Piroplasmosis in Italian Standardbred Horses: 15 Years of Surveillance Data

PIROPLASMOSIS IN ITALIAN STADARDBRED HORSES: 15 YEARS OF SURVEILLANCE DATA

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Abstract

This study aimed to document the prevalence of chronic equine piroplasmosis (EP) in poorly performing Standardbred racehorses and to explore associations between the disease and sex, age and hematological parameters. Blood was collected between 2004 and 2018; blood cell counts were performed using a cell counter analyser, biochemical parameters using a photometer, and serum proteins using agarose gel electrophoresis. Blood smears were prepared, coloured with a modified Giemsa, and an experienced technician identified the presence of protozoa. The horses were categorised into piroplasmosis positive (PP) and piroplasmosis negative (PN). The studied population included 520 horses (142 female, 27.6%; CI 23.8% - 31.7%), with a median age of 4 (IQR 3 to 8) years. The prevalence of EP was 9.3% (CI: 6.9% - 12.1%). There was no association between the infectious status and signalment. In Italian poorly performance Standardbreds, chronic piroplasmosis caused mild normocytic, normochromic anemia, hyperbilirubinemia, thrombocytopenia and hypergammaglobulinemia, as reported in literature. However, our findings suggests that blood analysis needs to be interpreted with caution as there were cases with overlap between parameters in PP and PN horses, and normal ranges. Hence, in cases of poorly performing Standardbreds living or recently moving into an EP endemic region, blood cytology should be performed as a first step in differential diagnosis procedures to exclude chronic EP as one possible cause for poor performance. Further diagnostic tests (i.e. PCR, ELISA) are also recommended because correct diagnosis is vital to ensure the criteria of "lack of disease" in the principle of good health.

Keywords: poor performance; hematology; diagnosis; health; welfare

1. Introduction

Equine piroplasmosis (EP) is a blood-borne disease caused by *Babesia caballi* and *Theileria equi* [1]. Worldwide, EP has an economic impact on the horse industry, and horses must be tested for EP before travelling internationally [2]. EP is endemic in tropical and subtropical areas, including all of the Mediterranean Region [1]. However, some countries (e.g. USA) are EP free and movements of horses is the highest risk factor for spreading the disease into these areas [3]. EP is endemic in Italy; whist *Theileria equi* has been identified the most prevalent species, some horses were found infected with *Babesia caballi, Babesia canis, Theileria annae, Theilleria sergenti* and *Theileria buffeli* [4, 5]. EP can manifest in hyperacute, acute, subacute and chronic forms [6]. The acute form causes fever, lethargy, petechiations on mucous membranes, icterus, pigmenturia and severe anemia, and the hyperacute form can be fatal [7]. Hyperacute case are usually due to *T. equi* affecting naïve horses introduced into an EP endemic region [6]. Horses with subacute form show less severe clinical signs, with similarities to the acute form [5]. Chronic infection, mainly due to *T. equi* or *B. Caballi,* results in non-specific signs, including lethargy, partial anorexia, weight loss, and poor performance [6, 7]. Chronic EP may be difficult to diagnose and consequently the prevalence underestimated.

Poor performance is a common problem in racing horses. A definitive diagnosis is often difficult to reach and requires a complete evaluation, including laboratory tests, respiratory scoping, cardiac examination and dynamic evaluation on the high speed treadmill [8, 9]. Furthermore, it has been reported that poor performing racehorses were kept in training, and were over trained in the hope of improving performance [10]. Appropriate differential diagnostic procedures, and avoiding strenuous exercise of an animal that could be affected by a systemic and/or infectious disease, would be vital to safeguard the principle of "good health" within the welfare quality system [11]. Whist EP is endemic in Italy and its chronic form is difficult to diagnose as the clinical signs are unreliable [6], it was hypothesized that chronic EP could be one of the agents associated with poor

62 performance in Italian Standardbred racehorses, and should be included as a differential diagnosis
63 in this population.
64 To the best of the authors' knowledges, there are no studies investigating the epidemiology of
65 chronic piroplasmosis in Standardbred horses in Southern Italy. The aims of this study were
66 therefore to document the prevalence of chronic piroplasmosis in poorly performing Standardbred
67 racehorses and to explore associations between the infectious status, horse signalment and
68 hematological parameters.
69

70 **2. Materials and Methods**

71 We reviewed the results of 520 complete blood counts of Standardbred horses that were examined
72 from 2004 to 2018 for poor performance.
73 The horses' blood was sampled and physical examinations were carried out in accordance with the
74 EU Directive 2010/63/EU regarding the protection of animals used for scientific purposes. Physical
75 examination and blood sampling procedures were explained to horse owners and written informed
76 consent was obtained to use the collected data.

77 **2.1 Horses, blood sampling and**

78 All the horses were stabled and trained in Southern Italy, under traditional management and training
79 regime [12]. Briefly, horses were usually kept at the race track or in private horse stables and
80 housed in a single box, without access to pasture and with limited access to paddocks (maximum 2
81 hours/daily). The diet was based on commercial food (40%) and oaten hay (60%). Horses were
82 under intense training and were raced at least twice monthly. Veterinary examination was requested
83 by the trainer if the horse displayed poor performance in either training or racing.
84 The same veterinarian (BP) collected blood samples from the external jugular vein in two tubes
85 with and without anticoagulant (EDTA) of the vacutainer system (Becton Dickinson. Franklin
86 Lakes, NJ). Blood was collected in the morning, before feeding and training, while the horses were
87 at rest and unrestrained in their stalls. The blood samples were collected at least three days after

racing or strenuous exercise; the day before sampling each horse was trained slowly (40 minutes at 4-5 m/sec). The horses were not receiving concurrent medication (e.g. corticosteroid or anti-inflammatory).

2.2 Blood analysis

After collection, blood samples were kept at 4°C and analysed within 4 hours at the Laboratory of the Department of Emergency and Organ Transplantation (Bari University, Italy). Hematocrit (HCT) values were determined after centrifugation at 12000 rpm/16128 CFT for five minutes and read on a hematocrit reader card [13]. An air-dried smear of fresh blood was also prepared and stained with a Romanowsky stain, specifically the Giemsa Stain. The blood films were placed in absolute methyl alcohol for 10 min and air-dried, covered with Giemsa diluted with neutral distilled water 1:10 for 60 min. and then washed [14]. Light microscopy was used to identify the organisms (i.e. *Babesia caballi* and *Theileria equi*) within the erythrocytes (Fig. 1) by an experienced technician (RL), and the samples were categorised into piroplasmosis positive (PP) and piroplasmosis negative (PN).

Blood cell counts (BCC) were performed using the Abbott cell counter analyser CELL DYN 3700 (Veterinary Multi-species Hematology System, Abbott, Chicago, IL). The following parameters were recorded: red blood cells (RBC) ($x10^6/\mu l$), hemoglobin (Hb) (g/dl), mean corpuscular volume (MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl), red blood cell distribution width (RDW)(%), white blood cells (WBC) ($x10^3/\mu l$), neutrophils (N) (%), lymphocytes (L) (%), monocytes (M) (%), eosinophils (E) (%), and basophils (B) (%), platelets ($x10^3/\mu l$).

Serum was obtained by centrifugation (1600 x g for 15 min) and the following parameters were determined: total serum proteins (TP, g/dl), aspartate aminotransferase (AST, IU/L), alanine transaminase ALT (IU/L), gamma-glutammiltransferasi (GGT, IU/L), alkaline phosphatase (ALP, IU/L), creatine kinase (CK, IU/L), lactate dehydrogenase (IU/L), total bilirubin (mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), urea (mg/dl), creatinine (mg/dl), glucose (mg/dl), calcium (mg/dl),

phosphorus (mg/dl), magnesium (mg/dl), choride (mEq/L). Biochemical parameters were assessed with ASSEL reagents and the SEAC photometer with interferential filters. Serum protein electrophoresis was performed on agarose gel, according to the Helena BioSciences method [15].

2.3 Treatment and follow-up

Piroplasmosis positive horses (PP) were treated with imidocarb proprionate (2.2 mg/kg, Carbesia®, 85 mg/ml), twice at 48 hours' interval, as suggested in literature [1]. Follow-up of PP horses was carried out at 4 weeks after the initial blood sampling occasion. Follow-up included a clinical examination and the same blood analysis.

2.4. Statistical analysis

Descriptive statistics was performed on signalment data and haematological, biochemical and assay parameters. Categorical data were presented as numbers, percentages and 95% confidence intervals (CI), whist continuous data were presented as medians and interquartile ranges (IQR). Associations between parameters and infection status (piroplasmosis positive (PP) and piroplasmosis negative (PN)) determined using non-parametric Wilcoxon Rank sum test. The association between positive animals and possible risk factors (age, sex) was evaluated using chi square test. All statistical analysis were performed using Genstat 19th edition and P value was set at 0.05.

3. Results

In total 520 horses were sampled, of which 142 (27.6%; 95% CI 23.8% to 31.7%) were female. At the time of sampling, horses were a median of 4 years of age (IQR 3 to 8; maximum 10). The prevalence of EP was 9.2% (95% CI 6.9% to 12.1%). No association was found between the infectious status and age or sex (P=0.205, and P=0.572, respectively).

3.1 Haematology

Table 1 shows the results of BCC in PP and PN horses. PP horses showed mild normocytic, normochromic anemia, and thrombocytopenia. No difference were present in the white blood cells.

3.2 Biochemical parameters

Table 2 shows the results of the biochemical profile in PP and PN horses. PP horses showed hyperbilirubinemia, and their GGT, ALT and CK levels were lower than those of PP horses. However, the liver and muscle enzymes activities of both PP and PN horses were within the normal range. Glycaemia was higher in PP than in PN ($P<0.001$). There were no differences between PP and NP horses and the other variables.

3.3 Electrophoresis of proteins

Table 3 shows the results of the serum protein electrophoresis in PP and PN horses. PP horses showed an increase in alpha 2 and gamma globulins in comparison with NP horses and normal range. There were no differences between PP and NP horses and the other variables.

3.4 Follow-up

PP horses were reported by trainers to have returned to acceptable performance by the time of follow-up. Light microscopy was negative and blood parameters were within the normal range [16,17].

4. Discussion

The current study investigated the prevalence of chronic EP in poorly performing Standardbred racehorses and explored associations between the infection status, horse signalment and hematological parameters. The prevalence of EP in the studied population was nearly 10% and while sex and age were not associated with EP, significant associations between infection status and RBC, PLT, bilirubin and globulin levels were found. Further, after being treated for EP, horses returned to their previous level of performance in training. This finding supports our hypothesis that in an EP endemic region, EP should be considered as one of the possible causes of poor performance and should be included as a first step of the differential diagnosis procedures. “Lack of disease” is one of the criteria of good health in the Animal Welfare Quality system, and offering the

appropriate diagnosis is one of the duties of veterinarians, who should always work safeguarding the health and welfare of their patients, avoiding protracted and useless sufferance [11, 20].

Numerous studies have been published regarding the epidemiology and distribution of EP in specific countries and regions [6, 7]. In Italy, EP prevalence ranges from 8.5% [21] to 86.4% [5].The prevalence varied depending on the experimental design, sample population and diagnostic testing procedure. In a study conducted in Piedmont Region, Northwestern Italy, the prevalence of EP in a population of 135 horses randomly selected was of 17% using semi-nested PCR [4]. In a study conducted on 412 horses reared in Central and Northern Italy the seroprevalence was of 68.4%, with 3.1% and 9.4% of samples positive to microscopy and PCR, respectively [22]. In a population of 303 horses living in Abruzzo and Marche (Central-Southern Italy) the prevalence EP was 14.2% using PCR and 18.7% using indirect immunofluorescence, with the majority of cases due to *T. equi* [23]. In addtion, one-quarter of the infected horses showed poor performance [23]. Our results fall within the prevalence range previously reported for the country, but to our knowledge this is the first study dealing with only racing Standardbreds with poor performance living in Southern Italy.

Standardbreds in racing in Southern Italy live in single stalls. Breed has been identified as a risk factor, with trotters were found to be less likely to be infected in comparison with Italian heavy draught horses [22]. Grazing, presence of ticks on the animals, group housing and riding into bushes have been also identified as risk factors for EP [22, 24]. Therefore, it could be supposed that the prevalence reported in our study was lower than in other studies because the target population could have less exposure to the natural infection. However, it should be considered that the transmission of *T. equi* can occur also iatrogenically through inappropriate mixing of the infected and uninfected blood [6]. This occurs during the poor or unregulated practices, such as needle sharing and "blood doping". Blood doping was implicated as the cause of an outbreak of EP in Florida among racing horses in 2008 [25]. It is possible that poor practices could have been one of the cause of

188 transmission in our racing horses, and these practices should be avoided and considered a threat for
189 the health and the welfare of Standardbreds in Italy.

190 No association between the infectious status and sex and age was found in our study. This is in
191 agreement with many other studies [24, 26, 27]. However, Moretti et al. [22] found that females
192 were infected more often than males and it is well known that naïve young animals were more
193 likely to develop clinical acute forms [6]. Our study focussed on chronic form of EP and this could
194 be the reason why we did not find sex or age as risk factors. Associations were found with RBC,
195 PLT, bilirubin, and globulins. Standardbreds affected by chronic EP developed mild normocytic,
196 normochromic anemia, thrombocytopenia, hyperbilirubinemia and hypergammaglobulinemia.
197 Anemia and thrombocytopenia were expected and in agreement with the literature [27, 28].
198 However, during the chronic form the effects could be very mild, so it is important to interpret the
199 RBC values taking into account the range of normality for this breed [16]. Further, some of the PP
200 horses had RBC values within the lower part of the normal range. EP usually causes a hemolytic
201 anemia, which explains the hyperbilirubinemia found in our and other studies [5]. In disagreement
202 with literature [5, 24], PP horses did not have increased enzyme activity. Instead, PP had lower
203 GGT, ALP and CK activity levels than NP horses. This could be due to the fact that NP were
204 affected by another cause of poor performance. However, since both PP and NP horses had enzyme
205 activity within the normal range, the difference found may be statistically significant, but not
206 biologically significant, and consequently not clinically relevant. The common clinical sign and
207 criterion to be included in the population investigated was poor performance, which was the reason
208 why our specialists were called by the trainers. This should be taken in account interpreting our
209 haematological results and could be the reason why overlaps between the parameters in PP and PN
210 horses occurred. The increase in γ globulins is in line with the chronic form of the infection [29, 30]
211 and with the response of the equine immune system to EP infection [6]. It is worth noting that in
212 endemic region, seropositive inapparent carriers are at risk of developing clinical EP in case of

concurrent disease or stress such as strenuous exercise [1]. Hence, considering that the relationship between the infection with *T. equi*, stress and immune system is complex and not completely defined [31], ensuring appropriate management and welfare standards to racing horses should be recommended to minimise the prevalence of EP and subsequent poor performance syndrome and the hematological alterations described.

A limitation of the current study was that EP was diagnosed using light microscopy. Within the erythrocyte, *B. caballi* typically appears as 2 large (pear-shaped) merozoites, and during clinical infection the percentage of erythrocytes parasitized is less than 1%. The *T. equi* merozolites are smaller (Fig. 1) and the percent of infected erythrocytes during clinical diseases is usually between 1% and 5% but can reach 20% [6]. As the current study investigated chronic EP, and it has been reported that microscopy is less sensitive than PCR [22], our prevalence is most likely underestimated. The competitive inhibition enzyme-linked immunosorbent assay (cELISA) has been one of the regulatory tests prescribed by the OIE for international horse transport [2]. Considering that PCR and ELISA are now a routine variable in commercial laboratory [25], in case of a horse with a haematological picture referrable to EP resulted negative to blood cytology, knowing that it could be a possible false negative at microscopy, PCR and ELISA should be requested. However, light microscopy remains a useful, simple and cheap screening and should be recommended as a first step in the differential diagnosis procedure.

Another limitation is that this study only looks at EP as one possible cause of poor performance, without excluding other possible chronic diseases, and does not note the reason for poor performance in horses not diagnosed with EP. Further, we did not differentiate between *B. caballi* and *T. equi* infection, which was not always possible by microscopy and could have been done using serology or using a specific polymerase chain reaction. As the study was conducted over a 15 year time period, it was impossible to store and retest the samples to differentiate between species.

Notwithstanding those limitations, this study has increased our knowledges of EP in a specific target population..

5. Conclusions

The prevalence of chronic form of EP was of 9.3% in poorly performing Standardbred racehorses living in Southern Italy**.** Accurate diagnostic procedures were required to identify cases of poorly performing horses suffering from this form of piroplasmosis to ensure the criteria of "lack of disease" in the principle of good health, and therefore maintain good welfare. Blood tests and blood cytology should be recommended as a first step of diagnosis when a Standardbred living in a EP endemic zone is not performing well. However, in the event that the organisms are not seen in the blood smear but hematology results show mild anemia, hyperbilirubinemia and hypergammaglobulinemia, further laboratory tests (i.e. ELISA and PCR) should be performed to reach an accurate diagnosis.

Conflicts of Interest

The authors declare no conflict of interest

Author contribution

Conceptualization: BP, RL, GR; Methodology: BP, RL, GR, CDT; Formal Analysis: CDB, RL, GR; Data Curation: BP, SMR ; Writing-Original Draft Preparation: BP; Writing-Review & Editing: GR, SMR; Supervision: GR; Funding Acquisition: BP, GR.

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329 **Table 1**. Blood cell count results of poor performing Standardbred horses (n=520) with (PP) and
330 without (NP) chronic form of equine piroplasmosis. Results are expressed as median and
331 interquartile range (IQR)

	PP (n=48)	**PN (n=472)**	**NR***	**P**
RBC (x10^6/µl)	7.61 (IQR 6.71-8.71)	8.67 (IQR 7.89-9.82)	8.4-10.4	**<0.001**
Hb (g/dl)	12.0 (IQR 10.9-13.5)	13.4 (IQR 12.2-14.5)	12.7-15.7	**<0.001**
Hct (%)	32 (IQR 20-36)	37 (IQR 34-40)	36-42	**<0.001**
MCH (pg)	15.8 (IQR 15-16.4)	15.5 (IQR 14.7-16.3)	13.3-17.5	**0.025**
MCV (fl)	42.8 (IQR 41.4-43.9)	42.2 (IQR 39.9-43.9)	39.3-44.6	0.120
MCHC (g/dl)	36.8 (IQR 36.5-37.4)	37 (IQR 36.4-37.3)	36.3-37.5	0.927
RDW (%)	21.2 (IQR20.5-22.4)	22.6 (IQR 21.5-24.1)	17-20	**<0.001**
WBC (x10^3/µl)	8.2 (IQR 6.9-9.91)	8.2 (IQR 7.2-9.6)	6.51-9.59	0.927
N (%)	57.1 (IQR49-62)	59.8 (51.6-66.9)	54.31-68.3	0.085
L (%)	37.5 (IQR 31-46)	35.9 (IQR 29-43)	25.15-37.7	0.294
E (%)	1 (IQR 0-1.5)	1 (IQR 0.1-2)	0.74-1.8	0.066
M (%)	2 (IQR1-4.8)	2 (IQR 1-4.7)	2-8.11	0.994
B (%)	0 (IQR 0-0.2)	0 (IQR 0-0.2)	0-0.4	0.932
PLT (x10^3/µl)	99.5 (IQR 81.1-125)	114 (92.1-139)	100-225	**0.001**

332 * Normal Range [16,

333333

334 **Table 2.** Biochemical profile results of poor performing Standardbred horses (n=520) with (PP) and

335 without (NP) chronic form of equine piroplasmosis. Results are expressed as median and

336 interquartile range (IQR).

	PP (n=48)	**PN (n=472)**	**NR**	**P**
Total Protein (g/dl)	6.7 (IQR6.4-7.1)	6.8 (IQR 6.4-7.1)	6.27-7.1	0.986
AST (IU/L)	357 (IQR 253.8-471.2)	399 (IQR 310-585.5)	335-623	0.052
ALT (IU/L)	15 (IQR 9.7-20.2)	12 (IQR 8-18)	7-15	0.154
GGT (IU/L)	12.5 (IQR 8-12)	16 (IQR 12-22)	6-36	**0.001**
ALP (IU/L)	288 (IQR 227.5-329.5)	325.5 (IQR 259-405)	278-490	**0.003**
CK (IU/L)	149 (IQR 108-191)	172.5 (128-237.5)	116-316	**0.007**
LDH (IU/L)	530 (IQR 415.5-721.5)	527 (IQR 401-662)	394-680	0.635
Bilirubin (mg/dl)	3.92 (IQR 2.91-5.57)	2.63 (IQR 2.05-3.48)	1-3	**<0.001**
Cholesterol (mg/dl)	83 (IQR 71-96.5)	80 (IQR72-89.7)	63-97	0.277
Triglycerides (mg/dl)	21.3 (IQR 15.5-39)	25 (IQR17.5-35.2)	17-45	0.388
Urea (mg/dl)	33.4 (IQR 27.0-36.8)	30.7 (IQR 25.9-36.4)	22-38	0.313
Creatinine (mg/dl)	1.4 (IQR 1.3-1.6)	1.5 (IQR1.3-1.6)	1.1-1.7	0.445
Glucose (mg/dl)	110 (IQR 95-121)	96 (IQR 84-106)	75-115	**<0.001**
Calcium (mg/dl)	11.1 (IQR 10.5-11.7)	11.3 (IQR 10.7-11.8)	9.5-12	0.259
Phosporus (mg/dl)	3.4 (IQR2.9-3.8)	3.6 (IQR 3-4.1)	3.1-4.4	0.062
Magnesium (mg/dl)	2.0 (IQR 1.8-2.2)	2.0 (IQR 1.9-2.3)	1.9-2.4	0.159
Chorine (mEq/L)	106.4 (IQR72-111.2)	108.3 (IQR101.9-115)	94-102	0.134

337 * Normal Range [17,

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339 **Table 3**: Serum protein electrophoresis results of poor performing Standardbred horses (n=520)
340 with (PP) and without (NP) chronic form of equine piroplasmosis. Results are expressed as median
341 and interquartile range (IQR).

	PP	PN	NR*	P
Albumin (%)	46.5 (IQR43.3-50.7)	47.3 (43.7-50.6)	45-60	0.660
α1 (%)	2.4 (IQR 1.7-3.1)	2.2 (IQR 1.8-3.4)	4-6	0.324
α2 (%)	13.4 (IQR 11.6-13.4)	13.3 (IQR 11.9-14.7)	7-13	**0.002**
β1 (%)	8.0 (IQR 6.6-9.1)	8.5 (IQR 7.4-9.3)	7-9	0.217
β2 (%)	5.5 (IQR 4.3-7.2)	5.6 (IQR4.5-7.0)	4.5-8.5	0.894
γ (%)	24.37 (IQR 21.12-27.04)	22.6 (IQR 19.74-25.50)	8-22	**0.020**

342 * Normal Range [15, 19]

343

344 Fig 1. Examples of blood smear microscopy

Table 1. Blood cell count results of poor performing Standardbred horses (n=520) with (PP) and without (NP) chronic form of equine piroplasmosis. Results are expressed as median and interquartile range (IQR)

	PP (n=48)	PN (n=472)	NR*	P
RBC (x10^6/µl)	7.6 (IQR 6.71-8.71)	8.7 (IQR 7.89-9.82)	8.4-10.4	**<0.001**
Hb (g/dl)	12.0 (IQR 10.9-13.5)	13.4 (IQR 12.2-14.5)	12.7-15.7	**<0.001**
Hct (%)	32.0 (IQR 20-36)	37.0 (IQR 34-40)	36.0-42.0	**<0.001**
MCH (pg)	15.8 (IQR 15-16.4)	15.5 (IQR 14.7-16.3)	13.3-17.5	**0.025**
MCV (fl)	42.8 (IQR 41.4-43.9)	42.2 (IQR 39.9-43.9)	39.3-44.6	0.120
MCHC (g/dl)	36.8 (IQR 36.5-37.4)	37.0 (IQR 36.4-37.3)	36.3-37.5	0.927
RDW (%)	21.2 (IQR20.5-22.4)	22.6 (IQR 21.5-24.1)	17-20	**<0.001**
WBC (x10^3/µl)	8.2 (IQR 6.9-9.91)	8.2 (IQR 7.2-9.6)	6.5-9.6	0.927
N (%)	57.1 (IQR49-62)	59.8 (51.6-66.9)	54.3-68.3	0.085
L (%)	37.5 (IQR 31-46)	35.9 (IQR 29-43)	25.1-37.7	0.294
E (%)	1.0 (IQR 0-1.5)	1.0 (IQR 0.1-2)	0.7-1.8	0.066
M (%)	2.0 (IQR1-4.8)	2.0 (IQR 1-4.7)	2-8.1	0.994
B (%)	0 (IQR 0-0.2)	0 (IQR 0-0.2)	0-0.4	0.932
PLT (x10^3/µl)	99.5 (IQR 81.1-125)	114 (92.1-139)	100-225	**0.001**

* Normal Range [16, 17]

Table 2. Biochemical profile results of poor performing Standardbred horses (n=520) with (PP) and without (NP) chronic form of equine piroplasmosis. Results are expressed as median and interquartile range (IQR).

	PP (n=48)	PN (n=472)	NR	P
Total Protein (g/dl)	6.7 (IQR6.4-7.1)	6.8 (IQR 6.4-7.1)	6.3-7.1	0.986
AST (IU/L)	357 (IQR 253.8-471.2)	399 (IQR 310-585.5)	335-623	0.052
ALT (IU/L)	15 (IQR 9.7-20.2)	12 (IQR 8-18)	7-15	0.154
GGT (IU/L)	12.5 (IQR 8-12)	16 (IQR 12-22)	6-36	**0.001**
ALP (IU/L)	288 (IQR 227.5-329.5)	325.5 (IQR 259-405)	278-490	**0.003**
CK (IU/L)	149 (IQR 108-191)	172.5 (128-237.5)	116-316	**0.007**
LDH (IU/L)	530 (IQR 415.5-721.5)	527 (IQR 401-662)	394-680	0.635
Bilirubin (mg/dl)	3.9 (IQR 2.91-5.57)	2.6 (IQR 2.05-3.48)	1-3	**<0.001**
Cholesterol (mg/dl)	83 (IQR 71-96.5)	80 (IQR72-89.7)	63-97	0.277
Triglycerides (mg/dl)	21.3 (IQR 15.5-39)	25 (IQR17.5-35.2)	17-45	0.388
Urea (mg/dl)	33.4 (IQR 27.0-36.8)	30.7 (IQR 25.9-36.4)	22-38	0.313
Creatinine (mg/dl)	1.4 (IQR 1.3-1.6)	1.5 (IQR1.3-1.6)	1.1-1.7	0.445
Glucose (mg/dl)	110 (IQR 95-121)	96 (IQR 84-106)	75-115	**<0.001**
Calcium (mg/dl)	11.1 (IQR 10.5-11.7)	11.3 (IQR 10.7-11.8)	9.5-12	0.259
Phosporus (mg/dl)	3.4 (IQR2.9-3.8)	3.6 (IQR 3.0-4.1)	3.1-4.4	0.062
Magnesium (mg/dl)	2.0 (IQR 1.8-2.2)	2.0 (IQR 1.9-2.3)	1.9-2.4	0.159
Chorine (mEq/L)	106.4 (IQR 72-111.2)	108.3 (IQR101.9-115)	94-102	0.134

* Normal Range [17, 18]

Table 3: Serum protein electrophoresis results of poor performing Standardbred horses (n=520) with (PP) and without (NP) chronic form of equine piroplasmosis. Results are expressed as median and interquartile range (IQR).

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