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Abstract

There has been increasing interest in blood gas analysis in donkeys. “Point-of-care (POC) testing” is a diagnostic testing performed at or near the patient. The aim of the study was to assess the agreement between two POC blood gas analyzers in donkeys. Arterial and venous blood samples were collected from 17 donkeys and analyzed using a fully automated blood gas analyzer (ABL 700 Series Radiometer, Denmark) (RAD) and two POC blood gas analyzers (i-STAT® System, USA; VetStat®, Idexx, USA). The parameters revealed by all three devices were submitted to a canonical discriminant analysis, to evaluate which of them can discriminate the POC analyzers from RAD. On the basis of the discriminant analysis, we evaluated the best POC for each parameter registered, in comparison with RAD. Moreover, the results changed in relationship with the type of blood (venous or arterial blood). The agreement between i-STAT® and RAD was good for venous samples, while was poor for arterial samples. A poor agreement was found between VetStat® and RAD for both venous and arterial samples. The implementation of the number of subjects might lead to a better understanding of the potential role of the POCs in clinical setting. Finally, increasing population of the study would be recommended in order to set reference values.

Keywords	Blood gas analysis, donkey; Point-of-care testing; arterial blood gas analysis; venous blood gas analysis.

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To the Editor in chief

Journal of Equine Veterinary Science

here is our paper titled “Evaluation of two handheld point-of-care blood gas analyzers in healthy donkeys” authored by Bonelli et al.

This study was approved by the Ethical Committee, University of Pisa. This study was supported by funds from the University of Pisa (100%).

The manuscript has not been published elsewhere. Authors’ contribution to the manuscript is equally distributed and no conflict of interest exists.

Yours sincerely,

Dr. Francesca Bonelli

Highlights

- Point-of-care (POC) testing is a diagnostic testing performed near the patient
- The agreement between two POC blood gas analyzers have been evaluated in donkeys
- The i-STAT® had a good agreement with the standard analyzer (RAD) for venous sample
- The VetStat® had a poor agreement with the RAD for venous and arterial samples

Research article

Evaluation of two handheld point-of-care blood gas analyzers in healthy donkeys

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ABSTRACT

There has been increasing interest in blood gas analysis in donkeys. “Point-of-care (POC) testing” is a diagnostic testing performed at or near the patient. The aim of the study was to assess the agreement between two POC blood gas analyzers in donkeys.

Arterial and venous blood samples were collected from 17 donkeys and analyzed using a fully automated blood gas analyzer (ABL 700 Series Radiometer, Denmark) (RAD) and two POC blood gas analyzers (i-STAT® System, USA; VetStat®, Idexx, USA). The parameters revealed by all three devices were submitted to a canonical discriminant analysis, to evaluate which of them can discriminate the POC analyzers from RAD.

On the basis of the discriminant analysis, we evaluated the best POC for each parameter registered, in comparison with RAD. Moreover, the results changed in relationship with the type of blood (venous or arterial blood).

The agreement between i-STAT® and RAD was good for venous samples, while was poor for arterial samples. A poor agreement was found between VetStat® and RAD for both venous and arterial samples. The implementation of the number of subjects might lead to a better understanding of the potential role of the POCs in clinical setting. Finally, increasing population of the study would be recommended in order to set reference values.

Keywords: Blood gas analysis, donkey, Point-of-care testing, arterial blood gas analysis, venous blood gas analysis.

1. Introduction

Human relationships and interactions with equines have varied in the course of history according to human needs [1]. Donkeys (*Equus asinus*) were domesticated in Northeast Africa around 6,000-7,000 years ago and descended from wild asses evolved to live in inhospitable environments. Donkeys, as well as mules and horses, have traditionally been part of worldwide agricultural systems providing an essential transport, pack and draught resource as working animals [2]. Nowadays, donkeys still play a key role as working animals in developing countries. Also, donkeys are used for meat, milk productions, for social activities, for tourism and leisure [3]. Consequently, the interest in the welfare and diseases of this species is constantly increasing. Despite this, clinical research on donkeys needs to be in continual development because they show different reactions compared to horses in many conditions, including infectious diseases [4-9], and need specific diagnostic [10-15] and therapeutical approaches [16-18].

Recently, there has been increasing interest in blood gas analysis in donkeys, along with their growing popularity as companions. Blood gas analysis can help assess underlying disease processes and the severity of illness and can guide medical interventions [19] or can be used as a tool to assess the impact of a medical procedure on blood gas parameters [20]. Blood gas analysis can be performed on arterial or venous blood and provides data regarding blood pH, oxygen (pO_2) and carbon dioxide (pCO_2) partial pressures, total carbon dioxide (tCO_2), oxygen saturation (sO_2), but results often include also lactate, glucose, electrolytes, bicarbonate (HCO_3) and base excess [21]. Concerning clinical monitoring of blood gases, accuracy in point-of-care (POC) analyzers is required for optimal patient management. “Point-of-care testing” is a diagnostic testing performed at or near the patient. These analyses are

important in evaluating the emergency or critical care patient both in clinic and in field conditions [21].

The aim of the present study was to assess the agreement between two point-of-care blood gas analyzers and a conventional fully automated blood gas analyzer.

2. Materials and Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of the University of Pisa (45965/2016). The donkeys were owned by the Tuscany Equids Breeding Farm. The owner's written consent was obtained for all the donkeys included in this study. All the donkeys were considered healthy based on history, physical examination, and lung ultrasound. Moreover, a complete blood cell count (CBC) and clinical chemistry panel were carried out the day before of the protocol in order to exclude sick animals. Only donkeys with no clinical and hematological alterations were included.

A total of 18 donkeys, different in age and gender were enrolled in the present study.

2.1 Animals

All the donkeys enrolled, presented a normal history and no abnormalities at the physical examination and lung ultrasound, while one donkey showed a slightly decreased blood Hematocrit (HCT) and Hemoglobin (Hg), thus it was excluded from the study. The study population was composed by a total of 17 donkeys belonging to the Amiata donkey breed. Donkeys were 13 jennies and 4 jacks, aged between 4 to 16 year old, weighed 300 to 380 kg, and fed with *ad libitum* hay and water. All the jennies were kept in collective paddocks at the Veterinary Teaching Hospital, Department of Veterinary Sciences, Pisa University, while jacks were housed in single pens.

2.2 Sampling Procedures

The day before the working session, sampling order (arterial *vs* venous sample) was randomly chosen using a True Random Number Service [a]. The Random Number Generator was set with a minimum value of 1 and a maximum value of 100. Pair numbers would lead to always start first with arterial sampling, while odd numbers would lead to always start with venous sampling. The Generator came with "56", thus throughout the all study, blood collection always began from arterial sampling. The donkeys order was also randomly chosen using a Random Name Picker [b].

Three mL of arterial blood were collected by using anaerobic conditions from the common carotid artery, approaching the vessel in the ventral region of the neck, facing the front of the animal and entering the needle in the jugular furrow with a direction perpendicular to the skin with a depth of 3-4 centimeters with three different blood gas syringes (1 mL each) containing lyophilized heparin (Safe PICO Self-fill arterial sampler, Radiometer Medical ApS-Denmark). Subsequently, 3 mL of venous blood were obtained from the left jugular vein using the same syringes previously described for arterial samples. No clipping was performed, and alcohol only was rubbed on the skin for a better visualization of the vessels. All samples were collected by the same veterinarian (Operator 1), in accordance with good veterinary practice, and did not cause evident pain to the animals. All the samples were performed in conscious donkeys, only manually restrained. While the Operator 1 sampled the animals, an assistant (Operator 2) grab the samples and gave it to another veterinarian (Operator 3) which suddenly processed it.

2.3 Samples processing

All the arterial and venous blood samples were analyzed using a fully automated blood gas analyzer (ABL 700 Series Radiometer, Radiometer Copenhagen Medical ApS, 2700 Brønshøj, Denmark) (RAD) and two handheld point-of-care (POC) blood gas analyzers (i-

STAT® System, Abbott Laboratories, Abbott Park, Il, USA; VetStat®, Idexx, USA).

For the i-STAT® System, a CG8+ cartridge (Abbott Laboratories, USA) was used. This cartridge measured the following parameters: pH, partial pressure of CO_2 (pCO_2 expressed in mmHg), partial pressure of O_2 (pO_2 expressed in mmHg), sodium (Na^{++} expressed in mmol/L), potassium (K^+ expressed in mmol/L), calcium (Ca^{++} expressed in mmol/L) and hematocrit (HCT expressed in %), while the following parameters were calculated: hemoglobin (Hb expressed in gr/dL), base eccess (BE expressed in mmol/L), bicarbonate (HCO_3^- expressed in mmol/L), total CO_2 (tCO_2 expressed in mmol/L), and oxygen saturation (SO_2 expressed in %). For the VetStat® the "respiratory cartridge" was used. This cartridge measured the following parameters: pH, pCO_2 (mmHg), pO_2 (mmHg), Na^+ (mmol/L), K^+ (mmol/L) and Cl^- (mmol/L), while Hb (gr/dL), BE (mmol/L), Anion Gap (mmol/L), HCO_3^- (mmol/L), total CO_2 (tCO_2 expressed in mmol/L), SO_2 (%) were calculated. Finally, the automated blood gas analyzer assessed the following parameters by direct measurement: pH, pCO_2 (mmHg), pO_2 (mmHg), Na^+ (mmol/L), K^+ (mmol/L), Ca^{++} (mmol/L), Chloride (Cl^- expressed in mmol/L) and HCT (%), while Hb (gr/dL) $BE_{(ecf)}$ (mmol/L), Anion Gap (mmol/L), HCO_3^- (mmol/L), tCO_2 (mmol/L), and SO_2 (%) were calculated.

Both arterial and venous samples were analyzed immediately after collection using the same order. The samples were processed with the RAD gas analyzer, then with VetStat®, and at last with the i-STAT®. All the machines were situated adjacent to each other to ensure equivalent environmental operating conditions and were serviced according to manufacturers' instructions. Calibration and automatic sample integrity and quality checks were performed for all the blood gas analyzers according to manufacturers' instructions. Results from each device were printed out and stored. Analytical system, methodological and human errors were

recorded.

2.4 Statistical analysis

Data were transferred to a spreadsheet (Microsoft Excel 2011, Microsoft Corporation, Redmond, Washington, USA) and analyzed using two commercial softwares (Microsoft Excel 2011, Microsoft Corporation, Redmond, Washington, USA; GraphPad Prism 6.0, La Jolla, California, USA). The Shapiro-Wilk test was carried out to verify data distribution. Data did not show a Gaussian distribution; thus, results were expressed as median value with range (minimum and maximum value) and interquartile range (IQR).

The variables detected by all three devices (pH, PCO_2, PO_2, TCO_2, SO_2, Hb, BE, HCO_3^-, Na and K) were submitted to a canonical discriminant analysis (CDA) by SAS software (SAS Institute Inc., Cary, NC, USA) (the CANDISC procedure), a dimension reduction technique which performs both univariate and multivariate one-way analysis to derive canonical functions, i.e. linear combinations of the quantitative variables, that summarize the variation between groups. Given a classification character and several interval variables, CDA derives a set of new variables, called canonical functions (CAN), which are linear combinations of the original interval variables, as reported in the follow equation:

$$\text{CAN} = d_1X_1 + d_2X_2 + \ldots + d_nX_n,$$

where d_i are the canonical coefficients (CC) that indicate the contribution of each variable in composing the CAN, and X are the scores of the n original variables. CAN summarize the between-groups variation, highlighting their differences. In general, if k groups are involved in the study, k−1 CAN are extracted. In the present work, having only 3 groups (RAD, i-STAT® and VetSTAT®), two CANs were extracted. The effective separation between groups was assessed by using the Mahalanobis distance and the corresponding Hotelling's T-square test [22]. Briefly, the Mahalanobis distance takes into account the variable co-variances in

calculating distances between individuals in a multivariate vector space. The ordinary Euclidean distance is a special case of the Mahalanobis distance when variables have equal variances and zero covariances. The Hotelling's T-square test is an extension of the Student's t-test to the multivariate domain [23].

The ability of CAN to assign each device data to the 3 groups was calculated as the percent of correct assignment using the Discriminant Analysis [24]. In practice, the CAN is applied to each animal, thus obtaining a value called discriminant score. Then, the centroids of the 2 groups are calculated and, for each experimental unit, distances from the 2 centroids are evaluated. One experimental unit is assigned to the one of the three groups on the basis of the lowest distance from the 2 group centroids [24]. CDA and DA were applied on the arterial and venous data, separately.

The effect of device lecture on the parameters not detected by all three devices (HCT, Anion gap, Ca^{++}, Cl^{-}) were estimated by Kruskal-Wallis test and Dunn's multiple comparisons test as a post hoc. Statistical significativity was set at 0.05.

3. Results

Sampling procedures were carried out in 2 separate days, over a one-month period. The number of samples analyzed on day 1 and 2 were 26 (13 arterial and 13 venous blood samples for a total of 13 donkeys) and 10 (5 arterial and 5 venous blood samples for a total of 5 donkeys), respectively.

All the venous samples collected were analyzed (n=17) and included in the statistical analysis, while 6/17 arterial samples were excluded from the statistical analysis due to the alterations caused by erroneous sampling procedures. In particular, these samples presented a SO_2 values over 100%, probably meaning air contamination.

Median and IQR for venous and arterial blood gas parameters assessed with i-STAT® and VetStat® are reported in Tables 1 and 2, respectively.

3.1 Multivariate analysis

The extracted CANs significantly discriminated the 3 groups (P-value Hotelling's t-test < 0.0001), as demonstrated by Figure 1 and Figure 2 for venous and arterial samples, respectively. The 3 groups were clearly separated in both types of blood. For venous samples, the CAN1 variable markedly separated the i-STAT® (positive value) from the other groups (Figure 1). The original variables which accounted mostly for this discrimination were TCO_2 e HCO_3^- (Table 3). The separation of i-STAT® from the other groups by CAN1 was also observed in arterial samples, even if with the opposite sign (Figure 2). In this case, the variables showing a higher discrimination were PO_2, TCO_2, BE and Na (Table 3). Their different relative positions (i.e., the i-STAT® group was in the negative and positive side of the graph in arterial and venous samples, respectively) are due to an algebraic effect that is a quite common event when multivariate statistics are carried out on different data sets [25]. On the other hand, RAD was strongly separated from VetStat® by CAN2 in both types of blood (Figure 1 and 2). The variables most involved in this difference for venous blood were pH, PCO_2, BE, HCO_3^- and Na. On the contrary, for arterial blood the variables related for CAN2 were pH, PO_2, TCO_2, BE, Na, K (Table 3).

3.2 Non parametric analysis

Results concerning the effect of device lecture on the parameters not detected by all three devices (HCT, Anion gap, Ca^{++}, Cl^-) were reported in Table 4.

4. Discussion

The primary advantage of POC testing is the ability to obtain immediate results, minimizing the need to send and await sample results from a clinical pathology laboratory. Also, POC allows more frequent monitoring of critically ill patients, providing the chance to adjust patient treatment [21]. The use of both VetStat® and i-STAT® gas analyzers have been evaluated in horses and reference values have been set [26-30]. Due to the importance in evaluating POC monitors in the population in which they will be used, the aim of the present study was to assess the agreement between two POC blood gas analyzers and a conventional fully automated blood gas analyzer in healthy adult donkeys.

Blood gas analysis can be performed on arterial or venous blood samples; however, despite arterial blood sampling is considered a low-risk procedure, bleeding, infections and arterial injury might represent possible side-effects [31]. Samples collection and processing was easy to perform and feasible in field conditions for both venous and arterial samples.

Different implications of hematological and blood gas parameters have been evaluated in few studies concerning donkey foals [10,12,31] and adult donkeys [32-37]. Our results concerning venous i-STAT® pH were in line with literature [34,37], and slightly lower than studies in donkey foals at different ages (between 24 hours and 21 days of life) [12,31]. Arterial pH i-STAT® was slightly lower compared with one paper in foals [12]. VetStat® pH were slightly higher compared to venous values [31,34,37] and arterial values [12] from literature. These differences might be due to different ages (adults *vs* foals), breeds, gender or to the different devices used for the analysis. Venous and arterial pCO_2 values were similar for both devices with literature [12,31,34,37]. Both venous and arterial values of HCO_3^- obtained from both the devices were slightly lower compared to literature in donkey foals and adults [12,31,34,37]. To the best of authors' knowledge, it was possible to compare tCO_2 values only with literature

from donkey foals. Venous results from both devices and i-STAT® arterial tCO_2 were in line with previous studies [12,31], while VetStat® arterial tCO_2 were slightly lower compared with findings in donkey foals (Veronesi et al., 2014). Venous i-STAT® SO_2 and arterial i-STAT® and VetStat® SO_2 results were in line with literature [31,34], while venous VetStat® were higher [12]. This might be due to the difference in ages (adults *vs* donkey foals) of the two populations. HCT and Hb values were similar to what reported in literature about donkey foals [10,12,31], pregnant and lactating jennies [13] and adult donkeys [33-36], while were slightly lower compared with another study [32]. However, Lemma and Mages [32] performed their study in working donkeys and a slightly higher HCT and Hb compared with animals at rest might be possible as in horses [38]. Concerning electrolyte, venous Ca^{++} evaluated with the i-STAT® were slightly lower compared with literature [36], while Cl^- were slightly higher [12,32,34,36]. Venous i-STAT® Na^+ results were in line with literature, while results for venous VetStat® Na^+ were slightly higher [10,12,13,32,34,36]. Our results concerning venous K+ for both devices were slightly lower compared with other studies [10,12,13,32,34,36]. Differences might be due to different devices used, and different age, sex and gender of the populations. Venous BE and Anion gap results were slightly lower compared with literature [12,31,34], while arterial values were in line with others [12,31]. As already discussed, differences might be related to devices used and different study populations.

The agreement between i-STAT® and RAD was good for venous samples, while was poor for arterial samples. A poor agreement was found between VetStat® and RAD for both venous and arterial samples. These findings might be related to the low number of animals included and to the quite high number of arterial samples not processed for the erroneous handling. Increasing the study population, especially for arterial samples, might be useful in order to

better evaluate the agreement between the VetStat® and RAD for both venous and arterial samples and between i-STAT® and RAD for arterial parameters.

Values of tCO_2, HCT, HCO_3^- and Ca^{++} were different between i-STAT® and RAD, while other values were in agreement. This difference might be related to the methodology, because the i-STAT® works as a “dry chemistry” analyzer, while the RAD is considered a “wet chemistry” analyzer. Dry chemistry analyzers are easily operated and offer a wide range of clinical screening tests that can be performed in an economical, timely, and convenient manner especially in field conditions [39]. Dry clinical chemistry analytical procedures are similar to the established wet clinical chemistry procedures, however, some error tolerance between POC and reference methods’ results might be possible [40]. Further studies are needed in order to increase the study population and evaluate physiological range values especially for those i-STAT® that differ from RAD.

This study presented some limitations due to the low number of samples included and to the absence of a validation. Next step would be investigated the intra- and inter-assay coefficient of variation for both the POCs and to increase the number of animals sampled.

5. Conclusions

In conclusion, dry clinical chemistry analyzers are intended to perform screening tests that alert the practitioner to critical abnormalities. A strong agreement between the i-STAT® and the standard analyzer for most venous analytes has been found. The implementation of the number of subjects might lead to a better understanding of the potential role of the POCs in clinical setting. Finally, increasing population of the study would be recommended in order to set reference values.

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i-STAT®	Venous Median (n=17)	Venous IQR (n=17)	Arterial Median (n=11)	Arterial IQR (n=11)
pH	7.42	0.02	7.43	0.03
pCO_2 mmHg	39.00	2.82	39.35	4.72
pO_2 mmHg	36.00	4.00	100.50	10.50
tCO_2 mmol/L	27.00	1.25	27.00	1.75
SO_2 %	68.00	7.00	98.00	0.00
HCT %	31.0	4.00	32.50	5.50
Hb g/dL	10.51	1.57	11.05	1.85
$BE_{(ecf)}$ mmol/L	1.00	1.25	1.50	1.75
HCO_3^- mmol/L	25.83	1.20	25.70	1.45
Na^+ mmol/L	138.00	3.25	138.00	2.75
K^+ mmol/L	3.85	0.45	3.80	0.27
Ca^{++} mmol/L	1.70	0.06	1.71	0.11

Table 1 – Data concerning median and interquartile range for venous (n=17) and arterial (n=11) parameters assessed with i-STAT® in adult donkeys. Legend: Base $excess_{(extracellular\ fluid)}$ ($BE_{(ecf)}$), Interquartile Range (IQR).

VetStat®	Venous Median (n=17)	Venous IQR (n=17)	Arterial Median (n=11)	Arterial IQR (n=11)
pH	7.45	0.12	7.45	0.03
pCO_2 mmHg	40.00	4.00	35.50	4.72
pO_2 mmHg	45.00	6.00	100.00	20.00
tCO_2 mmol/L	26.10	1.95	24.50	1.62
SO_2 %	79.00	4.00	98.00	2.00
Hb g/dL	14.50	2.52	12.80	1.90
$BE_{(ecf)}$ mmol/L	1.85	1.57	0.50	1.60
Anion Gap mmol/L	18.23	1.50	18.65	1.15
HCO_3^- mmol/L	24.60	2.22	23.40	1.55
Na^+ mmol/L	149.00	3.00	149.00	3.75
K^+ mmol/L	3.90	0.50	3.95	0.37
Cl^- mmol/L	111.00	2.50	111.00	3.00

Table 2 – Data concerning median and interquartile range for venous (n=17) and arterial (n=11) parameters assessed with VetStat® in adult donkeys. Legend: Base excess$_{(extracellular\ fluid)}$ ($BE_{(ecf)}$), Interquartile Range (IQR).

	Venous blood		Arterial blood	
	CAN1	**CAN2**	**CAN1**	**CAN2**
pH	0.192	**0.783**	0.294	**0.912**
PCO_2 mmHg	0.148	**1.448**	0.139	-0.426
PO_2 mmHg	0.340	-0.368	**-0.843**	**-0.775**
tCO_2 mmol/L	**2.070**	0.127	**-1.498**	**0.964**
SO_2 %	-0.493	0.410	**0.675**	0.324
Hb g/dL	0.190	-0.163	-0.559	0.459
$BE_{(ecf)}$ mmol/L	-0.419	**2.271**	**1.008**	**-1.152**
HCO_3^- mmol/L	**-1.739**	**-3.566**	-0.357	-0.127
Na^+ mmol/L	0.422	**1.374**	**0.760**	**1.217**
K^+ mmol/L	0.588	-0.112	0.317	**0.986**

Tab. 3 - Scores of canonical discriminant analysis summarizing the between-groups variation for the variables detected by all three devices (pH, PCO_2, PO_2, tCO_2, SO_2, Hb, BE, HCO_3^-, Na^+ and K^+). Legend: CAN - canonical function; CAN1 - canonical functions discriminating between i-STAT® *vs* a fully automated blood gas analyzer (ABL 700 Series Radiometer, Denmark); CAN2 - canonical function discriminating between VetStat® *vs* a fully automated blood gas analyzer (ABL 700 Series Radiometer, Denmark); Base excess$_{(extracellular\ fluid)}$ ($BE_{(ecf)}$). The variables that have shown a discriminating action for a given CAN are shown in bold.

	Venous blood			P-value	Arterial blood			P-value
	RAD	**i-STAT®**	**VetStat®**		**RAD**	**i-STAT®**	**VetStat®**	
HCT %	31.0	42.2	-	**	32.0	40.8	-	*
Anion Gap mmol/L	-	7.3	18.5	***	-	7.6	18.6	***
Ca^{++} mmol/L	1.7	3.2	-	***	1.7	3.1	-	***
Cl^{-} mmol/L	-	106.0	111.0	***	-	105.0	111.0	**

Tab.4 - Effect of devices on parameter not revealed by all devices (HCT, Anion Gap, Ca^{++} and Cl^{-}) evaluated by Kruskal-Wallis test and Dunn's multiple comparisons test as a post hoc. Legend: "-" data not revealed; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Figure Legend

Figure 1 - Plot of Canonic variables for venous samples.

Figure 2 - Plot of Canonical variables for arterial samples.

Dear Editor,

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The authors declare that they have no competing interests.

Yours sincerely,

Dr Francesca Bonelli

Dear Editor,

This study was approved by the Institutional Animal Care and Use Committee, University of Pisa (n. 45965/2016). The owner's written consent was obtained for all the donkeys included in this study.

Yours sincerely,

Dr Francesca Bonelli

Dear Editor,

Here we declare the contribution of each author:

1) Francesca Bonelli: conducting the study, data analysis, drafting and revising the article for intellectual content, approval of the final version.
2) Fulvio Laus: conception and design of the study, data acquisition and approval of the final version.
3) Angela Briganti: data acquisition and approval of the final version.
4) Marilena Bazzano: data acquisition and approval of the final version.
5) Flavia Evangelista: conducting the experiment and approval of the final version.
6) Giuseppe Conte: data analysis and interpretation, approval of the final version.
7) Micaela Sgorbini: conception and design of the study, data acquisition, analysis and interpretation, revising the article for intellectual content, approval of the final version.

Yours sincerely,

Dr. Francesca Bonelli