Title Page

Use of a saliva-based diagnostic test to inform on tapeworm infection in horses in the UK

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Author's declaration of interests

The authors Corrine J. Austin and Kirsty L. Lightbody report an affiliation to a commercial entity with a financial interest in materials discussed in this manuscript.

Ethical animal research

Saliva samples were collected as part of a routine targeted deworming programme.

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Authorship

Kirsty L. Lightbody analysed the data, drafted and revised the manuscript. Jacqueline B. Matthews critically reviewed and revised the manuscript. Jeremy G. Kemp-Symonds, Bransby Horses Veterinary Consultant, contributed towards acquisition of the samples, prescribed anthelmintics and critically reviewed and revised the manuscript. Peter A. Lambert contributed towards data analysis and critically reviewed and revised the manuscript. Corrine J. Austin analysed the data and critically reviewed and revised the manuscript.

Summary

Background

Anthelmintic resistance combined with limited chemotherapeutic options has prompted a change in approaches to control of equine helminth infections. Targeted selective treatment (TST) strategies utilise diagnostics to reduce anthelmintic use by treating individuals with worm burdens or egg shedding levels above a set threshold. Whilst faecal egg count analysis has limitations for informing tapeworm treatment, a commercially available saliva-based diagnostic test accurately diagnoses horses with tapeworm infection.

Objectives

Evaluation of a saliva-based diagnostic test to identify horses naturally-infected with tapeworm and assess the impact of using the test to inform anthelmintic administration.

Study design

Retrospective longitudinal study.

Methods

Saliva was collected from horses (n=237) at a UK welfare charity from autumn 2015-autumn 2016. Horses diagnosed as positive for tapeworm infection using the EquiSal® Tapeworm test were anthelmintic treated according to weight. The number of horses that received anthelmintic treatment based on the test result was compared to an all-group treatment approach and the reduction in anthelmintic usage calculated. Incoming horses were also tested (n=143) and the information used to inform quarantine treatments.

Results

In autumn 2015, 85% of 237 horses tested received no anthelmintic and the majority (71%) of these remained below the treatment threshold throughout the study. Of the 69 horses that received treatment, seven required treatment following three subsequent tests, whilst >50% of horses administered with anthelmintic fell below the treatment threshold at the following test. No increase in tapeworm prevalence within the 237 horses was observed during the study despite a substantial reduction in the application of anti-tapeworm treatments. A total of 41% of incoming horses required anti-cestode treatment.

Main Limitations

Other management practices were not included in the analysis.

Conclusions

Compared to an all-group treatment strategy, the diagnostic-led approach used here considerably reduced application of anti-cestode anthelmintics. This could reduce selection pressure for anthelmintic resistance.

Introduction

Horses are exposed to a range of parasitic helminths when grazing. These include tapeworms (*Anoplocephala perfoliata*, *Anoplocephala magna*, *Paranoplocephala mamilliana*) and roundworms (cyathostomins, large strongyles, *Parascaris equorum*). Studies in the UK and Ireland have shown *A. perfoliata* to be present in 51-69% of horses examined [1, 2, 3, 4]. These parasites have been associated with colic, weight loss and colitis [5, 6, 7]. Previously, frequent all-group administration of anthelmintics was used to control equine helminths; however, interval treatment-based practices strongly selected for anthelmintic resistance, especially in cyathostomins and *P. equorum* [8, 9, 10]. A limited number of anthelmintic classes are available for treating equine helminths and with no new chemical classes in development, care must be taken to preserve efficacy of the currently-effective products [9, 10]. Targeted selective treatment (TST) strategies aim to reduce use of anthelmintics by exploiting diagnostics (for example, faecal egg count [FEC] analysis) to identify animals that require treatment to reduce shedding of worm eggs in faeces. In the UK, this has become relatively commonplace in worm control programmes [11, 12] and is of value for detecting nematode eggs but not cestode eggs; the excretion of *A. perfoliata* eggs is intermittent and eggs are unevenly distributed in faeces resulting in low sensitivity of coprological analysis [13]. To address this, a serum-based test to diagnose tapeworm was developed and has been commercially available for over a decade [14]. More recently, a non-invasive saliva ELISA test (EquiSal® Tapeworm[1]) [4], based on the same antigens, was developed to facilitate uptake of testing by horse owners. The EquiSal® Tapeworm test, validated by comparing antigen-specific antibody levels in 104 equine saliva samples with tapeworm burdens post-mortem, differentiated 'low' burdens (0 tapeworm) from 'moderate/high' burdens (>1 tapeworm) with 83% sensitivity and 85% specificity [4]. The pathological effects of tapeworm correlate with burden intensity and lesions are more severe in horses with >20 tapeworms [2, 15, 16]. In a previous study, all horses with a clinically-relevant 'high' burden (>20 tapeworms) were accurately diagnosed using this test [4]. In practice, diagnosis is based on a 'saliva score', with a score of <-0.09 assigned as 'low' and >0.6 assigned as 'moderate/high'. Anti-tapeworm treatment is recommended when a saliva score = or > -0.09 is obtained. Reported here, is the first study evaluating the utility of this test in naturally-infected horses and the impact that use of the test has on the number of praziquantel administrations applied over 11 months.

Materials and Methods

Sample population

Saliva samples (n=1,000) were collected from horses as part of a site-wide targeted treatment programme for tapeworm at a UK welfare charity. Samples were predominantly obtained in October/November 2015 ('autumn 2015', n=305), April/May 2016 ('spring 2016', n=328) and August/September 2016 ('autumn 2016', n=367) and were collected from horses of both sexes and various breeds over a wide age range. The horses were grazed in 28 fields and only stabled under exceptional circumstances. The fields were maintained by a variety of means, including manure collection in some fields and resting of paddocks. During the study, between autumn 2015 and autumn 2016, a total of 81 horses left the premises and 143 horses were introduced. This included 40 horses leaving before testing in spring 2016 and a further 41 leaving before testing in autumn 2016. Eighty-eight new horses arrived between autumn 2015 and spring 2016 and 85 of these were tested in spring 2016. A further 53 new horses arrived between spring 2016 and autumn 2016 and two horses returned to re-join the population. Saliva samples were collected using EquiSal® saliva collection kits[1] as per manufacturer's instructions. The samples were stabilised in preservative buffer (1x PBS, 0.05% Tween 20, 0.05% 5-bromo-5-nitro-1,3-dioxane (BND) and 0.05% sodium azide) and centrifuged at 3000 *x g* for 5 min prior to analysis.

Diagnostic testing

EquiSal® Tapeworm testing was carried out at Austin Davis Biologics[1] as described by Lightbody *et al.* [4], where each saliva sample was analysed in a test that utilised three ELISAs; the 'Specific' ELISA to detect IgG(T) specific to excretory/secretory 12/13 kDa tapeworm antigens, the 'Non-Specific Binding' (NSB) ELISA to determine levels of non-specific binding and the 'Total' ELISA to measure the total amount of IgG(T) in the sample [4].

Anthelmintic administration

Horses were treated for *Anoplocephala* infection based on the saliva score derived from the EquiSal® Tapeworm test, above. Horses diagnosed as 'low' (<-0.09) received no anti-cestode treatment and those diagnosed above the 1+ burden cut-off, indicated by a 'borderline' (-0.09-0.6) or 'moderate/high' (>0.6) saliva score, were administered with praziquantel-based products such as Equimax[2], Equest Pramox[3], Noropraz[4] and EquiTape[5] by attending staff. Horses were weighed using a weighbridge and were dosed with between 1-2.5 mg of praziquantel/kg as per manufacturers' instructions on the product used.

Analysis

Microsoft® Office Excel 2013[6] was used to record EquiSal® Tapeworm test dates and saliva scores for each horse. Horses were organised according to the number of tests carried out then sorted according to test dates. New arrivals, as well as horses that had left the premises, were identified. For the purposes here, only data from permanent residents between autumn 2015 and autumn 2016, referred to as the 'constant herd', were analysed using scatter plots in Microsoft® Office Excel 2013. EquiSal® Tapeworm saliva scores for individuals were plotted over the testing period and the number of anti-cestode anthelmintic administrations calculated based on the number of borderline or moderate/high diagnoses.

Results

The constant herd (n=237) comprised horses tested on all three occasions (n=215) and horses tested in autumn 2015 and autumn 2016, but not spring 2016 (n=22). Within this population were 113 geldings (48%) and 124 mares (52%) (Figure 1, panel A) with ages ranging from 1 year to 37 years with 38 (16%) aged between 1 to 5 years, 63 (27%) aged between 5 to 10 years, 36 (15%) aged between 11 to 15 years, 41 (17%) aged between 16 to 20 years, 38 (16%) aged between 21 to 25 years, 18 (8%) aged between 26 to 30 years and three (1%) aged over 30 years (Figure 1, panel B). The constant herd included a wide range of breeds including Thoroughbreds (TBs), Arabs and crosses (n=24), Cobs and crosses (n=65), Native ponies and crosses (n=139), Warmbloods and crosses (n=5) and Draught horses and crosses (n=4) (Figure 1, panel C).

Between autumn 2015 and autumn 2016, a total of 1,000 saliva samples were tested (Table 1). This included 305 horses tested between October and December 2015 (autumn 2015), 328 tested between March and June 2016 (spring 2016) and 367 tested between August and October 2016 (autumn 2016). Analysis of the test outputs revealed that 71% of the constant herd (168 horses) received no anti-cestode treatment between autumn 2015 and autumn 2016. Only 69 horses (29% of the constant herd) were administered a praziquantel-based anthelmintic during this period (Figure 2).

Testing in autumn 2015 showed that 202 horses (85%) from the constant herd fell below the treatment threshold and were diagnosed as having a low burden (below 1+ tapeworm cut-off) (Table 2). Analysis of saliva from the remaining 35 horses (15% of the constant herd) provided values categorised as borderline (n=17) or moderate/high (n=18) (above 1+ tapeworm cut-off). All of these were administered a praziquantel-based anthelmintic (Table 2).

When re-tested in spring 2016, 184 horses (86%) of the 215 constant herd horses sampled were diagnosed as low and did not require treatment (Table 2). Thirty-one horses (14%) were administered a praziquantel-based anthelmintic as they fell in the borderline (n=13) or moderate/high (n=18) categories (Table 2). Of the 31 horses identified as having scores above the treatment threshold, 15 had previously been below the treatment threshold (Figure 3, Panel A). The remaining 16 horses (7% of the constant herd) had previously been treated in autumn 2015, following borderline (n=4) or moderate/high (n=12) test results. The results obtained in spring 2016 indicated that 17 (52%) of 33 constant herd horses that received anti-cestode treatment in autumn 2015, fell below the treatment threshold and did not require further treatment (Figure 3, Panel B). Of the sixteen horses diagnosed above the treatment threshold in autumn 2015 and spring 2016, 12 had a lower saliva score in spring 2016 following anthelmintic treatment in autumn 2015 (Figure 3, Panel C). Only four horses had a higher saliva score in spring 2016 compared to autumn 2015 (Figure 3, Panel C).

In autumn 2016, 204 (86%) of the 237 constant herd horses were diagnosed as low (Table 2). Thirty-three horses (14% of the constant herd) had scores above the treatment threshold and were diagnosed as borderline (n=16) or moderate/high (n=17) (Table 2). Nineteen horses requiring anti-cestode treatment in autumn 2016 had previously been diagnosed as low in autumn 2015 and spring 2016 and had received no prior treatment, and two horses had been treated in autumn 2015, but were below the treatment threshold in spring 2016 (Figure 4, Panel A). Of the constant herd horses that were administered a praziquantel-based anthelmintic in spring 2016 (n=31), 19 horses (61%) were diagnosed as low and did not require further treatment in autumn 2016 (Figure 4, Panel B). Twelve horses were diagnosed as borderline or moderate/high in autumn 2016 following treatment in spring 2016 and included seven horses with lower saliva scores compared to the previous test and five with increased saliva scores (Figure 4, Panel C).

Overall, 168 horses (71% of the constant herd) remained below the treatment threshold at all three test time points. Thirty-six horses (15% of the constant herd) were diagnosed as low in autumn 2016, following a borderline or moderate/high result in autumn 2015 and/or spring 2016 (Figure 5, Panel A). The 33 horses (14% of the constant herd) with saliva scores above the treatment threshold in autumn 2016 included nineteen horses diagnosed as low in autumn 2015 and spring 2016 (Figure 5, Panel B) and 14 horses diagnosed as borderline or moderate/high in autumn 2015 and/or spring 2016 (Figure 5, Panel C). In total, only seven horses (3% of the constant herd) were diagnosed as borderline or moderate/high in all three tests (Figure 5, Panel D). This included four horses categorised as moderate/high in all tests, two categorised as moderate/high in autumn 2015 and spring 2016 and borderline in autumn 2016 and one categorised as moderate/high in autumn 2015 and borderline in spring and autumn 2016.

The gender, age and breed of horses in the constant herd and the number of borderline or moderate/high diagnoses obtained between autumn 2015 to autumn 2016 are shown in Table 3. Geldings and mares reported a similar proportion of borderline or moderate/high diagnoses (32/113 vs. 37/124), however younger horses required more treatments with 21/38 (55%) of 1-5 year old horses being diagnosed as borderline or moderate/high on at least one occasion. In addition, 10/38 (26%) of the 1-5 year old horses required multiple treatments following borderline or moderate/high diagnoses in two or three tests. Cobs and Cob crosses received the most treatments, with 32/65 (49%) reporting at least one borderline or moderate/high result.

Over the study, 141 new horses arrived and two horses returned to the site. Analysis of the test results, indicated that 35 (40%) of the 88 horses that arrived from autumn 2015 to spring 2016 and 23 (42%) of the 55 horses that arrived between spring 2016 and autumn 2016 were diagnosed in the borderline or moderate/high categories.

Discussion

As demonstrated for strongyle FEC-based TST-strategies [8, 11], the results here show that use of a diagnostic test to inform on the requirement to treat *Anoplocephala* infection, led to lower anthelmintic usage compared with an interval treatment strategy in which all horses were administered with an anti-cestode product in the spring and autumn. In total, 99 doses of praziquantel product were administered to the constant herd using this approach. This represents an 86% reduction in anthelmintic administration during the study period compared to an interval treatment strategy based on two annual treatments for all horses. Despite the reduction in anthelmintic use, tapeworm infection prevalence did not increase during the course of the study.

The majority of horses from the constant herd, diagnosed as being below the treatment threshold in autumn 2015, fell below this level and were diagnosed as low in subsequent tests. The apparent over-dispersed distribution of infection here is similar to previous reports on tapeworm infection intensity [18, 19, 20], as well as for other types of equine helminths [21, 22]. The fact that 168 horses were diagnosed as low on all three occasions suggests that some horses control *Anoplocephala* burden level, similar to results reported for nematode infections, where some horses repeatedly have low FEC values, even in the absence of anthelmintic treatment [17, 23, 24], or they were not exposed to infection.

Praziquantel is rapidly absorbed following oral administration and the drug and its metabolites are predominantly excreted within 24 hr [25]. Despite the lack of persistence of the drug, most horses that received praziquantel treatment in autumn 2015 or spring 2016, showed lower saliva scores in the subsequent test; with 54% and 61% of the horses treated in autumn 2015 and spring 2016 falling below the treatment threshold at the following test. This indicates that treatments were likely to have been effective or to have lowered the burden sufficiently to reduce the stimulation of antigen-specific IgG(T). The remaining 46% of horses that received treatment in autumn 2015 tested above the treatment threshold again in spring 2016 and 39% of horses treated in spring 2016, tested above the treatment threshold again in autumn 2016. It is possible that these horses had become re-infected as a previous study [4] reported that saliva scores of eight horses fell below the treatment threshold within five weeks of treatment and the scores of three remaining horses fell below the treatment threshold within three months, indicating that, in a re-test at six months, saliva scores above the threshold are suggestive of the acquisition of new infection. Notably, a substantial increase in tapeworm incidence was not observed in those horses diagnosed as low burden, as only 7% of untreated horses in autumn 2015 required treatment following testing in spring 2016 and similarly, only 11% of untreated horses in spring 2016 required treatment following testing in autumn 2016.

The patterns of infection and reinfection here highlight the value of biannual monitoring as the prevalence and frequency of tapeworm infections can be affected by factors such as grazing practice, pasture management, the presence of oribatid mite intermediate hosts, as well as climatic and environmental conditions [19, 26, 27, 28]. Prevalence and distribution of tapeworm infection will vary between seasons and between geographical locations; therefore regular biannual monitoring of individuals would identify those acquiring new infections allowing treatment at an early stage, reducing contamination of the pasture and limiting exposure of the rest of the herd, as well as preventing unnecessary use of anthelmintics. Regular monitoring will also identify those individuals which may be more prone to re-infection.

Over the study period, only seven horses (9% of the constant herd tested) received three doses of praziquantel-based anthelmintic due to borderline or moderate/high scores in all tests. This group included four Cob geldings, one Thoroughbred gelding, one Cob mare and a Native pony mare. In line with previous reports, no association between gender and tapeworm infection was observed in this study [19, 26]. Studies have also reported that tapeworm infection prevalence and intensity is not influenced by age or breed [2, 16, 18, 26]. Younger horses in this population (between 1-5 years) reported more borderline or moderate/high scores than older horses (>6 years) and although this may be suggestive of age-related exposure to infection or the development of acquired immunity [29], and is similar to the influence of age on strongyle infections [22, 30], additional research would be required to investigate and confirm age-related exposure as determined by saliva testing. The high number of Cobs and Cob crosses reporting borderline or moderate/high results throughout this study may be related to age rather than breed, as 40% of these horses were in the 1-5 year age group.

New horses joining the population may be responsible for pasture contamination. Here, 41% of new arrivals were diagnosed with a burden indicative of a requirement to treat. This proportion of test-positive horses is higher than that of the constant herd (approximately 15%) and may be reflective of horses of arriving from sites with unknown or questionable helminth control practices. This augments the need to enact appropriate quarantine procedures, either by testing or deworming, on all incoming individuals into populations.

The EquiSal® Tapeworm test [4] reports sensitivity and specificity of 83% and 85%, respectively, when a 1+ tapeworm cut-off is applied (low burden = 0 tapeworm, moderate/high burden = 1+ tapeworm). In comparison, coprological diagnosis of *Anoplocephala* infection is highly variable, in part due to the sporadic release of tapeworm eggs, poor distribution of eggs in faeces, the FEC technique used and the burden present [13, 20]. Modified FEC methods, such as the centrifugation/flotation technique, are most sensitive, reporting up to 61% sensitivity, however they are more time consuming and labour intensive than standard methods [7, 13, 18, 20, 31]. Sensitivity of the centrifugation/flotation technique can be increased to approximately 90% when diagnosing infections with >20 tapeworms present [18, 31]. When a 20+ tapeworm cut-off is applied to the EquiSal® Tapeworm test (low/moderate burden = 0-19 tapeworms, high burden = 20+ tapeworm), sensitivity is 86% [4]. Overall, the EquiSal® Tapeworm test shows similar sensitivity to modified FEC analysis when diagnosing high (20+) tapeworm burdens however, the saliva-based test displays higher sensitivity when diagnosing low (1+) burdens, which may include infections with immature tapeworms that would not be identified using FEC methodologies [18, 20, 28]. If saliva testing is undertaken twice a year and/or at least four months after anti-cestode treatment, then it is likely that antibodies, if detected, are due a current infection rather than a historic infection as it was reported that antigen-specific salivary antibodies of 11 horses reduced below the 1+ tapeworm cut-off within three months of treatment [4]. However, the dynamics of tapeworm-specific antibodies in the saliva will depend on the individual and whether reinfection occurs.

The use of diagnostic tests to predict *Anoplocephala* burden and hence inform anti-cestode treatments will reduce the frequency of chemicals, such as praziquantel and pyrantel, used in practice. This theoretically could reduce selection pressure for the development of drug resistance not just in cestodes but in nematodes too, as some products contain a combination of praziquantel and macrocyclic lactones. Resistance to macrocyclic lactones in nematodes is already a concern as shortened egg reappearance periods (ERP) following ivermectin and moxidectin use have been reported [10, 32, 33]. Additionally, resistance to pyrantel, a broad spectrum anthelmintic used to treat tapeworm infections, has also been reported in nematodes [8, 32, 34]. Although anthelmintic resistance has yet to be documented for *A. perfoliata* and other tapeworm species, it is not unforeseeable that, with the widespread application of regular blanket treatments, resistance would be inevitable. This study demonstrates that the saliva-based EquiSal® Tapeworm test holds promise in reducing treatment frequency in practice, which could help protect efficacy of anti-cestode anthelmintics for the future.

Manufacturer's details

1 Austin Davis Biologics Ltd., Great Addington, Northamptonshire, UK

2 Virbac, Carros, France

3 Zoetis, London, UK

4 Norbrook Laboratories Ltd, Newry, UK

5 Bayer, Newbury, UK

6 Microsoft, Redmond, WA, USA

Figure Legends

Figure 1. Demographics of the constant herd horses (n=237), including gender (A), age (B) and breed (C).

Figure 2. Anti-cestode treatments administered to constant herd horses (n=237) between autumn 2015 and autumn 2016. Ninety-nine doses of anthelmintic were administered to 69 horses, with 46 treated once, 16 treated twice and seven treated three times.

Figure 3. EquiSal® Tapeworm test saliva scores for constant herd horses tested in autumn 2015 and spring 2016. (A) Untreated horses that changed from a low to a borderline or moderate/high (n=15). (B) Horses treated in autumn 2015 that changed from a borderline or moderate/high result to a low result (n=17). (C) Horses treated in autumn 2015 that remained in either borderline or moderate/high result categories (n=16).

Figure 4. EquiSal® Tapeworm test saliva scores for constant herd horses tested in autumn 2015, spring 2016 and autumn 2016. (A) Untreated horses from spring 2016 that changed from low to a borderline or moderate/high result (n=21). (B) Horses treated in spring 2016 that changed from a borderline or moderate/high result to a low result (n=19). (C) Horses treated in spring 2016 that remained either in borderline or moderate/high result categories (n=12).

Figure 5. EquiSal® Tapeworm test saliva scores for constant herd horses tested in autumn 2015, spring 2016 and autumn 2016. (A) Horses treated in either autumn 2015 and/or spring 2016 that changed from a borderline or moderate/high result to a low result in autumn 2016 (n=36). (B) Untreated horses from autumn 2015 and spring 2016 that changed from a low result to a borderline or moderate/high result in autumn 2016 (n=19) (C) Horses treated in autumn 2015 and/or spring 2016 that remained in either borderline or moderate/high results categories in autumn 2016 (n=14). (D) Horses treated in both autumn 2015 and spring 2016 that remained in either borderline or moderate/high results categories in autumn 2016 (n=7).

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Tables

Table 1. Summary of numbers of horses analysed using the EquiSal® Tapeworm test between autumn 2015 and autumn 2016.

	Autumn 2015 horse numbers	Spring 2016 horse numbers	Autumn 2016 horse numbers
Constant herd horses tested three times	215	215	215
Constant herd horses tested twice	22	0	22
Non-constant herd horses tested	68	113	130
Horses arrived	-	88	53
Horses returned	-	0	2
Horses departed	-	40	41

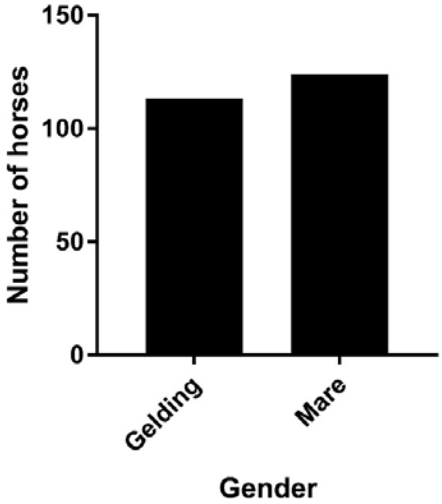
Table 2. EquiSal® Tapeworm test diagnosis and treatment recommendations. Below treatment threshold = saliva score <-0.09 ('low'); above treatment threshold = saliva score =>-0.09 ('borderline' or 'moderate/high').

	Autumn 2015		Spring 2016		Autumn 2016		
EquiSal® Tapeworm test Diagnosis	Constant herd horses	Constant herd horses (%)	Constant herd horses	Constant herd horses (%)	Constant herd horses	Constant herd horses (%)	Treated for tapeworm
Low	202	85	184	86	204	86	No
Borderline + moderate/high	35	15	31	14	33	14	Yes
Total	237		215		237		

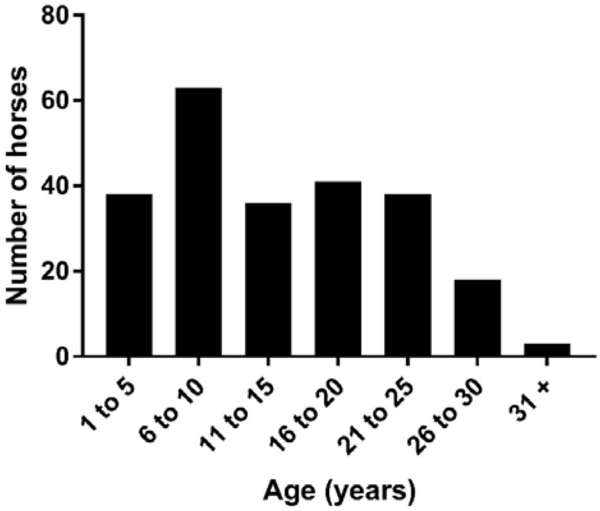
Table 3. Summary of the gender, age and breed of horses that remained low in all three EquiSal® tests between autumn 2016 and spring 2016 (n=168) and horses that were diagnosed as borderline (B) or moderate/high (M/H) in 1 test (n=46), 2 tests (n=16) and 3 tests (n=7).

	Category	B or M/H in 0 tests	B or M/H in 1 test	B or M/H in 2 tests	B or M/H in 3 tests
Gender	Gelding	81 (72%)	21 (19%)	6 (5%)	5 (4%)
	Mare	87 (70%)	25 (20%)	10 (8%)	2 (2%)
Age	1 to 5 years	17 (45%)	11 (29%)	6 (16%)	4 (10%)
	6 to 10 years	44 (70%)	14 (22%)	3 (5%)	2 (3%)
	11 to 15 years	31 (86%)	4 (11%)	1 (3%)	0 (0%)
	16 to 20 years	30 (73%)	9 (22%)	2 (5%)	0 (0%)
	21 to 25 years	28 (74%)	6 (16%)	4 (10%)	0 (0%)
	26 to 30 years	15 (83%)	2 (11%)	0 (0%)	1 (6%)
	30+ years	3 (100%)	0 (0%)	0 (0%)	0 (0%)
Breed	Thoroughbreds, Arabs and crosses	18 (75%)	5 (21%)	0 (0%)	1 (4%)
	Cobs and crosses	33 (51%)	18 (28%)	9 (14%)	5 (7%)
	Native ponies and crosses	110 (79%)	22 (16%)	6 (4%)	1 (1%)
	Warmbloods and crosses	3 (60%)	1 (20%)	1 (20%)	0 (0%)
	Draught horses and crosses	4 (100%)	0 (0%)	0 (0%)	0 (0%)

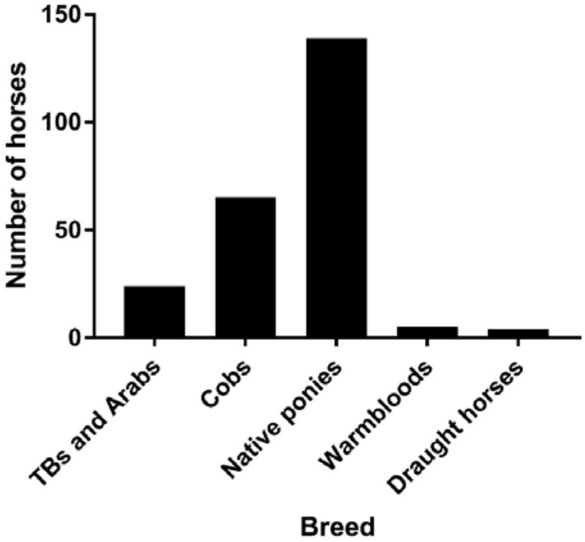
A.

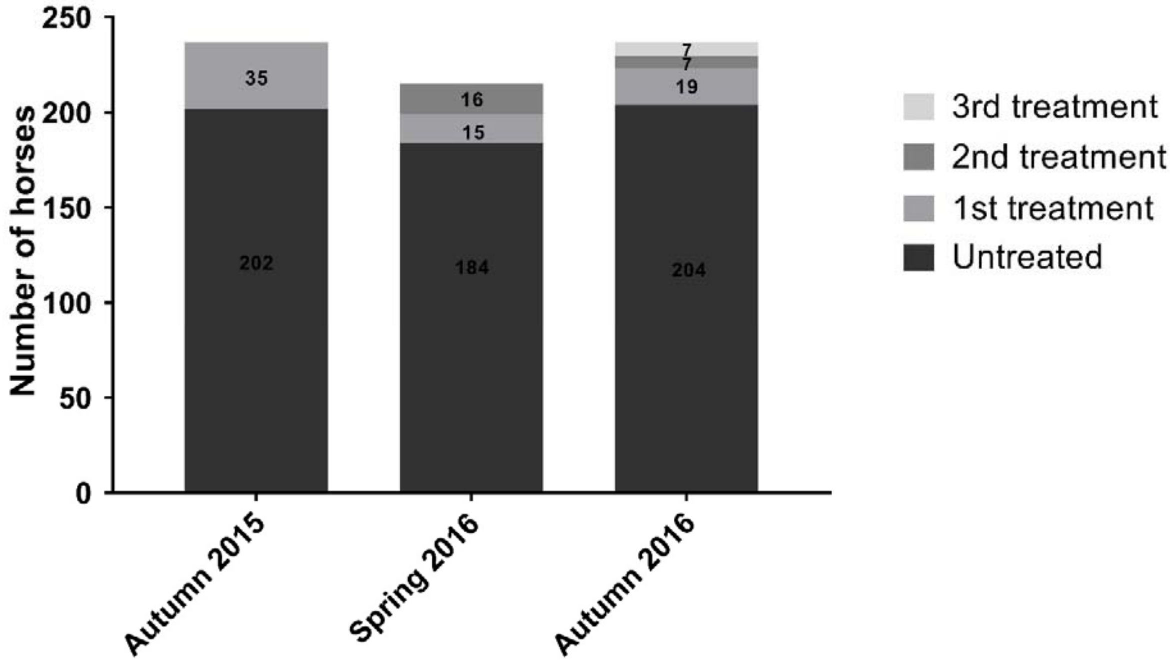

150
100
50
0
Number of horses
Gelding
Mare
Gender

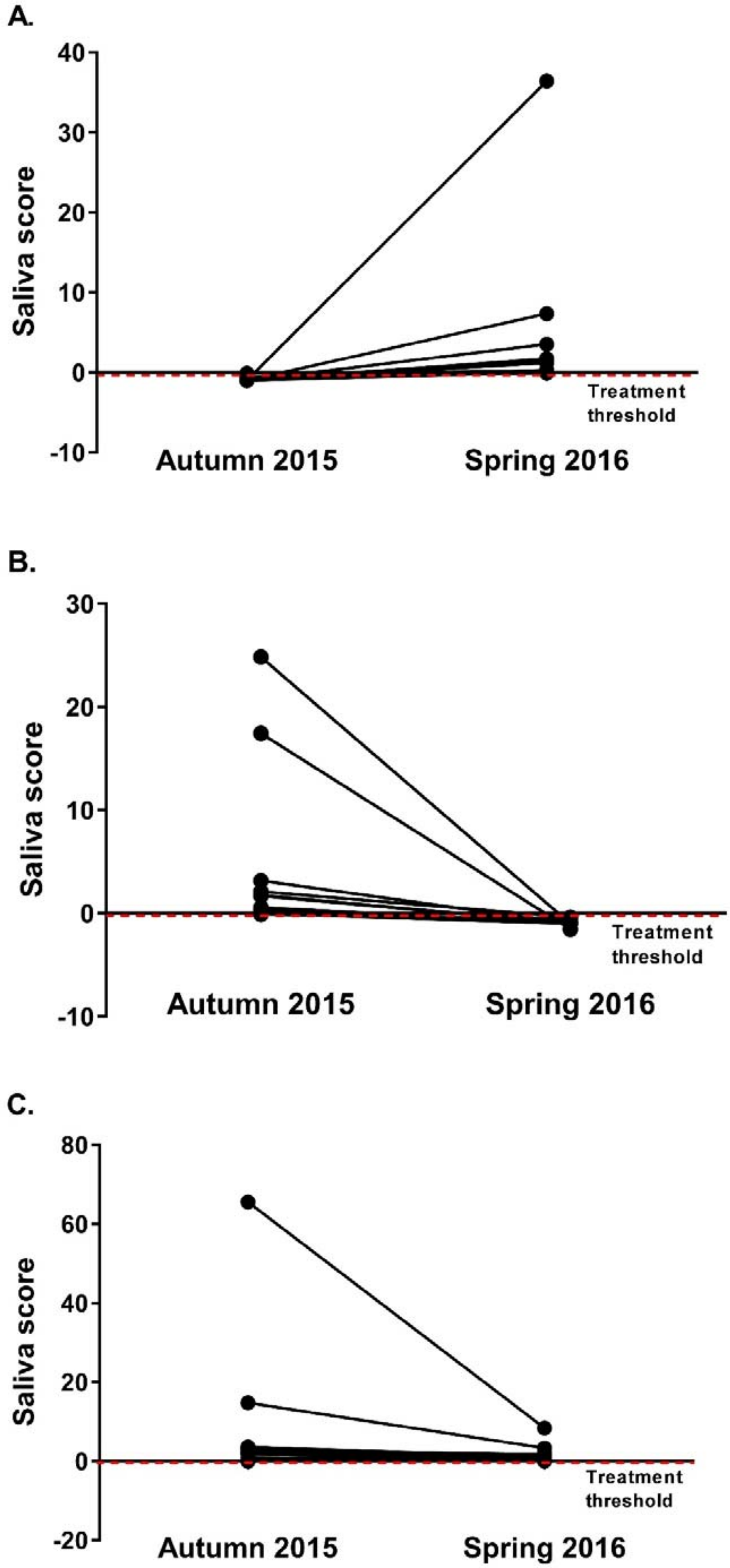
B.


80
60
40
20
0
Number of horses
1 to 5
6 to 10
11 to 15
16 to 20
21 to 25
26 to 30
31 +
Age (years)

C.

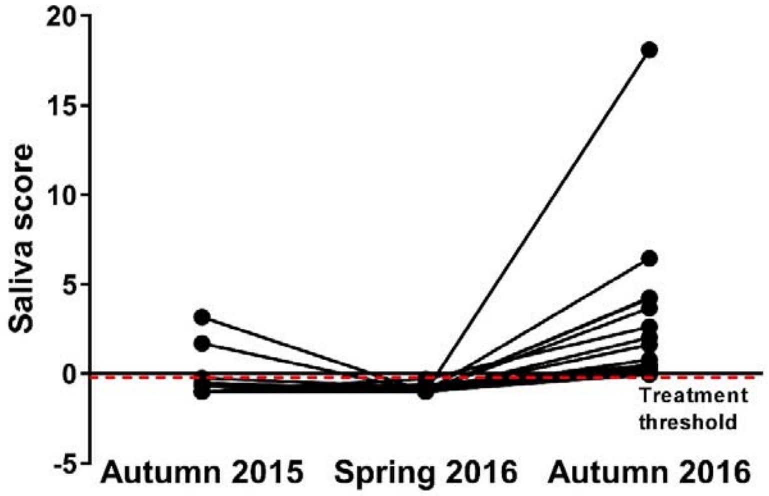

150
100
50
0
Number of horses
TBs and Arabs
Cobs
Native ponies
Warmbloods
Draught horses
Breed


Number of horses
250
200
150
100
50
0
35
202
16
15
184
7
7
19
204
Autumn 2015
Spring 2016
Autumn 2016
3rd treatment
2nd treatment
1st treatment
Untreated

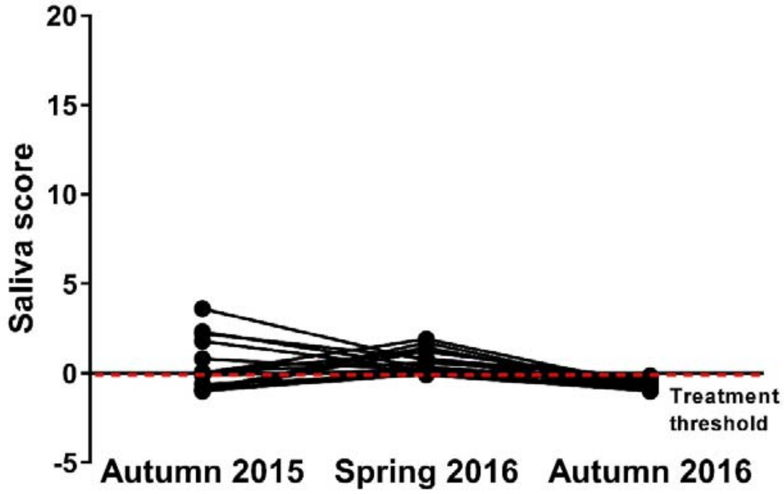

A.
40
30
20
10
0
-10
Saliva score
Treatment
threshold
Autumn 2015
Spring 2016
B.
30
20
10
0
-10
Saliva score
Treatment
threshold
Autumn 2015
Spring 2016
C.
80
60
40
20
0
-20
Saliva score
Treatment
threshold
Autumn 2015
Spring 2016

471

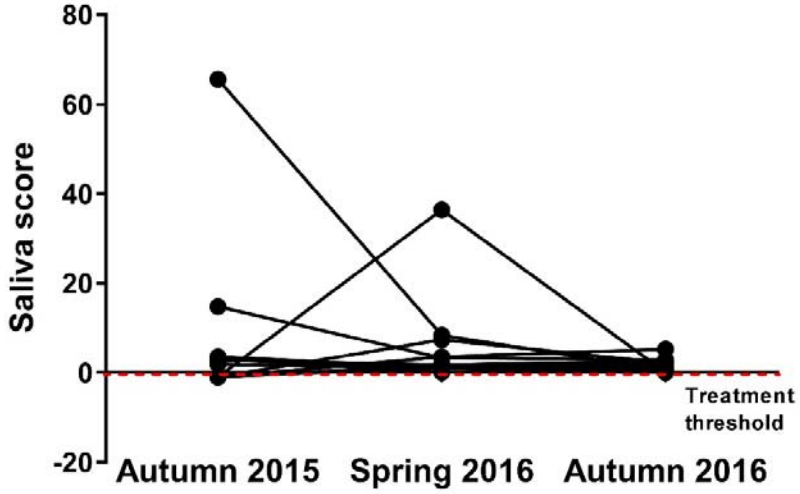
A.


20
15
10
5
0
-5
Saliva score
Treatment threshold
Autumn 2015
Spring 2016
Autumn 2016

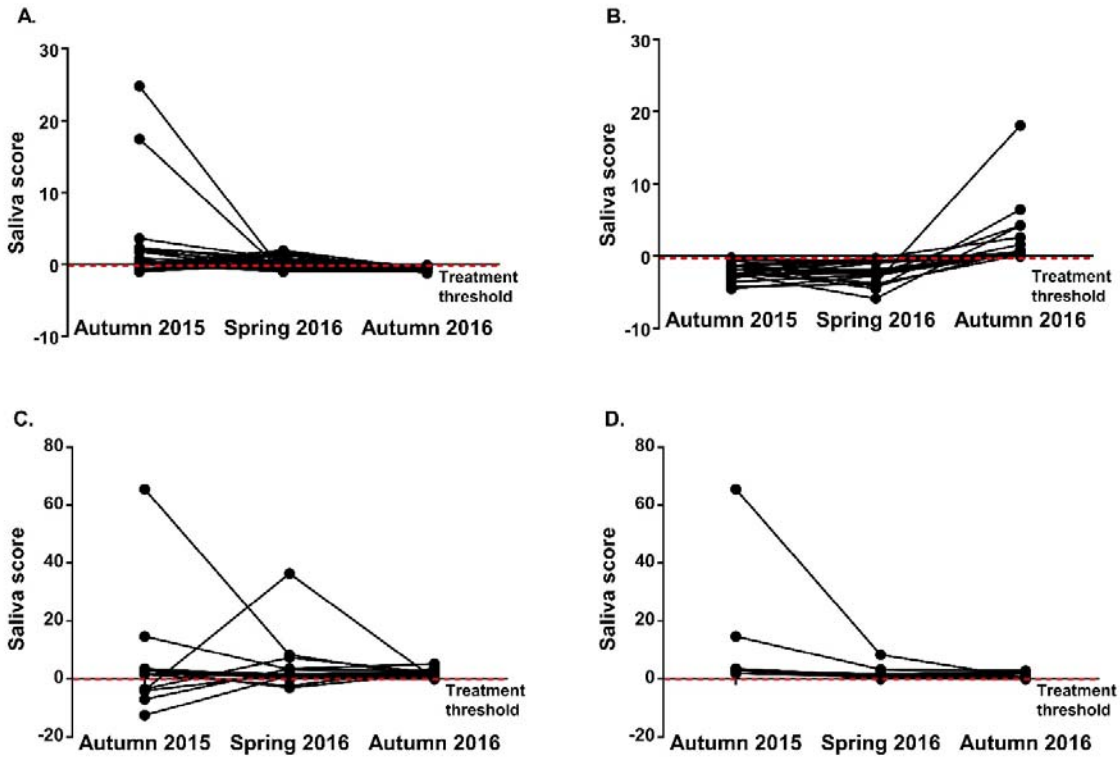
B.


20
15
10
5
0
-5
Saliva score
Treatment threshold
Autumn 2015
Spring 2016
Autumn 2016

C.


80
60
40
20
0
-20
Saliva score
Treatment threshold
Autumn 2015
Spring 2016
Autumn 2016

473



A.
B.
C.
D.
Saliva score
Autumn 2015
Spring 2016
Autumn 2016
Treatment threshold
30
20
10
0
-10
80
60
40
20
-20