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Physiological measurements and prevalence of lower airway diseases in Trotters with dorsal displacement of the soft palate

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Keywords: horse; equine; sport medicine; respiratory; muscle; Heart rate; DDSP

Summary

Reasons for performing study: **Dorsal displacement of the soft palate (DDSP) is one of the most common obstructive conditions of the upper respiratory tract in the racehorse. This condition has a complex aetiology which may be caused or exacerbated by pharyngeal inflammation. Additionally, lower respiratory airway diseases may be associated with DDSP thereby contributing to exercise intolerance in these horses.**

Objective: **The aim of this study was to measure physiological variables during a standardised exercise test and to assess the prevalence and consequences of lower respiratory airway disease in horses with DDSP.**

Methods: **A total of 46 horses were included in this study: 22 in the control and 24 in the DDSP groups. All horses performed a SET with measurement of heart rate (HR) and blood lactate concentration. One hour post exercise, respiratory samples were collected for cytological and bacteriological analysis.**

Results: **During exercise, the DDSP group had higher blood lactate concentration than the control group. According to BAL results, 50 and 63% of control and DDSP group horses, respectively, had evidence of inflammatory airway disease (IAD). In the DDSP group, 42% of horses had a syndrome of tracheal inflammation (STI) with 71% of this group having bacteria isolated at $>10^5$ CFU/ml.**

Conclusions: **Horses with DDSP showed evidence of a high prevalence of IAD and STI with an associated positive bacteriology in 55% of the cases. Even if DDSP is treated by surgery, the authors' recommendation would be to investigate the possibility of lower respiratory airway problems which may also be impacting the horse's performance and/or surgery efficiency.**

Introduction

Dorsal displacement of the soft palate (DDSP) is an important cause of poor performance in racehorses (Martin *et al*. 2000; Lane *et al*. 2006a). The condition occurs as a dynamic event during high intensity exercise resulting in exercise intolerance. The underlying aetiology of this condition is not clearly understood and many theories exist to explain the aetiology of this condition, which is probably multifactorial. One of the possible causes is a neuromuscular dysfunction of the structures controlling the position of the soft palate which may occur due to inflammation and/or infection of the upper airway. Also, DDSP causes modification in the respiratory pattern with increased expiratory impedance, decreased minute ventilation, hypoxia and hypercapnia. Therefore, there might be an influence of a concurrent respiratory inflammation and/or infection on the occurrence of DDSP or, *a contrario*, DDSP may result in greater prevalence of lower respiratory tract diseases.

The syndrome of tracheal inflammation (STI), inflammatory airway disease (IAD) and exercise-induced pulmonary haemorrhage (EIPH) are common conditions affecting the intermediate and lower respiratory tract of racehorses with each potentially leading to decreased performance (Couetil 2006; Holcombe *et al*. 2006). These diseases may also be associated with upper respiratory airway diseases and to other diseases contributing to poor racing performance (Couroucé-Malblanc *et al*. 2002; Richard *et al*. 2009a).

Examining the horse at rest, during and after exercise is useful in establishing the origin of poor performance. It also helps to evaluate the horse's fitness with the measurement of blood lactate concentration and heart rate during exercise. The physiological response to exercise evaluated by the calculation of V_4 (velocity for a 4 mmol/l blood lactate concentration) and V_{200} (velocity for a HR of 200 beats/min) was found to be correlated with the level of performance (Leleu *et al*. 2005).

The aim of this study was to measure physiological variables during a standardised exercise test and to assess the prevalence and impact of lower respiratory airway disease in horses with DDSP. This would then allow development of improved clinical approaches to the management of affected horses.

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Materials and methods

Horses

Two populations of horses were studied. The control group included 22 French Standardbred Trotters age 3–9 years (4.4 ± 1.6 years) (11 females, 5 males and 6 geldings) and weighing 448 ± 51.7 kg (mean ± s.d.). These horses were classified as good performers according to their racing records and the opinion of their trainers. These horses came from 8 different training stables. The DDSP group included 24 horses age 3–8 years (4.9 ± 1.6 years) (5 females, 15 males and 4 geldings) and weighing 487.4 ± 41.5 kg (mean ± s.d.). They had a history of exercise intolerance during strenuous exercise with or without a history of respiratory noise during exercise. They came from 24 different training establishments. All horses were in active race training.

Before the exercise tests were undertaken, control horses were given a detailed clinical examination plus haematology and serum biochemistry testing to ensure that they had no significant lameness, clinical or clinicopathological abnormalities. For the horses with DDSP, a detailed clinical examination was performed before referral for the exercise tests by the referring veterinarian. The initial presenting signs consistent with a diagnosis of DDSP were exercise intolerance resulting in loss of momentum or stopping during the exercise period and/or an expiratory noise during exercise (Franklin *et al*. 2004; Lane *et al*. 2006b).

Standardised exercise test

Control group: For the control group, the tests were performed either on a 1000 m sand track (n = 9 horses) or on a high speed treadmill (Säto I)[1] set at a 2.5% slope (Couroucé *et al*. 2000) at a training centre located at the Domaine de Grosbois (94 Boissy St Léger, France; n = 14 horses). When performing treadmill tests, each horse was given 3 acclimation runs on the treadmill on the morning before testing commenced, in a similar manner to that described by King *et al*. (1994). Then, the horses performed a standardised exercise test which utilised a 10 min warm-up step at 4.5 m/s followed by 3 steps each of 3 min at speeds of 8.4, 9.6 and 10.7 m/s. The treadmill was stopped for 1 min to allow positioning of the endoscope (Optomed endoscopie)[2] in the upper airway. Thereafter, the horse performed a fourth step of 1 min 30 s at maximal speed (from 10.8–13 m/s). This was followed by a 5 min recovery period at about 4 m/s (from 3.5–4.5 m/s according to the horse).

For track tests, the horses were harnessed and driven by their usual driver. Then, the horses performed a standardised exercise test which utilised the same protocol as on the treadmill: a 10 min warm-up step followed by 3 steps each of 3 min at the same speeds as described and then a fourth step of 1 min 30 s at maximal speed.

DDSP group: For the DDSP group, all tests were performed on the same high speed treadmill (Säto I)[1] set at 2.5% slope (Couroucé *et al*. 2000). The same procedure was followed: on the morning before testing commenced, each horse was given 3 acclimation runs on the treadmill. Then, the horses performed a standardised exercise test which utilised a 10 min warm-up step at 4.5 m/s followed by 3 steps each of 3 min at speeds of 8.2, 9.4 and 10.5 m/s. The treadmill was stopped for 1 min to allow positioning of the endoscope (Optomed endoscopie)[2] in the upper airway. Thereafter, the horse performed a fourth step of 1 min 30 s at maximal speed (from 10.8–13.4 m/s). This was followed by a 5 min recovery period at about 4 m/s (from 3.5–4.5 m/s according to the horse).

Radiographic examination

A lateral radiograph of the pharyngeal region was taken of horses in both groups to determine the length of the epiglottic cartilage by measuring from the body of the thyroid cartilage to the tip of the epiglottis (thyroepiglottic length).

Endoscopy

All horses in the DDSP group had an endoscopy at rest, during exercise and post exercise. For those in the control group, all horses exercised on the treadmill also had an endoscopy at rest, during exercise and post exercise. For horses exercised on the track, they only had a post exercise endoscopy. Based on these examinations, dynamic upper respiratory airways abnormalities were identified. Additionally, assessment of the pharyngeal mucosa was undertaken in each horse and a grade for the degree of pharyngitis assigned (Auer *et al*. 1985).

Measurements

Heart rate (HR) was measured throughout the test using a heart rate meter (Polar S810i and CS600)[3]. The CS600 was also used on the track to measure velocity. Blood samples were collected from the jugular vein into tubes containing fluoride oxalate for subsequent determination of whole blood lactate as described by Dubreucq *et al*. (1995).

Collection of blood samples post exercise

Tracheal wash (TW) and bronchoalveolar lavage (BAL) samples were collected 1 h after the end of the standardised exercise test and subjected to bacteriological (TW) and cytological (TW and BAL) examinations. The horses were sedated with romifidine (0.4 ml/100 kg) with additional restraint afforded by application of a nose twitch. The TW was performed with 20 ml of sterile isotonic saline instilled *via* a sterile catheter (EMAC 800, Mila)[4] introduced via the endoscope with the catheter positioned at the most ventral point of the trachea (tracheal puddle). The endoscope was disinfected prior to performing each TW. Then, the TW sample was placed in a sterile serum tube for bacteriological analysis and in an EDTA tube for cytological analysis (Frank Duncombe Laboratory, Caen-France). Bronchoalveolar lavage was performed with a 9 mm diameter BAL tube (Equine BAL catheter)[5] passed via the ventral nasal meatus into the lower airways until the tube could not be further advanced. Once the tube was wedged in a bronchus, a cuff at the distal end of the tube was inflated with 5 ml of air to form a seal with the bronchus and 120 ml sterile saline were infused and immediately aspirated. Aliquots of lavage fluid were placed in EDTA tubes and submitted for cytology (Laboratoire Départemental Frank Duncombe). The May Grunwald Giemsa stain was applied to slides prepared by cytocentrifugation (Cytospin, Shandon-France) and differential cell counts were performed by counting a minimum of 200 cells. The number of each cell type was expressed as a percentage of total nucleated cells (including epithelial cells) and leucocyte count.

Data analysis

Using regression and exponential analysis, respectively, the speeds at HR 200 beats/min (V_{200}) and at blood lactate concentration 4 mmol/l (V_4) were calculated as described previously (Dubreucq *et al*. 1995; Couroucé 1999). V_4 is an indicator of aerobic capacity (Persson 1983).

Case definition

The following definitions were adopted:

- Inflammatory airway disease (IAD): Post exercise BALF cytology having >15% neutrophils or >2% mast cells or >1% eosinophils or any combination of these 3 criteria (Couetil and Denicola 1999; Couroucé-Malblanc *et al*. 2002; Robinson and Hoffman 2003; Couetil *et al*. 2007).
- Syndrome of tracheal inflammation (STI) (Couetil *et al*. 2007): cytological examination of TW revealing >20% neutrophils.
- Exercise-induced pulmonary haemorrhage (EIPH), Cytological examination of BALF in which >20% of macrophages were haemosiderophages (Newton and Wood 2002; Richard *et al*. 2009a).
- Bacterial infection: isolation of bacteria in numbers $\geq 10^5$ colony-forming units/ml. When more than 3 bacteria were found it was considered as nonsignificant and as a contamination of the sample. Also, as described by Richard *et al*. (2009b), distinction was made between common pathogens and likely contaminants. Only common pathogens were taken into account to consider bacterial infection (Richard *et al*. 2009b).
- Locomotor disturbance: irregularity/lameness graded ≥ 2 (adapted AAEP grading scale, Richard *et al*. 2009a).

In order to assess the impact of lower respiratory airway disease in horses with DDSP, this group was divided into 3 groups: Horses with only DDSP (age = 3.7 ± 0.8 years), horses with DDSP and IAD (age = 5.5 ± 1.6 years) and horses with DDSP, IAD and EIPH (age = 4.3 ± 1.5 years).

Statistical analysis

All results are presented as mean ± s.d. A multifactorial analysis (NCSS statistical software) was performed to analyse a potential influence of age and gender on the differences observed in DDSP prevalence (pharyngeal inflammation scoring, physiological measurements during exercise). For comparison of the different variables between the control and DDSP group, a Student *t* test was performed. The level of statistical significance was set at $P<0.05$.

Results

Clinical, endoscopic and cytological evaluation

Considering the multifactorial analysis, there was an effect of age on pharyngeal lymphoid hyperplasia, young horses showing higher grades than older horses ($P<0.05$). However, there was no effect of age on V_4 and V_{200}. Also, there was no effect of sex on pharyngeal lymphoid hyperplasia and on physiological variables V_4 and V_{200}.

Considering control and DDSP groups, no significant difference was found between groups for the grading of pharyngeal lymphoid hyperplasia.

All the horses in both groups had an epiglottic cartilage length of >9 cm.

In the DDSP group, 20 of the 24 horses (83.3%) were presented with a history of a respiratory noise during exercise. However, all demonstrated DDSP during exercise on the treadmill. Five horses had a palatal instability in association with the DDSP (rostral displacement of the soft palate). Also, in the DDSP group, one horse had a right laryngeal hemiplegia, one horse vocal cord collapse and one horse bilateral aryepiglottic fold collapse. Only 2 of the 24 horses (8.3%) showed a DDSP at rest during nasal occlusion (Table 1a). In the control group, endoscopy during exercise was performed in 13 horses. None of these horses had evidence of palatal instability and/or DDSP (Table 1b).

In the DDSP group, when considering the neutrophils expressed as a percentage of the total nucleated cell count, 10 horses (41.7%) had STI. When considering the neutrophils expressed in percentage of the leucocyte count, 14 horses (58.3%) had STI. Furthermore, 15 horses showed IAD with 15 showing more than 15% neutrophils in the post exercise BALF, 6 showed more than 1% eosinophils and 2 more than 2% mast cells in this fluid. Also, of these 15 horses, 4 had more than 20% of macrophages that were haemosiderophages (Table 1a). Whatever the expression of the results (percentage of total nucleated cell count or percentage of leucocyte count), the same 15 horses showed IAD (Table 1a).

Concerning other diseases, 4 horses showed low graded lameness during high speed exercise and 2 had *grade 2* right side holosystolic heart murmurs at rest.

For the control group, 45.4% of the horses showed IAD but none had evidence of STI. Whatever the expression of the results (percentage of total nucleated cell count or percentage of leucocytes count), the same horses showed IAD and no horse showed STI. None had evidence of EIPH or locomotor or cardiac diseases (Table 1b).

Respiratory samples for cytology

For BAL, comparison between the DDSP (n = 24 horses) and the control groups (n = 22 horses) were made. Results are expressed as percentage of total nucleated cell count and percentage of leucocyte count. Total nucleated cell count in BAL fluid collected from the DDSP horses was significantly lower (325 ± 221 cells/mm^3) than in controls (559 ± 303 cells/mm^3). Considering, respectively, total nucleated cell and leucocyte counts, the percentage of neutrophils was significantly higher in horses with DDSP (19.4 ± 13.5% for nucleated cell count and 19.7 ± 13.5% for leucocyte count) compared to control horses (11.8 ± 6.4% for nucleated cells count and 12.1 ± 6.6% for leucocyte count - Fig 1). In the DDSP group, 4 horses had evidence of EIPH. In the control group, no horse had evidence of EIPH. In both groups, there were free erythrocytes in the BAL fluid.

For TW, comparison was performed between horses in the DDSP group (n = 24 horses) and the 9 horses in the control group which had a tracheal wash performed. The percentage of neutrophils was significantly higher and percentage of macrophages significantly lower in DDSP than in control horses. For percentage given in total nucleated cell count it was 20.8 ± 19.0 vs. 3.7 ± 2.3 for neutrophils and 37.0 ± 19.3 vs. 60.0 ± 17.7 for macrophages in, respectively, DDSP and control group. For percentage given in leucocyte count it was 27.2 ± 21.2 vs. 4.3 ± 2.4 for neutrophils and 54.7 ± 21.1 vs. 80.1 ± 6.7 for macrophages

TABLE 1a: Prevalence of different diseases in the DDSP group

					STI				IAD			
					Ne		Ne		Eo	Mast cells		
N	Age	Pharyngitis	Rest endoscopy (nasal occlusion)	Exercise endoscopy	% TNC	% LC	% TNC	% LC	%TNC and LC	%TNC and LC	EIPH	Other
1	5	2	DDSP	DDSP + VCC	**49**	**50**	**28**	**28**	0	0	0	HM
2	6	0	DDSP	DDSP + RLH	2	9	7	7	0	0	0	–
3	8	0	Normal	DDSP	6	9	14	14	0	0	0	–
4	5	0	Normal	DDSP	2	2	**20**	**20**	0	0	0	–
5	3	2	Normal	DDSP + PI	10	**32**	12	12	0	2	0	–
6	3	0	Normal	DDSP + PI	**32**	**34**	**23**	**24**	0	0	**81**	–
7	6	3	Normal	DDSP	**55**	**84**	**61**	**62**	**3**	0	6	Loc
8	6	1	Normal	DDSP	**29**	**37**	**24**	**24**	**1**	0	6	–
9	3	3	Normal	DDSP	12	12	10	10	0	1	0	Loc
10	3	4	Normal	DDSP + ADAF	17	**21**	3	3	0	1	0	–
11	4	3	Normal	DDSP	2	13	**15**	**21**	0	0	**28**	–
12	3	3	Normal	DDSP	3	4	5	5	0	1	4	–
13	4	4	Normal	DDSP	16	**24**	7	7	0	1	0	–
14	4	3	Normal	DDSP + PI	18	**38**	14	14	**4**	0	**41**	–
15	5	2	Normal	DDSP	1	8	12	12	0	1	0	–
16	7	0	Normal	DDSP	**62**	**62**	**30**	**30**	0	0	10	Loc
17	4	0	Normal	DDSP	3	5	**28**	**28**	**1**	1	0	Loc
18	7	0	Normal	DDSP	5	8	**15**	**15**	**8**	**3**	4	–
19	5	2	Normal	DDSP	**22**	**24**	**22**	**22**	0	2	2	HM
20	5	0	Normal	DDSP	**20**	**22**	**15**	**15**	**1**	0	13	–
21	4	2	Normal	DDSP	7	7	7	7	0	1	0	–
22	3	3	Normal	DDSP	**28**	**34**	**19**	**19**	0	0	0	–
23	8	0	Normal	DDSP	**44**	**45**	**47**	**47**	0	1	0	–
24	6	0	Normal	DDSP	**45**	**50**	**20**	**20**	0	2	**31**	–
Percentage of horses			8.3% DDSP rest		42% STI	58%STI			62.5% IAD		16.7% EIPH	

With % TNC = percentage of total nucleated cells, % LC = percentage of leucocyte count, VCC = vocal cord collapse; RLH = right laryngeal hemiplegia; PI = Palatal instability; ADAF = Axial deviation of aryepiglottic folds; HM = right side holosystolic heart murmur (*grade 2*); Loc = Locomotor disturbance during exercise. With Ne, Eo, Mas cells expressed as percentage of total nucleated cell count (TNC) and EIPH: haemosiderophages expressed as a percentage of total macrophages; The highlights design the horses that show STI (neutrophils >20% in the TW) or IAD (neutrophils >15%, Eo > 1%, Mast cells 3% in the BAL).

TABLE 1b: Prevalence of different diseases in the control group

					STI Ne		IAD				
N	Age	Pharyngitis	Rest endoscopy (nasal occlusion)	Exercise endoscopy	% TNC	% LC	Ne	Eo % TNC	Mast cells	EIPH	Other
1	9	0	Normal	Normal	ND	ND	12	0	**4**	15	–
2	6	0	Normal	Normal	ND	ND	**18**	0	2	0	–
3	6	0	Normal	Normal	ND	ND	**21**	0	1	2	–
4	6	0	Normal	Normal	ND	ND	14	0	1	2	–
5	6	0	Normal	Normal	ND	ND	13	0	0	8	–
6	4	1	Normal	Normal	ND	ND	13	0	1	2	–
7	4	0	Normal	Normal	ND	ND	**16**	0	**3**	0	–
8	4	1	Normal	Normal	ND	ND	**19**	0	**5**	0	–
9	3	2	Normal	Normal	ND	ND	ND	ND	ND	0	–
10	3	3	Normal	Normal	ND	ND	12	0	2	0	–
11	3	4	Normal	Normal	ND	ND	14	0	1	0	–
12	3	3	Normal	Normal	ND	ND	**16**	0	2	0	–
13	3	2	Normal	Normal	ND	ND	13	0	2	0	–
14	3	1	Normal	ND	2	3	6	**1**	0	0	–
15	4	3	Normal	ND	8	9	2	0	1	0	–
16	4	4	Normal	ND	5	5	4	0	0	0	–
17	4	3	Normal	ND	0	0	**26**	0	1	2	–
18	7	0	Normal	ND	5	6	6	0	0	1	–
19	4	4	Normal	ND	4	4	4	**2**	0	1	–
20	3	4	Normal	ND	3	4	8	0	1	4	–
21	3	2	Normal	ND	2	2	8	0	2	0	–
22	4	2	Normal	ND	4	4	3	**1**	1	0	–
Percentage of horses					0% STI	0% STI		45.4% IAD		0% EIPH	

With % TNC = percentage of total nucleated cells, % LC = percentage of leucocyte count, LLH II_2 = left laryngeal hemiplegia *grade* II_2; LAC = left arytenoid collapse. With Ne, Eo, Mas cell expressed as percentage of total nucleated cell count (TNC) and EIPH: haemosiderophages expressed as a percentage of total macrophages. ND, Not done; The highlights design the horses that show STI (neutrophils >20% in the TW) or IAD (neutrophils >15%, Eo > 1%, Mast cells 3% in the BAL).

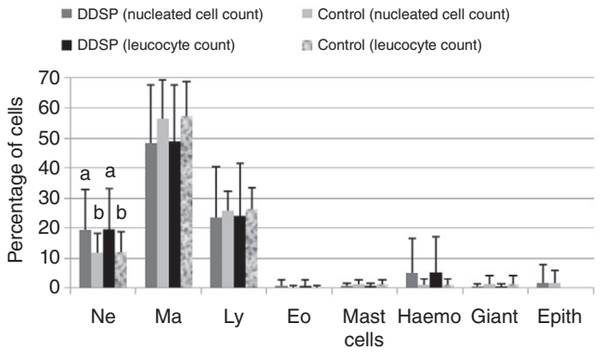

Fig 1: Mean ± s.d. percentage of different cell types harvested by bronchoalveolar lavage expressed in percentage of total nucleated cell count and in percentage of leucocyte count. Ne = neutrophils, Ma = macrophages, Ly = lymphocytes, Epith = epithelial cells, Eo = eosinophils, haemo = haemosiderophages and Giant = Giant cells. Values with different superscripts are significantly different at P<0.05.

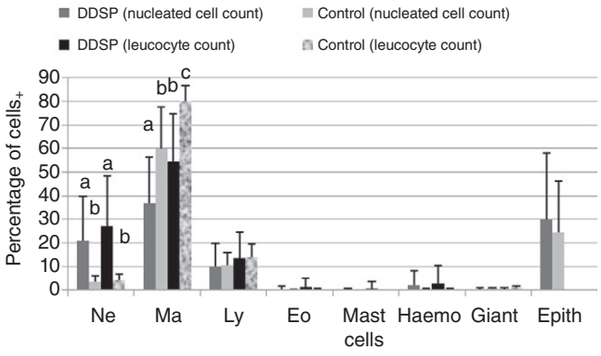

Fig 2: Mean ± s.d. percentage of different cell types harvested by tracheal wash expressed in percentage of total nucleated cell count and in percentage of leucocyte count. Ne = neutrophils, Ma = macrophages, Ly = lymphocytes, epith = epithelial cells, Eo = eosinophils, Haemo = haemosiderophages and Giant = Giant cells. Values with different superscripts are significantly different at P<0.05.

in, respectively, DDSP and control group (Fig 2). Considering the different cell counts, there was a significant difference for macrophages considering total cell count vs. leucocyte count.

Overall when BAL and TW cytology results, expressed in percentage of total nucleated cells count were compared, it was found that the percentage of lymphocytes and mast cells were significantly higher in BAL compared to TW for both groups. The percentages of macrophages and haemosiderophages were significantly higher in BAL compared to TW for horses in the DDSP group. There was no significant difference for the percentages of neutrophils between BAL and TW samples for both groups (Table 2a). However, there was a significant difference for the percentages of neutrophils between BAL and TW samples for DDSP group when considering cytology results expressed as a percentage of leucocyte count (Table 2b).

Respiratory samples for bacteriology

In the DDSP group, 7 (26%) horses had TW samples that yielded no bacteriological growth. For the other 17, mixed bacterial growths occurred, with 15 horses showing 1 or 2 different bacteria and more than 10^5 colony forming units (CFU/ml) and with 2 showing 3 or more bacteria. In the control group, 2 (22%) TW had no bacterial growth. For the remaining 7 horses in this group, mixed bacterial growth occurred both with yields below 10^5 CFU/ml. The predominant bacteria isolated are indicated in Table 3. The percentage of neutrophils in TW relative to the number of bacteria (expressed in CFU/ml) is shown in Figure 3. In the control group, neutrophil percentage was always below 10%. In the DDSP group, there was no significant difference between neutrophil percentages according to number of bacteria (no growth = neutrophils of 24.5% (± 15.9); <100 000 CFU/ml = neutrophils of 20.9% (± 20.8) and ≥100 000 CFU/ml = neutrophils of 30.2% (± 24.5%).

Physiological measurements

In order to compare measurements collected under the same conditions of exercise (on the high speed treadmill), physiological measurements were compared between all the horses in DDSP group and 13 horses in the control group. Treadmill speeds including peak velocity were the same (no differences) for both groups. For blood lactate concentration, there was no significant difference for step 1, 2 and 3 between control and DDSP groups. Also, there was no significant difference for V_4 between control (V_4 = 10.5 ± 0.4 m/s) and DDSP groups (V_4 = 10.3 ± 1.0 m/s). However, for step 4 during which endoscopy was performed, there was a significantly higher blood lactate value in DDSP horses compared to control group horses (Fig 4). For V_{200}, there was no significant difference between control (V_{200} = 10.5 ± 0.7 m/s) and DDSP group (V_{200} = 10.3 ± 0.8 m/s).

If the DDSP group was further subdivided according to absence of IAD, presence of IAD only and presence of IAD and EIPH, those with IAD and with IAD and EIPH (V_4, respectively, 10.0 ± 1.1 m/s and 9.3 ± 0.4) showed significantly lower V_4 compared to the control group and compared to horses with DDSP only (V_4 = 10.8 ± 0.6) (Fig 5).

Follow-up of horses

Follow-up of treatments was carried out for the horses in DDSP group. A total of 16 of the 24 horses had a tie-forward surgery. Of these 16 horses, 6 also had a medical treatment (3 received corticosteroids and antibiotics with an inhalation mask; 3 received a throat spray with glycerin, corticosteroids, DMSO). A total of 8 horses had no surgery: 6 had corticosteroids with or without antibiotics by systemic or inhalation administration and 2 had no medical treatment.

For the 24 horses, a follow-up of racing performances with total earnings at the 31st of March 2010 was made. For 2 horses, it was not possible to evaluate racing earnings as these horses were Italian horses trained in France, and these horses had surgery. To our knowledge, for the 22 other horses, horses with surgery (n = 14) had mean earnings ± s.d. of 82,729 ± 76,192 euros while horses without surgery and with medical treatments (n = 6) had 69,870 ± 77,874 euros. Two of the horses in this group also had orthopaedic evaluation and treatments. There was no significant difference in race earnings between the 2 groups. The 2 horses that had no surgical and no medical treatment had no race earnings.

TABLE 2a: Cytology analysis comparison between DDSP and control group for BAL and TW with results expressed as a percentage of total nucleated cell count (TNCC)

	DDSP group (n = 24)		Control group (n = 9)	
	BAL	TW	BAL	TW
Neutrophils %	19.4 ± 13.5[a]	20.8 ± 19.0[a]	7.4 ± 7.3[a]	3.7 ± 2.3[a]
Macrophages %	48.4 ± 19.2[a]	37.0 ± 19.3[b]	65.1 ± 10.1[a]	60.0 ± 17.7[a]
Lymphocytes %	23.5 ± 17.0[a]	10.2 ± 9.7[b]	24.9 ± 6.4[a]	10.6 ± 5.4[b]
Epithelial cells %	1.6 ± 6.3[a]	30.0 ± 28.0[b]	0[a]	24.6 ± 21.8[b]
Eosinophils %	0.8 ± 1.9[a]	0.5 ± 1.2[a]	0.4 ± 0.7[a]	0.1 ± 0.3[a]
Mast cells %	0.7 ± 0.9[a]	0.2 ± 0o.5[b]	0.7 ± 0.7[a]	0[b]
Haemosiderophages %	5.1 ± 11.5[a]	2.3 ± 6.1[b]	0.7 ± 1.0[a]	0.2 ± 0.4[a]
Giant cells %	0.5 ± 0.8[a]	0.3 ± 0.6[a]	0.7 ± 0.7[a]	0.7 ± 0.5[a]

Values are mean ± s.d. Values with different superscripts are significantly different between BAL and TW for, respectively, DDSP and control group at P<0.05.

TABLE 2b: Cytology analysis comparison between DDSP and control group for BAL and TW with results expressed as a percentage of leucocyte count (LC)

	DDSP group (n = 24)		Control group (n = 9)	
	BAL	TW	BAL	TW
Neutrophils %	19.7 ± 13.5[a]	27.2 ± 21.2[b]	7.4 ± 7.3[a]	3.7 ± 2.3[a]
Macrophages %	48.9 ± 18.9[a]	54.7 ± 20.1[a]	65.1 ± 10.1[a]	80.1 ± 6.7[b]
Lymphocytes %	24.1 ± 17.4[a]	13.7 ± 10.8[b]	24.9 ± 6.4[a]	14.0 ± 5.5[b]
Eosinophils %	0.8 ± 1.9[a]	1.4 ± 3.5[a]	0.4 ± 0.7[a]	0.2 ± 0.5[a]
Mast cells %	0.7 ± 0.9[a]	0.7 ± 2.8[b]	0.7 ± 0.7[a]	0[b]
Haemosiderophages %	5.3 ± 11.8[a]	3.0 ± 7.5[b]	0.7 ± 1.0[a]	0.3 ± 0.5[a]
Giant cells %	0.5 ± 0.8[a]	0.4 ± 0.7[a]	0.7 ± 0.7[a]	0.9 ± 0.7[a]

Values are mean ± s.d. Values with different superscripts are significantly different between BAL and TW for, respectively, DDSP and control group at P<0.05.

TABLE 3: Main bacteria isolated in TW in DDSP and control groups

	DDSP (n = 24)	Control (n = 9)
Positive bacteriology	17 horses (71%)	7 horses (78%)
Enterobacteriaceae (*Pantoea* spp, *Klebsiella* sp., *E. Coli*)	9 horses (53%)	4 horses (57%)
Pseudomonas sp.	5 horses (29%)	2 horses (28%)
Streptococcus (zooepidemicus, equisimilis)	4 horses (23%)	6 horses (86%)
Actinobacillus sp.	5 horses (29%)	1 horse (14%)

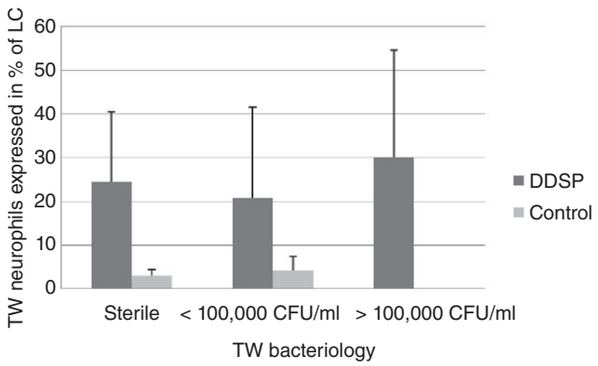

Fig 3: Mean ± s.d. percentage of neutrophils expressed as a percentage of TW leucocytes count according to the number of bacteria in both DDSP and control groups.

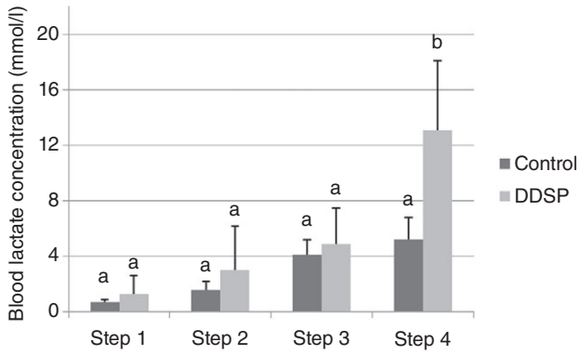

Fig 4: Mean ± s.d. for blood lactate concentration expressed in mmol/l for each step of the exercise test in both DDSP and control group. Values with different superscripts are significantly different at P<0.05.

Discussion

Dorsal displacement of soft palate (DDSP) is the most common cause of dynamic obstruction identified during treadmill exercise in racehorses (Martin *et al*. 2000; Lane *et al*. 2006a). Most affected horses make an abnormal noise during exhalation during a fast work (a 'snoring' noise). However, in approximately 30% of horses with DDSP, this noise is not reported (Franklin *et al*. 2004). In our study, in the DDSP group, 83.3% of the horses were presented with a history of a respiratory noise during exercise which is in accordance with previous studies (Franklin *et al*. 2004; Lane *et al*. 2006b). However, we did not record the presence of abnormal noise during high speed treadmill exercise.

Resting endoscopic examination has limited value in determining if a horse will experience DDSP during exercise.

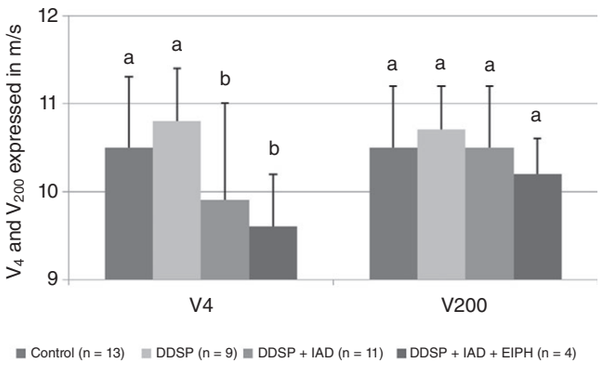

Fig 5: Mean ± s.d. V_4 and V_{200} expressed in m/s in both DDSP and control group. Values with different superscripts are significantly different at P<0.05.

Although Holcombe *et al*. (1996) have reported that clinicians can create negative intrapharyngeal pressures during nasal occlusion, equivalent to negative pressures experienced during high-speed exercise, many horse that displace at rest do not displace during high speed exercise and the reverse is also true (Parente and Martin 1995; Ferrucci *et al*. 2004; Tan *et al*. 2005; Lane *et al*. 2006b). This was the case in this study as only 2 of the 24 horses (8.3%) showed a DDSP during nasal occlusion at rest. Also, it has been suggested in previous reports that some abnormalities observed at rest may point to a diagnosis of DDSP during exercise. These include prolonged spontaneous dorsal displacement, displacement and/or billowing in response to nasal occlusion or swallowing, ulceration of the caudal border of the soft palate and/or the presence of a hypoplastic or flaccid epiglottis. In our study, only one of the 24 horses had ulceration of the caudal border of the soft palate and 2 had a flaccid epiglottis at rest. These 2 horses also demonstrated displacement of the soft palate in response to nasal occlusion. Also, all horses in the DDSP group had a normal length epiglottis as measured on a standardised lateral radiograph of the pharyngeal region according to Linford *et al*. (2002). The authors suggest that this rules out epiglottic hypoplasia as a cause of DDSP during exercise in these horses. This was already reported by Rehder *et al*. (1995) who showed that in a population of DDSP horses the epiglottic size was within normal limits.

Considering upper airway inflammation occurrence, there is in racehorses, a high prevalence of pharyngeal lymphoid hyperplasia (PLH). In a population of 1005 Thoroughbred horses, Saulez and Gummow (2009) showed that 63% of the horses had a *grade 2–4* PLH, with *grades 3* and *4* occuring in younger racehorses. This is in accordance with our study in which 54.2% of the horses also had a *grade 2–4* PLH, with young horses showing significantly higher grades of PLH. Age may be a risk factor for DDSP as young horses show higher grades of PLH and as upper airway inflammation may lead to DDSP.

IAD has recently been redefined (Couetil *et al*. 2007) as a nonseptic inflammation affecting horses of any age with a definitive diagnosis based on BALF cytology. The cytological profile of IAD is characterised by an increase in the total nucleated cell count with mild neutrophilia or alternatively increased mast cell or eosinophil percentages (Couetil *et al*. 2007).

A distinction has recently been made between IAD and the syndrome of tracheal inflammation (STI) but the relationship between the 2 conditions is unknown. One of the reasons for this distinction may be the poor correlation found between TW and BALF cytology (Malikides *et al*. 2003; Allen *et al*. 2006). Therefore, there is a consensus that the best chance of arriving at a correct diagnosis of intermediate and lower respiratory tract inflammation is afforded by performing both TW and BAL (Malikides *et al*. 2003; Hodgson 2006; Ramzan *et al*. 2008). In the present study, both TW and BAL were performed and there was a significant difference for the percentages of macrophages, haemosiderophages, mast cells, epithelial cells and neutrophils between TW and BAL.

The differential cell count may be performed on either leucocytes or on nucleated cells which includes epithelial cells (Hewson and Viel 2002). In a recent review on laboratory findings in respiratory fluids, Richard *et al*. (2009b) presented reference values from various studies for TW and BALF cytology. However, the results are difficult to compare as the methodology varies from one study to the other and presentation of the results differs, some authors including epithelial cells and some not. In order to evaluate the incidence of epithelial cells inclusion or not on other cells percentage (macrophages, haemosiderophages, lymphocytes, neutrophils, eosinophils, mast cells, giant cells), the results in this study were presented with both total nucleated cell count (TNCC) and leucocyte count (LC).

There have been no published accepted values for the different cell types observed in the TW cytology, although it was reported that in young, clinically normal racehorses, the neutrophil count was <20% of the leucocytes in 73–80% of horses examined (Sweeney *et al*. 1992a; Christley *et al*. 2001). In the current study, control group horses showed 4.3% (± 2.4) neutrophils as part of the leucocyte count and 3.7% (± 2.3) when considered as part of the total nucleated cell count. Regardless of how results were expressed, all horses in the DDSP group had >20% neutrophils in TW samples. In addition to the differential cell count, the morphological description of the cells may also be informative. This has been shown in a recent study by Fortier *et al*. (2009) which studied the potential role of BAL and TW in the detection of equine herpesvirus. In BAL, exfoliated epithelial cells and ciliocytophtoria were associated with PCR evidence of EHV-2 and EHV-5 virus-types in the BAL. When examining TW's, the detection of EHVs was correlated with the polymorphonuclear neutrophil count. However, in the current study, no PCR was performed on the respiratory samples. Also, there were no cell abnormalities in the various respiratory samples.

Exercise may also result in changes in BAL and TW cytology, as high speed exercise may lead to a higher number of neutrophils (Couetil and Denicola 1999; Malikides *et al*. 2007). Therefore, the most convenient time for collection of a tracheal wash sample to obtain the most diagnostically useful information might be after a suitable 'washout' period of at least 1–2 h post exercise (Malikides *et al*. 2007). In our study, respiratory sampling was performed 1 h after a maximal exercise. Also, the amount of neutrophils in a BALF is inversely proportional to the quantity of saline instilled (Sweeney *et al*. 1992b; Richard *et al*. 2009b). Instillation of a 120 ml volume may explain the higher neutrophils amount compared to other studies in which saline volumes were more important. In this study, horses in the control group had a neutrophil percentage in the BAL >10%: neutrophils = 11.8% ± 6.4 when considered as a percentage of nucleated cell count and 12.2% ± 6.6 when considered as a percentage of the leucocyte count. This is in accordance with a previous study in which neutrophils percentage for the control group was 15% for a BAL collected under the same conditions (Couroucé-Malblanc *et al*.

2002). Because of the methodology used (120 ml saline instilled and fluids samples taken 1 h after exercise) and because of these results, our definition of IAD was based on ≥15% neutrophils in the BALF with STI defined as there being ≥20% neutrophils in a TW sample. The neutrophils percentage is expressed as a percentage of leucocyte count.

Tracheal inflammation associated with mucus accumulation is known to have a negative impact on performance (Holcombe *et al*. 2006). However, in the present study, tracheal endoscopy was performed 1 h after exercise which will have an influence on the presence of mucus and as such the visual tracheal mucus score. This is why no mucus score was ascribed in the present study. However, the authors suggest that a tracheal lavage score as described by Ramzan *et al*. (2008) may have been utilised in an attempt to better differentiate horses according to the variation in mucus characteristics (e.g. accumulation, viscoelasticity).

Exercise-induced pulmonary haemorrhage (EIPH) was defined according to the definition adapted from Newton and Wood (2002). This involved a cytological diagnosis based on examination of BALF in which >20% of macrophages were haemosiderophages. In the current study, 26.7% of horses in the DDSP group with IAD had EIPH. In contrast, no horses in the control group had EIPH as defined by Newton and Wood (2002). However, free red cells were found in the BALF of all horses thus leading to the suspicion of some form of recent pulmonary haemorrhage in these animals.

Care should be taken when interpreting bacterial cultures taken from the intermediate or lower respiratory tract. Since many bacteria detected also belong to the commensal flora of the upper airways (Robinson and Derksen 1998), isolation of bacteria in TW may represent infection, transient intermediate airway colonisation or contamination of the sample. A comparison of percutaneous (transtracheal) aspiration and transendoscopic tracheal wash using a guarded sterile catheter, as used in this study, found no significant difference between both techniques with respect to sample contamination (Christley *et al*. 1999). In a longitudinal study performed over 3 years in Thoroughbreds, 22% of the TW samples had no bacteriological growth (Wood *et al*. 2005). This is similar to the current study in which 27% of the horses had no bacteriological growth. Isolation of bacteria from TW samples has previously been associated with lower airway inflammation in racehorses (Chapman *et al*. 2000; Christley *et al*. 2001; Wood *et al*. 2005). The chances of airway inflammation being present are significantly greater with increased numbers (CFU/ml) of *Streptococcus zooepidemicus* (Burrell *et al*. 1996; Wood *et al*. 2005) and *Actinobacillus* spp. (Wood *et al*. 2005). However, only TW and not BAL was performed in these earlier studies. Therefore, there may be a relationship between tracheal infection and STI but not between tracheal infection and IAD (Couetil *et al*. 2007). In the current study, 66.7% of horses with DDSP had evidence of intermediate and/or lower respiratory tract disease with 44–59% of them having STI (8.3% had only STI with 91.7% having STI and IAD) and 62.9% with IAD. For horses with evidence of STI, 75% of them had a positive bacteriological culture with bacteria isolated $\geq 10^5$ CFU/ml. This is in accordance with a previous study showing 80% prevalence of IAD in horses with $\geq 10^5$ CFU bacteria/ml isolated (Wood *et al*. 2005) and with the hypothesis that aerobic bacteria are significantly associated with lower airway inflammation. However, other workers suggest caution in interpreting these results because inflammation of the tracheobronchial tree for any reason may leave it more susceptible to colonisation by oropharyngeal bacteria. These bacteria may, therefore, be a consequence of lower airway inflammation, rather than its cause. Nonetheless, because they are potential pathogens, it is likely that they may contribute to the worsening and prolongation of IAD. However, there was also a high prevalence of IAD in the control group (50%). These horses had no STI and when there was bacteriological growth this occurred at $<10^5$ CFU/ml. From these results, we suggest that even if there was a high prevalence of IAD in both groups, there was a higher prevalence of STI and a higher number of bacteria isolated from horses with DDSP compared to controls.

Various authors have shown that respiratory abnormalities can limit oxygen exchange (Persson 1983; Morris 1991; Persson and Lindberg 1991; King *et al*. 1994; Couroucé-Malblanc *et al*. 2002). DDSP is considered a common cause of poor performance in racehorses. It leads to an expiratory obstruction that subsequently results in decreased ventilation, hypoxaemia and exercise intolerance (Rehder *et al*. 1995; Holcombe *et al*. 1998). IAD is frequently diagnosed as a cause of poor exercise performance. However, the true effect of IAD on performance is incompletely documented. Many horses undergoing investigation for poor performance have signs of IAD. However, given the high prevalence of IAD, the clinical relevance of this finding remains uncertain. Nyman (2003) failed to identify an obvious difference in IAD-affected horses, compared to healthy horses, with regard to arterial blood gas changes. In contrast, others have found that in horses with IAD, gas exchange is impaired during exercise these horses exhibiting worsening of exercise-induced hypoxaemia (Couetil and Denicola 1999; Couroucé-Malblanc *et al*. 2002; Sanchez *et al*. 2005). Furthermore, it has been shown that IAD horses have evidence of a degree of airway obstruction (Couetil *et al*. 2001). Post exercise, horses with IAD have higher blood lactate concentration than control horses (Couetil and Denicola 1999). Hence, there is experimental evidence that IAD induces physiological changes in exercising horses that may have a negative impact on performance. In this study, horses with DDSP had a higher blood lactate concentration for step 4 which was performed at maximal velocity compared to control horses. This appears to be in accordance with the occurrence of DDSP which happens more frequently at the end of a maximal exercise such a race. Interestingly, the authors found that horses with DDSP and IAD and also DDSP, IAD and EIPH showed higher blood lactate concentration and thus lower V_4 values compared to control. This was not the case with horses with DDSP only. It has been previously shown that V_4 may be assimilated to the aerobic capacity of the horse and is significantly related to race performance in the horse and particularly in trotters (Leleu *et al*. 2005). Therefore, we suggest that horses with both DDSP and lower respiratory diseases such as IAD with or without EIPH are more exercise intolerant at submaximal and maximal exercise intensity.

In the present study, 4 horses showed subclinical locomotor diseases (lameness) and 2 showed a *grade 2* right sided holosystolic murmur which was, in both cases, a murmur consistent with tricuspid regurgitation which is frequent in racehorses and seldom associated with poor performance (Young *et al*. 2008). This underlines the fact that poor performers may have several concomitant problems. Horses with evidence of upper respiratory disease may show concurrent lower respiratory tract disease (STI and/or IAD and/or EIPH) with or without bacterial isolation and other problems such as cardiac and/or muscular and/or locomotor problems (Martin *et al*. 2000; Couroucé-Malblanc *et al*. 2002;

Richard *et al*. 2009b). Therefore, we recommend that a thorough and careful examination of the horse is of great importance in horses with poor performance, especially as in this series where we studied DDSP. Tack modifications such as the use of a bit that maintains the position of the tongue, tongue-ties and the figure-of-eight noseband are traditional approaches that may be of value in reducing the occurrence of DDSP (Franklin *et al*. 2002; Barakzai and Dixon 2005). Also, the treatment of any concurrent diseases may be necessary before undertaking surgical treatment for DDSP. We suggest that in any horse, with or without upper airway dysfunction, that has evidence of active upper, intermediate or lower respiratory airway inflammation, the initial therapy should focus on decreasing this inflammation by medical therapy. Also, as seen previously, presence of bacterial colony may be associated with STI and IAD. Because they are potential pathogens, it is likely that they may contribute to the worsening and prolongation of lower airway inflammation. Therefore, in these cases, it seems important to have an antibiotic medical therapy associated with corticosteroids to decrease inflammation. If the horse suffers from a subclinical locomotor disease, it is also important to undertake a thorough orthopaedic evaluation and to treat the horse as some anectodal reports indicate that the associated pain is considered to be the key factor leading to DDSP. In support of this premise, 2 of the horses in this study with locomotor disease received treatment for their orthopaedic problem and thereafter did not have occurrence of DDSP.

In conclusion, this study demonstrated that exercise intolerance in horses with DDSP may be exacerbated by concomitant underlying lower respiratory tract problems such as STI with or without the presence of bacteria in the trachea and/or IAD and/or EIPH. Our study also showed that a high prevalence of IAD occur in horses performing well (control group). However, these controls animals had no evidence of STI and, when they had bacteria in the TW, they were isolated at numbers of $<10^5$ CFU/ml. In contrast, horses with DDSP had a high prevalence of STI with 75% of the cases having bacteria isolated at $\geq 10^5$ CFU/ml. These horses also had a high prevalence of IAD with 23.5% of these cases having EIPH. As DDSP is a complex disease with various possible causes, a careful examination at rest, during exercise and after exercise should be done on horses thought to have this disease. According to follow-up of the horses in this study and as poor performance may have a number of various components, we recommend that this examination should include endoscopy before, during and after exercise with evaluation of upper respiratory airway at rest (response to nasal occlusion) and during exercise and by scoring the degree of pharyngitis and tracheal mucus. It should also include tracheal wash after exercise (with tracheal lavage score and bacteriology and cytology analysis) and bronchoalveolar lavage after exercise (for cytological analysis). If any inflammation of upper and/or lower respiratory airway exists, medical treatment of this is recommended before scheduling attempted a surgical correction of DDSP. Also, even if respiratory problems appear to be the most important problem, a careful evaluation of cardiac, muscular and locomotor systems should be performed with adequate treatment afforded especially in cases of orthopaedic disease.

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Conflicts of interest

The authors have not declared any potential conflicts.

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