Assessment of grades of airway mucus, cytology of tracheal aspirates and serum amyloid A (SAA), fibrinogen (Fbg) and pulmonary surfactant protein D (SP-D) measurements were performed. Relationships between age, gender and racing careers were also investigated to understand the characteristics of LRTD in racehorses. Mean age was significantly higher in non-coughing group compared to coughing group. Existence of racing career and number of starts were significantly greater in non-coughing group compared to coughing group. On the other hand, grades of airway mucus were significantly higher in coughing group compared to control group. Percentages of neutrophils in tracheal aspirates were significantly higher in coughing group compared to non-coughing and control groups. SAA, Fbg and SP-D were higher in coughing group compared to non-coughing and control groups indicating that condition of coughing group is in the acute phase. Positive rate of inflammatory airway disease was significantly higher in coughing and non-coughing groups compared to control group. It was concluded that carrying out comprehensive evaluation including investigation on SAA, Fbg and SP-D analysis with airway assessment to Thoroughbred racehorses which were suspected to have LRTD are useful procedure to understand the pathological condition which aid to initiate appropriate treatment, prognosis judgment or to advise trainers to consider altering training regimen.*

**Key words:** cough, inflammatory airway disease, lower respiratory tract, poor performance, Thoroughbred racehorse

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## Introduction

Respiratory tract diseases are second most common cause of poor performance in racehorses after

musculoskeletal disease [12, 14, 16]. Amongst respiratory tract disease, lower respiratory tract disease (LRTD) and inflammatory airway disease (IAD) have been discusses as a common cause of poor performance, interruption of training and premature retirement in racehorses [3, 5, 13, 15]. LRTD is characterized with increased tracheal mucus accumulation and prolonged episode in average of 15.5 days [13]. Although its aetiopathogenesis is not fully

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**Table 1.** Gender and age of studied Thoroughbred racehorses

Group	Gender			Ratio	Age (Years old)
	Male	Female	Total		
non-coughing	13	7	20	1.86	3.3 ± 0.9 <sup>a</sup> (2–5)
coughing	37	29	66	1.28	2.6 ± 1.0 <sup>a</sup> (2–7)
control	5	4	9	1.25	2.9 ± 1.8 (2–7)
Total	55	40	95	—	—

Gender: Geldings are included in male. Ratio: Ratio of male to female. <sup>a</sup> Significant differences among same alphabet (P<0.01). Age is indicated as mean ± SD (range).

understood, it have been indicated that infection plays an important role [3, 4, 17]. On the other hand, IAD is a syndrome, more tightened definition of LRTD which affects young racehorses and clinical presentation of IAD include cough, accumulation of secretions in trachea, nasal discharge, poor performance and prolonged recover after exercise. Horses with IAD have increased proportions of inflammatory cells, in particular neutrophils, mast cells and/or eosinophils in airway secretions obtained by tracheal aspirates or bronchoalveolar lavage (BAL) [4, 5, 8]. Many research has been undertaken regarding etiology and risk factors of IAD and LRTD [5, 13, 15], however, treatment decisions are still determined largely by clinician/client experience, anecdotal and economic factors. Therefore, effective management such as specific treatment regimes and encompassing drug use are longed in the clinical practice.

Racehorses suspected to have LRTD can be clinically classified into two groups, coughing group and non-coughing group. If any scientific differences could be found between these clinical symptoms, it will provide useful information to assess the conditions or to evaluate the prognosis of the disease. The purpose of this study is to assess the usefulness of tracheal endoscopic and cytological findings, measuring serum amyloid A (SAA), fibrinogen (Fbg) and pulmonary surfactant protein D (SP-D) to understand the conditions and the characteristics of LRTD in racehorses.

## Materials and Methods

### *Classification of studied racehorses*

Eighty-six Thoroughbred racehorses which involvement of upper respiratory tract abnormality was strongly denied by endoscopy at rest and suspected to

have LRTD were used in this study. Nine Thoroughbred racehorses without respiratory abnormality were used as controls (Table 1). All 95 racehorses in this study had been stabled at the facility managed by Japan Racing Association at times, which no epidemic infectious diseases had occurred.

Eighty-six Thoroughbred racehorses were classified based on major complaint and clinical symptoms into two groups, coughing group and non-coughing group representing clinical LRTD using medical data from Japan Racing Information System (JARIS).

Racing careers were also searched by the same system. Survey items were existence or nonexistence of racing career prior to examination, number of starts prior to examination, days from the latest start prior to examination, and days from examination to nearest start after examination. The data of control group were eliminated from the racing career survey since none of the racehorses had any starts at the time of survey.

### *Endoscopic examination of trachea and tracheal aspirates*

Video endoscopy of trachea was performed with restraint provided by nose twitch more than 2 hr after the training. Endoscopic findings of trachea were evaluated as previously reported for tracheal mucopus (TM) into following grading system [6]; Grade 0: none of findings, Grade 1: seed mucus near the rima glottides, Grade 2: continuous mucus by width of less than 15 mm, Grade 3: continuous mucus by width of more than 15 mm. Tracheal aspirates were collected via video endoscope forceps channel with 30 ml of 0.9% sterile saline solution. Slide preparations were made by cytocentrifugation fluid (Shandon Cytospin<sup>®</sup>4 Cytocentrifuge, Thermo Electron Corporation, U.S.A.) with 1 ml of sample treated with cytologic fixative fluid, Cytospin collection fluid (Thermo Electron Corporation, U.S.A.). Prepared slide was stained by stain solution (Diff-Quick 16920, Sysmex, Hyogo) and differential cell counting was performed. Two

**Table 2.** Racing careers of studied Thoroughbred racehorses

Group	Racing career		Number of starts (Starts)	Days from the latest start (Days)	Days to nearest start (Days)
	More than one start (%)	None (%)			
non-coughing	65.0 <sup>a</sup>	35.0 <sup>b</sup>	6.7 ± 8.0 <sup>c*</sup>	44.5 ± 58.7	144.3 ± 177.4
coughing	34.8 <sup>a</sup>	65.2 <sup>b</sup>	2.7 ± 6.0 <sup>c</sup>	40.1 ± 76.2	91.4 ± 134.6

Racing career: Existence or nonexistence of racing career prior to examination. Number of starts: Numbers of starts prior to examination. Days from the latest start: Mean days from the latest start prior to examination. Days to nearest start: Days from examination to nearest start after examination. <sup>a,b</sup> Significant differences among same alphabet (P<0.01). <sup>c</sup> Significant differences among same alphabet (P<0.05). \*Data are indicated as mean ± SD.

hundred cells on representative area were counted, and percentages of neutrophils, lymphocytes, eosinophils, macrophages and respiratory tract epithelial cells were recorded. Horses with > 20% neutrophils in tracheal aspirates were diagnosed as IAD [4].

#### Blood examination

Blood samples were collected from jugular vein at the time of endoscopy. Collected bloods were conventionally prepared for plasma and serum. Fbg, SAA and SP-D concentrations were obtained from plasma and serum, respectively, as previously described [2, 7, 9].

#### Statistical analyses

$\chi^2$  test was used to analyze grades of TM and ratio of male to female, Wilcoxon signed-ranks test was used to analyze data between two groups and Kruskal-Wallis test and Scheffe's F test was used to analyze data among three groups. Value of P<0.05 were considered significant.

## Results

#### Classification of studied racehorses

Sixty-six racehorses and 20 racehorses representing clinical LRTD were classified into coughing group and non-coughing group based on major complaint and clinical symptoms, respectively (Table 1). Nine racehorses without respiratory abnormality were used as control group.

#### Gender and age

Ratio of male to female was higher in non-coughing group compared to coughing or control groups,

however, the difference was not significant (Table 1). Mean age was significantly higher on non-coughing group compared to coughing group (Table 1).

#### Racing careers

Racing career among non-coughing group (65.0%) was significantly higher (P<0.01) compared to those of coughing group (34.8%) (Table 2). Numbers of starts prior to examination were significantly higher (P<0.05) in non-coughing group (6.7 ± 8.0 starts) compared to coughing group (2.7 ± 6.0 starts). Days from the latest start prior to examination and days from examination to nearest start after examination were greater in non-coughing group compared to coughing group, however, the difference was not significant (Table 2).

#### Endoscopic examination of trachea

Grades of TM were higher in order of coughing group, non-coughing group and control group (Table 3). There was significant difference between coughing group and control group (P<0.01) (Table 3).

#### Tracheal aspirates

Percentages of neutrophils in tracheal aspirates were significantly higher (P<0.01) in coughing group compared to non-coughing and control groups (Table 4). Positive rate of IAD was significantly higher (P<0.01) in coughing group (91.8%) and non-coughing group (60.0%) compared to control group (0.0%) (Table 4).

#### Blood examination

SAA, Fbg and SP-D of coughing group showed higher values compared to other two groups, however, the differences were not significant (Table 4). The horse that clinical condition was severe tended to be high value in blood examination.

**Table 3.** Relationship of the prevalence of TM to each group

Group	Total	Grade				P-value ( $\chi^2$ test)
		0	1	2	3	
non-coughing	20	4	10	4	2	
coughing	66	5	22	23	16	a
control	9	4	4	1	0	a

Data are expressed number of horses. <sup>a</sup> Significant differences among same alphabet (P<0.01).

**Table 4.** Results of tracheal aspirates and blood tests of studied Thoroughbred racehorses

Group	Tracheal aspirates		SAA ( $\mu\text{g}/\text{ml}$ )	Fbg ( $\text{mg}/\text{dl}$ )	SP-D ( $\text{ng}/\text{ml}$ )
	Neutrophils (%)	IAD positive (%)			
non-coughing	31.0 $\pm$ 16.5 <sup>a*</sup>	60.0 <sup>c</sup>	0.5 $\pm$ 0.6	255.3 $\pm$ 68.1	0.6 $\pm$ 2.4
coughing	52.1 $\pm$ 25.3 <sup>a,b</sup>	91.8 <sup>d</sup>	52.9 $\pm$ 156.3	311.7 $\pm$ 123.2	4.9 $\pm$ 12.3
control	12.3 $\pm$ 2.9 <sup>b</sup>	0.0 <sup>c,d</sup>	0.2 $\pm$ 0.4	254.1 $\pm$ 20.0	0.0 $\pm$ 0.0

<sup>a-d</sup> Significant differences among same alphabet (P<0.01). \*Data are indicated as mean  $\pm$  SD.

## Discussion

To seek the usefulness of endoscopic examination, cytologic examination of tracheal aspirates, and measuring SAA, Fbg and SP-D to assess the conditions and the characteristics of LRTD in racehorses, studied racehorses were classified into coughing group and non-coughing group based on major complaint and clinical symptoms, respectively. Since coughing is known to be a relatively specific indicator of LRTD and are potential causes of pneumonia, pleuritis, viral and bacterial respiratory infections, exercise-induced-pulmonary-hemorrhage and chronic obstructive pulmonary disease [1, 10, 13, 16], the authors speculated that the pathological condition in horses presenting coughing may be different to horses which are not presenting cough. Therefore, studied horses were classified in to two groups to clarify the difference of these pathological conditions, which are important in clinical practice. Also, investigation on the differences of age, gender and racing careers between the groups were carried out since we expected that it would provide useful information regarding horseracing practice.

In the comparison among gender, ratio of male to female was higher in non-coughing group compared to coughing group, however, the difference was not significant. Since the ratio of male and female in Japan Racing Association registered racehorses are approximately 1.3:1.0, the ratio of male in the non-coughing group is considered to be relatively high. In

addition, our results that mean age was significantly higher on non-coughing group compared to coughing group supports the previous reports that ratio of male are higher in elder horses and airway inflammation is most common in young horses [4, 13].

On the other hand, ratio of racehorses with racing career among non-coughing group was significantly higher compared to those of coughing group. In addition, numbers of starts prior to examination were also significantly higher in non-coughing group compared to coughing group. This result was considered to attribute to the higher age of non-coughing group compared to coughing group.

Grades of TM were higher in order of coughing group, non-coughing group and control group, however, there was significant difference only between coughing group and control group. This result suggests that more mucus exist in the trachea of racehorses in the coughing and non-coughing groups compared to control group and the grade of tracheal mucus relate with clinical symptom.

In this study, we selected tracheal aspirates to evaluate LRTD because less invasive method was preferable for all of the studied racehorses prepared to race. The use of BAL technique is limited in the field since collected sample only reflects the small area of the lung and videoendoscope over 3 m length with local anesthesia is required. On the other hand, tracheal aspirates can evaluate broad condition of the LRTD without local anesthesia via normal endoscope. During tracheal aspirates, any difficulties such as

neither excitement nor breakage of the equipment were observed. It was confirmed that tracheal aspirates is a safe technique to apply to Thoroughbred racehorses.

Mean percentages of neutrophils in tracheal aspirates were higher in order of coughing group ( $52.1 \pm 25.3\%$ ), non-coughing group ( $31.0 \pm 16.5\%$ ) and control group ( $12.3 \pm 2.9\%$ ). Mean percentages of positive rate of IAD were higher in order of coughing group (91.8%), non-coughing group (60.0%) and control group (0.0%). These results suggest that LRTD and IAD are involved in horses presenting coughing and poor performance [3, 5, 14, 17] as well as IAD exists in Japan reported by Kusano *et al.* [11]. Also, it has been revealed that coughing in young horses is a relatively specific indicator of LRTD. According to findings of SAA, Fbg and SP-D showing higher values in coughing group compared to other two groups, it was suggested that the condition of coughing group is in the acute phase [2, 7, 9]. This acute phase may attribute to stress induced by strenuous training or other factors related to stable management, however, we could not identify the relationships with these risk factors in this study. Antibiotics and anti-inflammatory agents are requisite for such cases.

In conclusion, carrying out comprehensive evaluation including investigation on SAA, Fbg and SP-D analysis with airway assessment to Thoroughbred racehorses suspected to have LRTD are useful procedure to understand the pathological condition which aid to initiate appropriate treatment, prognosis judgment or to advise trainers to consider altering training regimen.

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