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Full Length Research Paper

A cross-sectional sero-survey of some infectious diseases of working equids in Central Ethiopia

M. Getachew[1, 2*], F. Alemayehu[2], C. Chala[2], B. Amare[2], D. Kassa[3], F. Burden[1], R. Wernery[4] and U. Wernery[4]

[1]Donkey Sanctuary, Sidmouth, Devon EX10 0NU, UK.
[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, Donkey Health and Welfare Project-Donkey Sanctuary, Ethiopia.
[3]Wollo University, P.O. Box 1145, Dessie, Amhara regional state, Ethiopia.
[4]Central Veterinary Research Laboratory (CVRL), P. O. Box 597, Dubai, United Arab Emirates.

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Despite the large numbers of working equids and their significant contribution towards communities as well as the national economy, the attention given to study the health and welfare problems of working equids in Ethiopia is quite minimal. The main objective of this study was to investigate the sero-prevalence of some important infectious diseases infecting working equids and asses their spatial distribution in the different agro-ecological zones of Ethiopia. Sera collected from 1007 equids selected by simple random sampling technique were tested using the Office Internationale des Epizootic (OIE) approved serological tests. The overall sero-prevalence for all equids were 10.5% (n=288) for African horse sickness (AHS), 0.7% (n=997) for Dourine, 3.8% (n=982) for Glanders, 0.1% (n=1002) for equine infectious anaemia (EIA), 13.5% (n=208) for equine herpes virus 1 (EHV-1), 88% (n=208) for equine herpes virus 4 (EHV-4) and 65% (n=20) for Piroplasmosis. Significant interspecies (P=0.001) and spatial (P=0.01) variations were observed for AHS, Glanders, Dourine and EHV-1. However, the age and sex of the animals had no significant effect on the prevalence of the tested diseases. Infection of equids with more than one infectious disease was diagnosed. Out of the 208 equids tested for herpes, 11.1% were sero-positive for both EHV-1 and EHV-4. Co-infections of AHS and EHV-1 (1%), AHS and EHV-4 (4%), Glanders and EHV-1 (2%) and Glander and EHV-4 (7.7%) were also observed. This study has shown not only the high prevalence of some of the infectious diseases in the equine population but their wide distribution across the different agro-ecological zones of the central regions of Ethiopia.

Key words: Working equids, infectious diseases, epidemiology, sero-prevalence, Ethiopia.

INTRODUCTION

The world equine population is estimated at 44 million donkeys, 11 million mules and 59 million horses (FAOSTAT, 2012). More than 97% of the world's donkey and mule populations, and over 72% of the world's horse population is found in developing countries specifically kept for draft purpose. These vast numbers of working

*Corresponding author E-mail: m.getachew06@yahoo.co.uk. Tel: +44 7818592548/+251911089648.

Table 1. Number of equids examined from the different agro-ecological zones in central regions of Ethiopia.

Agro-ecological zone	Altitude range*	No. of equids examined			Total
		Donkeys	Mules	Horses	
Highlands (Dega)	2400-2850	347	40	258	645
Midlands (Weyna Dega)	1500-2400	197	11	2	210
Lowlands (Kola)	≤1500	123	0	29	152
Total		667	51	289	1007

*Meters above seas level.

equids play crucial roles in both urban and rural areas, providing agricultural energy and transport, and in many cases, the sole means of generating income for their resource-limited owners (Fielding and Pearson, 1991; Swaan, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350, 000 mules (FAOSTAT, 2012) specifically kept for work. Despite their huge numbers and significant contribution to the communities and the national economy (Behnke and Metaferia, 2011), the attention given to study the health aspects of working equids in Ethiopia is quite minimal. Among the multiple health and welfare problems affecting working equids, infectious diseases are one of the major constraints to their productivity and work performance; this often leads to high morbidity and mortality (El Idrissi and Lubroth, 2006; Knottenbelt, 2009). Apart from studies made on some parasitic infections (Getachew, et al., 2010; Hagos, et al., 2010; Getachew, et al., 2012; Gizachew, et al., 2013) and few studies on African horse sickness and Epizootic lymphangitis (Asfaw, et al., 2012; Ayelet et al., 2013) information on other equine infectious diseases is virtually unknown in Ethiopia. The present study was therefore conducted to investigate the prevalence of some infectious diseases in working equids and their spatial distribution in the central regions of Ethiopia.

MATERIALS AND METHODS

Study areas

The study covered areas of the central regions of Ethiopia and targeted the rural and sub-urban of Addis Ababa and Akaki, eastern and northern shewa districts of Ormia region, and southern Wollo zone of the Amhara region, representing different agro-ecological zones. Based on the traditional agro-climatic classification of Ethiopia, the animals were sampled from 'Dega', highland; 'Weyna Dega', midland and 'Kola', lowland areas (Table 1). Geographically the study areas covered latitude and longitude ranges of 08^0 09'472" to 11^0 09' 810" North and 38^0 05'248" to 39^0 61'740" East. Within the target districts one to two villages or peasant associations were purposively selected based on their road accessibility. Lists of equine owners were then made to help random selection of animals.

Study animals

A total of 1007 working equids including 667 donkeys, 289 horses and 51 mules were randomly selected using a simple random sampling technique (Table 1) from the list of owners generated. Only a maximum of two equids were sampled from each owner having more than three equids. Because of differences in the distribution of the equine species in the different agro-ecological zones and availability of animals during sampling periods, it was not possible to collect equal numbers of samples from each equine species. More than 85% of the equine population of the study areas were donkeys. This was particularly the case in mules. Mules are mostly found in highland areas and owned by limited number of people; they are very expensive. However, the total number of equids sampled was more than the required sample size calculated based on the assumption of 50% expected prevalence and 95% confidence interval with a 5% precision level (Thrusfield 2007). All equids were kept by resource-limited communities under extensive traditional management system. As per the information collected the sampled equids were not vaccinated or treated against any of the diseases under investigation. The study included both sexes (male and female) and all age groups (> 6 months) of equids. Age of the animals was estimated on the basis of their dentition according to Crane (1997) and Evans (2001). The study was conducted by the Donkey Sanctuary (DS) project based in Ethiopia in collaboration with CVRL from September 2005 to April 2006.

Blood sampling and storage

Five to ten ml of blood samples were collected from the jugular vein into 10ml plain test tubes and transported under cold-chain using cool-box according to OIE (2013b) to the diagnostic lab at the Addis Ababa University, College of Veterinary Medicine and Agriculture (CVMA). The samples were allowed to clot overnight at room temperature, and sera were separated and stored at -20 ^oC until processed. The sera were then exported under cold-chain protocol to the Central Veterinary Research laboratory (CVRL) to be analysed. CVRL is OIE accredited diagnostic laboratory in Dubai, United Arab Emirates (UAE).

Laboratory testing

Sera samples were investigated for African horse sickness (AHS), Dourine, equine infectious anaemia (EIA), Glanders, equine herpes viruses (EHV-1 and 4) and equine Piroplasmosis (*Theileria equi* and *Babesia caballi*) at CVRL using the OIE approved serological tests (OIE, 2013b). OIE approved serological tests used to analyse the different diseases are shown in Table 2. To reduce nonspecific reactions, donkey sera were inactivated at 62 ^oC for 30 min for complement fixation test (CFT). Because of the insufficient sera-samples due to variation in the amount of blood taken and the recovered sera during separation, it was not possible to test each serum for all diseases.

Data analysis

Twenty-four and fourteen sera-samples which showed doubtful or

Table 2. Serological tests used to investigate the different infectious diseases of equids in central regions of Ethiopia.

Disease[a]	Serological tests[a]
African horse sickness (AHS)	Competitive Enzyme-Linked Immunosorbent Assay (cELISA)
Glanders and Dourine	Complement Fixation Test (CFT)
Equine infectious anaemia (EIA)	Agar-gel immunodiffusion (AGID)
Equine herpes virus (EHV-1&4)	Indirec t-ELISA
Equine Piroplasmosis (*T. equi* and *B. caballi*) (IFAT)	Immunofluorescence antibody test

[a]OIE approved serological tests (OIE, 2013b. All diseases were diagnosed at CVRL, OIE accredited diagnostic laboratory in Dubai.

inconclusive results (OD = 0.1- 0.2) for EHV-1 and EHV-4, respectively, were excluded from the analysis. It was not possible to repeat the test due to shortage of sample. The association of sero-prevalence of the tested diseases and the different agro-ecological zones, equine species, age and sexes were evaluated by multivariable binary logistic regression analysis. Where appropriate, a pair-wise contrast analysis (R library Gremisc) was performed between these variables (risk factors) that were found to be significant. Descriptive statistics were performed using data analysis tools in Excel© (Microsoft, 2007) and Minitab© statistical software (Minitab release 14), whereas all statistical analyses were carried out in R (R Development Core Team 2009). The significance level for all statistical tests was set to $p<0.05$.

Results

The number of equids examined and percentage serologically positive for the tested diseases are shown in Table 3. Significant interspecies differences were observed for AHS, Glanders, and EHV-1 ($p = 0.001$). Mules showed a higher sero-prevalence of AHS when compared to donkeys and horses, while Glanders was significantly higher in horses than in donkeys and mules ($p = 0.001$). Similar sero-prevalence of EHV-4 was observed among the different species of equids, while that of EHV-1 showed a significantly higher sero-prevalence in donkeys ($p = 0.001$). Compared to other diseases tested, EHV-4 showed a significantly higher sero-prevalence in all species of equids ($p = 0.001$). There was no significant variation in sero-prevalences of the tested diseases among the different age groups ($p=0.580$) and sexes ($p=0.320$) of equids. Infection of equids with more than one infectious disease was diagnosed. Out of the 208 equids tested for herpes 11.1% were infected with both EHV-1 and EHV-4. Co-infection of AHS and EHV1 (1%), AHS and EHV-4 (4%), Glanders and EHV-1 (2%) and Glander and EHV-4 (7.7%) were also observed. Although low numbers of equids were examined for Piroplasmosis, the overall sero-prevalence of *T. equi* (50%) was higher than that of *B. caballi* (15%). The number of equids examined and sero-prevalence of the tested diseases in the different agro-ecological zones are shown in Table 4. Significant variation of sero-prevalence of AHS, Glanders and Dourine was observed in the different agro-ecological zones. The sero-prevalence of dourine was significantly higher ($p = 0.001$) in the mid-lowland, while that of AHS and Glanders was higher in the highland regions ($p = 0.01$). Although, the numbers of equids examined for EHV-1 and piroplasmosis were not sufficient to statistically compare among the different regions, higher sero-prevalences of EHV-1, *T. equi* and *B. caballi* were found in the mid-lowland, lowland and highland regions, respectively. Similar sero-prevalence of EHV-4 was found across the different agro-ecological zones.

DISCUSSION

The present study has shown not only the prevalence of the different infectious diseases in the equine population but also their distribution across the different agro-ecological zones of the central regions of Ethiopia.

African horse sickness (AHS)

The present finding of AHS in all species is similar with the finding by Kassa (2006). However, a higher sero-prevalence of AHS was reported by Bitew, et al. (2011) in equids and by Teshome, et al. (2012) in donkeys and mules. These studies were conducted in regions where AHS is endemic and frequent outbreaks are reported compared to our study areas. This could be the reason for the observed differences in sero-prevalence. Although, the sensitivity and specificity of the tests they used was not specified, the methods of diagnosis could also be a factor. Although, the present study and studies by Bitew, et al. (2011) and Teshome, et al. (2012) have shown a high sero-prevalence of AHS in donkeys in Ethiopia, no clinical cases were reported in these equine species, unlike in horses. However, frequent outbreaks of suspected cases of AHS with typical clinical signs of the mixed form were observed in donkeys in Kenya; eighteen sera samples from some of these cases were diagnosed at CVRL in Dubai and revealed 10 (55.6%) sero-positives for AHS (M Getachew, unpublished data). Although, it is generally accepted that donkeys, particularly African donkeys, are resistant and most infections are sub-clinical

Table 3. Number of each equine species examined and % serologically positive for the different diseases tested in the central regions of Ethiopia.

Disease	Equids	% sero-positive	95% CI*
AHS	Donkeys (n =165)	8.5	4.24-12.76
	Mules (n =18)	33.3	26.42-40.18
	Horses (n = 45)	8.9	0.58-17.22
	Total (n = 288)	10.5	6.52-14.48
Dourine	Donkeys (n = 662)	1.1	0.31-1.89
	Mules (n = 51)	0	-
	Horses (n = 284)	0	-
	Total (n = 997)	0.7	0.18-1.22
Glanders	Donkeys (n = 657)	0.2	0.14-0.54
	Mules (n = 48)	0	-
	Horses (n = 277)	12.3	8.4-16.17
	Total (n = 982)	3.8	2.6-5.0
EHV-1	Donkeys (n = 104)	20.2	12.48-27.92
	Mules (n = 4)	0	-
	Horses (n = 100)	7	2.0-12.0
	Total (n= 208)	13.5	8.86-18.14
EHV-4	Donkeys (n = 104)	84.6	82.37-86.83
	Mules (n = 4)	100	100-100
	Horses (n = 100)	91	85.39-96.61
	Total (n = 208)	88	83.58-92.42
EIA	Donkeys (n = 662)	0.2	-0.68
	Mules (n = 51)	0	-
	Horses (n = 289)	0	-
	Total (n= 1002)	0.1	-0.4

*95% confidence interval.

clinical (Mellor and Hamblin, 2004; Guthrie, 2007; OIE, 2013a), the epidemiological role they may play is not well known. Moreover, their susceptibility to the 9 different strain of AHSV has not been investigated. Given the current high sero-prevalence, the large donkey population and absence of vaccination against AHS in donkeys in Ethiopia, and the fact that they could act as silent reservoirs like zebras do, and may play a significant role for the virus transmission to other equids (Hamblin et al., 1998; Guthrie, 2007) consideration should be given to the inclusion of donkeys in the vaccination programme. The practice of vaccinating in horses, irregular vaccination of mules and no vaccination in donkeys (Aklilu et al., 2014; Ayelet et al., 2013) could be one of the major risk factors of having frequent outbreaks of AHS in horses in Ethiopia.

Dourine

Dourine, caused by *Trypanosoma (T) equiperdum*, is among the Non-Tsetse Transmitted Animal Trypanosomosis (NTTAT) (OIE, 2013a). Although, low in sero-prevalence, the finding of dourine only in donkeys in the present study is quite interesting. Dourine was reported only in horses from the highland regions of Ethiopia (Gari et al., 2010; Hagos et al., 2010). However, these studies did not include donkeys and other studies by Mekuria et al. (2010) and Eyob et al. (2011) were focused on detecting tsetse transmitted trypanosomosis. The present finding from the mid-lowland region is the first report, showing the spread of the disease from the highland to the mid-lowland regions. This could be associated with the movement of animals through trade routes of equids from the highland to mid-lowland regions. As *T. equiperdum* is a sexually transmitted disease, and donkeys are more resistant than horses and may remain as apparent carriers, it can spread not only among the donkey population but also to horses in areas where mule breeding is practiced. Although, the area of study was not inhabited by camels, and Surra, caused by *T. evansi*, seem to be restricted to arid and semi-arid areas of Ethiopia where it is endemic in camel population (Tefera and Gebreab, 2004; Kassa et

Table 4. Number of equids examined from the different agro-ecological zones and % serologically positives for the different diseases tested in central regions of Ethiopia.

Disease	Agro-ecological zone	% sero-positive	95% CI*
AHS	Highland (n =188)	9.6	5.39-13.81
	Lowland (n = 40)	15	3.93-26.07
Dourine	Highland (n = 636)	0.2	-0.7
	Midland (n = 210)	2.9	0.63-5.17
	Lowland (n = 151)	0	-
Glanders	Highland (n = 621)	4.5	2.87-6.13
	Midland (n = 210)	1.4	-3.18
	Lowland (n = 151)	2.4	-4.88
EHV-1	Highland (n = 159)	12	6.69-17.05
	Midland (n = 19)	31.6	10.7-52.5
	Lowland (n = 30)	10	-21.48
EHV-4	Highland (n = 159)	88.1	83.07-93.13
	Midland (n = 19)	84.2	67.8-100.0
	Lowland (n = 30)	90	9.26-100.0
EIA	Highland (n = 641)	0	-
	Midland (n = 210)	0.5	-1.9
	Lowland (n = 151)	0	-

*95% confidence Interval

al., 2011), now-a-days dromedaries are forced to move to the mid-lowland areas in search for feed during the dry season. These might predispose equids in the region to Surra complicating the diagnosis. Because *T. equiperdum* is genetically and antigenically similar to *T. evansi*, and CFT cannot differentiate between the two infections (Claes et al., 2003). The current finding therefore, should be cautiously interpreted. This indicates the need of further epidemiological studies to establish the extent of the distribution of dourine and surra in the different agro-ecological zones and their prevalence in the equine population in general and donkeys in particular using highly sensitive and specific diagnostic methods that can differentiate between the two infections (Sumbria et al., 2014).

Glanders

Apart from fragmented reports (OIE, 2013a), and anecdotal information based on clinical signs, the epidemiology of Glanders has not been previously investigated in Ethiopia. This is the first report of serological evidence of the disease in Ethiopia. The serological finding of this disease in all agro-ecological zones shows its wide distribution among the equine population in the central regions of the country. Donkeys are reported to be the most susceptible equids often developing the acute form of the disease, which is often fatal within days (Wernery, 2009; Khan et al., 2012). Survival bias could be the reason for the low sero-prevalence in donkeys as compared to in horses in the present study. The crowding condition of equids in public places, such as market, flour mills, traditional ceremonies and the trade routes across the country with poor hygienic and stressful conditions could be the major factors for its spread. Moreover, the highly prevalent harness inflicted wounds and fly population may play a significant role in the transmission of the disease among equids. As CFT can give some false-positive or false-negative results (OIE, 2013b), the present serological finding of Glanders should be interpreted carefully. Study made by Turnbull et al. (2002) showed that donkeys which were positive with CFT were later confirmed to be negative with the Mallein test and at necropsy, indicating a cross-reaction to other related microorganisms. *Burkholderia mallei* and *Burkholderia pseudomallei* are antigenically closely related and cross-reactivity was noted (Anuntagool and Sirisinha, 2002; Wernery, 2009). Studies have shown that combined use of CFT and Mallein test, or CFT with a more specific and complement independent tests increases the detection rate of Glanders (Neubauer et al., 2005; Khan et al., 2012). Therefore, further epidemiological study using a more sensitive and specific tests are recommended to

establish the true prevalence of Glanders among working equids in Ethiopia to help develop strategic control programme.

Equine infectious anemia (EIA)

Although EIA has a worldwide distribution (OIE, 2013a; van Maanen, 2013), there is no previous report as to its presence in Ethiopia. Despite the conducive environment for the insect vectors in the low lying and swampy areas in the mid-lowland regions, from where some of the samples were collected, the finding of only one sero-positive donkey (0.2%) out of the 1002 sera-samples tested may show that this disease is rare in the study areas. Moreover, we could have seen more infection prevalence given the possibility that once equids are infected they become carriers and remain infectious for life and with rare exception yield a positive serological test result (OIE, 2013b; Issel et al., 2013). Similar studies conducted in UAE (Turnbull et al., 2002), in Bulgaria (Chenchev et al., 2011) and Turkey (Ataseven and Arslan, 2005) showed the absence of this disease in the donkey population.

Equine herpes viruses

The finding of high sero-prevalence of both EHV-1 and EHV-4 in the different agro-ecological zones shows the wider distribution of these viruses among working equids in the study areas. Similar high sero-prevalence of both herpes infections was reported in Ethiopia (B. Endebu, unpublished data). As EHV4 and EHV1 establish persistent lifelong latent infections and ELISA is type specific (Crabb and Studdert, 1995; Patel and Heldens, 2005), and vaccination against any of the herpes virus is unknown in Ethiopia, the present finding indicates the natural occurrence of these viral infections in the equine population of the study areas. Working equids are often exposed to stressful and harsh conditions from overworking, malnutrition, parasitic infections and to other management factors. This may predispose them to frequent relapse and diseases as herpes viruses establish lifelong infection. It is known that EHV-4 and EHV-1 cause acute respiratory disease, viral-induced abortion and myeloencephalitis, respectively, in equids throughout the world (Patel and Heldens, 2005; Pronost et al., 2012). The high sero-prevalence of EHV- 4 and EHV-1 in the present study areas might be one of the major reasons for the high respiratory problems and abortion rates seen in working equids, particularly in donkeys (Donkey health and welfare project, Ethiopia, personal communication). An outbreak of a neurological syndrome in donkeys in the Amhara region of Northern Ethiopia was recently reported, which was later diagnosed to be due to EHV-1 (T. Worku, personal communication). The present high sero-prevalence of both EHV-1 and EHV-4 shows the need to conduct further epidemiological researches to determine the extent of its distribution and associated problems in different parts of the country to help develop sound control and prevention strategies.

Equine piroplasmosis

The present study showed high sero-prevalence of both *T. equi* and *B. caballi* although the numbers of samples examined were small. Studies made by Gizachew et al. (2012), Mekibib et al. (2010) and Tefera et al. (2011) showed a much lower prevalence of these parasites. This variation could be attributed to differences in the geographical location and methods of diagnosis employed. Apart from study by Gizachew et al. (2012), the others studies were based on parasitological methods. Given the difficulty in demonstrating the parasites in blood, especially when the parasitaemia is low in carrier animals, false negatives are common using parasitological methods. As *T.equi* can be transmitted transplacentally from carrier mares to offspring (Chhabra et al., 2012), a thorough investigation using serological and molecular methods is required in combination with parasitological diagnosis (George et al., 2011). The high sero-prevalence in the present study indicates the presence of suitable tick vectors capable of transmitting the parasites (Pegram et al., 1981; Gizachew et al., 2012) and high degree of exposure of equids to the infection. Therefore, further epidemiological studies are required to more precisely determine the extent of the distribution of this pathogen within the equine population in different agro-ecological zones.

Conclusions

The present study has shown from high sero-prevalence to rare presence of the investigated infectious diseases in the equine population with a wide spatial distribution. Although, not investigated here, the high presence of multiple infectious diseases could contribute to the low productivity, low work performance and early demise observed in working equids in these areas. This further indicates the potential of these infectious diseases in causing welfare problems and economic losses. The wide distribution of most infectious diseases in the donkey population needs further investigation. Donkeys are particularly neglected when it comes to disease investigation, control and prevention. Although reported to be resilient to the effect of many infectious pathogens, they can be carriers and serve as source of infections for other equids. The high population density of equids, particularly donkeys, in public places, such as markets, local flour mills, watering points and ceremonial events,

and increased movement of animals between different regions due to trade could contribute to transmission and hence the high sero-prevalence, multiple infections and wide spread of most infectious diseases in the different agro-ecological zones. These pose special threat and demonstrate some important considerations in the practice of disease control.

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Conflict of Interest

The author(s) have not declared any conflict of interests

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Full Length Research Paper

Isolation and identification of aerobic bacterial species from upper respiratory tract of cart horses in Central Ethiopia

Tesfaye Debelu[1], Nigatu Akililu[2], Tesfaye Sisay[1] and Fanta Desissa[2*]

[1]College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Ethiopia.
[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, SPANA Project, P. O. Box 34, Bishoftu, Ethiopia.

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A cross sectional study to isolate and identify aerobic bacterial species from upper respiratory tract of horses was conducted from October, 2009 to April, 2010. Forty eight apparently healthy horses (APHH) and 56 horses with respiratory tract diseases (HRTD) brought to Society for protection of animals abroad (SPANA) clinics in Central Ethiopia were randomly selected. Swab samples were collected aseptically from nasopharynx of the horses. Isolation and identification of the bacteria was carried out following the recommended standard procedures. A total of 270 bacteria were recovered from the sample taken from both groups. Of the total isolates, 65.9% were Gram positives and the remaining 34.1% were Gram negatives. Bacterial species isolated in order of dominance include: *Bacillus* species, *Streptococcus* species, *Staphylococcus* species, *Escherichia coli*, *Pasteurella* species, *Micrococcus* species, *Bordetella* species, *Pseudomonas* species, *Actinobacillus* species and *Rhodococcus equi*. *Actinobacillus* species and *Rhodococcus equi* were only isolated from APHH. Despite the percentage variation of the isolates, there was no statistically significant ($p>0.05$) variation among the isolates with respect to altitude and health status of horses except significant variation of *Staphylococcus* species ($p\leq0.05$) between low and high altitudes. In conclusion, the study showed that wide variety of aerobic bacterial species inhabiting the upper respiratory tract of horses with similar distribution of the bacteria in APHH and HRTD suggesting bacteria which reside in the URT of healthy animals might cause opportunistic infections. It is recommended that clinicians should consider the dominance of Gram positives primarily as a cause of upper respiratory tract diseases in horses.

Key words: Bacterial species, horses, respiratory tract, Central Ethiopia, SPANA.

INTRODUCTION

Respiration is a cellular activity and respiratory tract is the organ that permits respiration to take place. The movement of air through the respiratory tract is achieved by the creation of pressure gradients during inspiration and expiration.

*Corresponding author. E-mail: fantadesissa@yahoo.com.

The primary functions of the nasal cavity and paranasal sinuses of the upper respiratory tract (URT) are to conduit air flow, warm, humidify air and remove particulate debris from inspired air prior to exposure to the lower respiratory tract. Equines are obligatory nasal breathers. Due to this fact, problems of the upper respiratory tract are more critical in these species than other domestic animals (Art et al., 2002). Respiratory disease is multifactorial resulting from a complex interaction of parasitic, bacterial, viral, management factor and environmental conditions. The lungs are continuously exposed to air that contains dust, bacteria, fungi, viruses and various noxious agents. Body defense against these potentially harmful materials is controlled by a complex of protective mechanisms (Jerome, 1993).

Most respiratory diseases in horses are originated from lesions located in the respiratory airways and lung (Quinn et al., 2002; Sellon and Long, 2007). The major causes of these respiratory diseases are microbial organisms. Foreign objects inhaled during feeding and oral drenching of horses with traditional medicines are predisposing factors for respiratory diseases (Quinn et al., 2002). The upper airway of healthy horses contains many bacterial flora including *Streptoccus equi subsp.zooepidemicus, Pasteruella spp., E coli, Actinomyces spp.,* and *Streptococcus spp.* Typically, horses with infectious lower airway diseases are infected with one of these bacteria, consistent with the concept that the contamination of the lower respiratory tract originates from the upper airways (Sellon and Long, 2007). In Ethiopia, despite the crucial role of cart horses in the livelihood of the owners, cart horse drivers and the economy of the country as a whole, the impact of respiratory diseases on their health and welfare and the potential microbes involved in the diseases have not been well addressed. Moreover, respiratory disease is one of the major health problems in society for protection of animals abroad (SPANA) Ethiopia project intervention areas being treated empirically (SPANA, 2008). Therefore, the objective of the study was to isolate and identify aerobic bacterial species from the upper respiratory tract of apparently health horses and horses with respiratory tract diseases in three selected SPANA project intervention towns of Central Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in three towns of Central Ethiopia namely, Debre Zeit, Debre Brihan and Adama from October, 2009 to June, 2010. Debre-Zeit and Adama are lowland areas whereas Debre Brihan represents highland altitude. Debre Zeit is 45 km South East of Addis Ababa, located 9^0N and 4^0E at an altitude of 1850m above sea level. The rainfall is bimodal. It receives an annual rainfall of 1151.6 mm with a mean minimum and maximum temperature of 8.5^0C and 30.7^0C, respectively and a mean relative humidity of 61.3%. The cart horse population in the town is 1170 and the number of carts is 585. Adama town is located in Eastern Shoa zone of Oromia regional state 100 km South east of Addis Ababa at $8^0$32'N and 39^0 and 17`E. It has an altitude of 1622 m in the Great Rift Valley and receives an annual rainfall ranging from 400mm to 800mm and a temperature of 13.9^OC to 37^OC. There are 1580 cart horses and 790 carts in the town. Debre Berhan is located in Amhara regional state, in Central highlands of Ethiopia, 130 km North East of Addis Ababa at 9^0 36^0 N and 39^0 38'E with an altitude of 2780m above sea level. The average annual rainfall is 950 mm and the average minimum and maximum temperature is 17.6^0c to 22.5^0c in August and June, respectively. The mean relative humidity is 68.2%. There are 7114 cart horses and 357 carts in the town (NAMSA, 2003).

Study population

Cart horses that were presented to SPANA Ethiopia clinics at Debre Zeit, Debre Berhan and Adama towns during the study period were the target population. The study population was forty eight apparently health horses (APHH) and fifty six horses with respiratory tract diseases (HRTD) brought to the clinics. Apparently healthy horses (APHH) were horses with no obvious signs of diseases: no sores, no history of respiratory diseases, normal range of vital signs, body condition score 2 and above in a 1 to 5 scale brought to the clinic for regular deworming. Horses with respiratory tract diseases (HRTD) were horses with obvious signs of respiratory diseases: nasal discharges, with or without fever, dyspnoea, anorexia, respiratory distress, history of respiratory diseases and abnormal lung sounds.

Study design

A cross sectional study design was employed and the study population was selected using simple random sampling technique. All the study animals were subjected to a thorough physical and clinical examination prior to sampling.

Sample collection

Each study animal was individually identified, restrained by its owner and kept fixed. The nares and the external parts of the nose were cleaned using 70% ethyl alcohol. After disinfection, sterile cotton tipped swab in a sterile test tube having a length of 20 to 25cm was inserted into the nasopharynx, rotated and rubbed back and forth against the nasopharyngeal wall gently and carefully to take a nasopharyngeal swab sample. The swab was replaced back into the sterile test tube to which a transport medium, 5ml of tryptone soya broth, was added and tightly closed with a stopper. After labeling, the test tube containing the swab sample was kept in an ice box, shipped to a Microbiology laboratory and incubated aerobically overnight until culturing takes place on the subsequent day.

Isolation and Identification

The broth cultured samples, which were incubated overnight aerobically were thoroughly agitated and mixed. A loopful of broth culture was taken and streaked over an identified Petri dish plates containing blood agar base supplemented with 7% sheep blood. At least, two cultures were made from each specimen. The remaining samples in the test tubes were put as a sample pool source inside a refrigerator at $+4^0$C until the end of the investigation process. From culture positive plates representative of colonies were further streaked on blood agar, MacConkey agar and tryptic soya agar. Pure colonies again further transferred into slant nutrient agar for

further tests. The isolates were identified based on their growth characteristics, primary identification and secondary biochemical tests. All cultures were incubated aerobically at 37^0c for 24 to 48hrs (Quinn et al., 2002).

Data analyses

SPSS Version 16 as a statistical package was used to see variation, nature and proportion of isolates between APHH and HRTD. Descriptive statistics (percentage) was used to summaries the generated data. P value less than 0.5 was used to see the significance level.

RESULTS

All the samples collected for aerobic bacterial isolation yielded at least one isolate. A total of 270 bacterial isolates belonging to 10 genera were recovered from the entire samples with an average recovery rate of 2.6 bacteria per sample. Gram positives were recovered more often than Gram negative bacteria. Among the isolates, 178 (65.9%) were Gram positives and 92 (34.1%) were Gram negatives bacteria. The overall isolated bacterial species isolated in order of dominance include: *Bacillus* species (20%), *Streptococcus* species (17%), S*taphylococcus* species (16.7%), *E.Coli* (12.6%), *Pasteurella* species (11.5%), *Micrococcus* species (11.1%), *Bordetella* species (4.8%), *Pseudomona* species (3%), *Actinobacillus* species (2.2%) and *Rhodococcus equi* (1.1%) (Table1, 2 and 3). Almost all the isolates were recovered from both groups of horses except *Rhodococcus equi* and Actinobacillus species which were only isolated from APHH. The overall recovery rate of bacterial isolates in APHH was 52.2% (39% from highland and 61% from lowland horses) and 47.8% (32.6% from highland and 67.4% from lowland horses) in HRTD.

DISCUSSION

The present study has shown the presence of a wide variety of bacterial species in the upper respiratory tract of APHH and HRTD brought to SPANA Clinics at Adama, Debre Zeit and Debre Berhan towns of Central Ethiopia. Despite variation in the percentage of bacterial isolates, there was no statistically significant ($p> 0.05$) variation among the isolates except *Staphylococcus* species with respect to altitude and health status of the animals. *Bacillus* species, the predominant bacteria recovered in this study, were isolated from the upper respiratory tract of cart horses at a rate of 20%. Several researchers have isolated *Bacillus* species at different rate of recovery from different species of animals (Shemsedin, 2002, Tesfaye, 2004, Yimer, 2007, Desissa et al., 2009). Most Bacillus species are saprophytes and they are widely distributed in air, soil and water (Quinn et al., 2002). The presence of *Bacillus* organisms in nasopharyngeal cavity might reflect and associated with deep grazing behavior horses which exposed them to contaminate the cavity with the saprophytic bacilli in soil. *Streptococcus* species were encountered as second dominant bacteria. It was isolated at the rate of 17% equally from both APHH and HRTD. Streptococci are widely distributed in nature and commensal on the upper respiratory tract of equine species which way be potentially pathogenic (Carter and Chengappa, 1991, Quinn et al., 2002). As a matter of the fact, isolation of streptococcus species from horses with respiratory syndromes indicates the role of these bacteria as a primary opportunistic pathogen in respiratory tract infections following any stressful conditions. *Staphylococcus* species were isolated at a rate of 16.7% (17% from APHH, 16% from HRTD, 77%). Statistically significant variation in number of *Staphylococcus* species ($p<0.05$) between lowland and high altitudes, with high isolation rate from lowland horses was observed. The variation might attributable to horses in the lowlands are under continuous environmental stresses such as high environmental temperature during daytime that profoundly amplifies the impact of work stress which eventually leads to immunosupression. A related study indicated that *Staphylococcus auerus* was isolated from horses with cases of pneumonia (Sweeny et al., 1998). Coagulase negative *staphylococci* (CNS) were isolated as the dominant species of bacteria in upper respiratory infections of foals (Boguta et al., 2004). Coagulase positive *staphylococci* can be involved as opportunistic bacteria following pathogenic role of stress conditions such as viral infections and other causes of infection in immunosuppressed hosts (Quinn et al., 2002). *Escherichia coli* was the leading isolate among Gram negatives with an isolation rate of 12.6% (17% from both APHH and HRTD). It has been suggested that *E. coli*, which is usually harmless in their natural habitat, could cause disease when they gain access to other sites or tissue leading to pulmonary and urogenital infections (Pelczar et al., 1986).

Pasteurella species were the second dominant among the population of Gram negative bacteria isolated at a rate of 11.5% (12% from APHH, 14% from HRTD). Similar study conducted on sheep showed the isolation rate of pasteurella as high as 68.6% from tonsil tissue (Tesfaye, 2004) and at the rate of 8.7% from the lung of camels (Shemsedin, 2002). *Mannhemia hemolytica*, a normal flora of the upper respiratory tract, may play a secondary role after the primary initiating agent suppressed the host defense mechanism and favors the multiplication of *pasteucella* species leading to bronchopneumonia (Aiello and May,1998). *Micrococci* species were isolated at a rate of 11.1%. *Micrococci* are non-pathogenic species of upper respiratory tract of domestic animals (Quinn et al., 2002). Hence, the isolation of the bacteria from HRTD does not necessarily indicate their role in upper respiratory tract *diseases. Bordetella* species were isolated at a rate of4.8% (5% from APHH, 3% from HRTD.

Table 1. Bacterial species isolated from apparently healthy cart horses, Central Ethiopia.

Altitude	Isolated Species	Number	Percentage
Highland	*Streptococcus* species	10	41
	Micrococcus species	10	66
	Staphylococcus species	7	29
	Pasteurella species	6	50
	E. coli	7	41
	Bacillus species	7	25
	Actinobacillus species	2	33
	Bordetella species	4	50
	Pseudomonas species	2	40
		55	**39**
Lowland	*Streptococcus* species	14	59
	Micrococcus specie	5	34
	Staphylococcus species	17	73
	Pasteurella species	6	50
	E. coli	10	59
	Bacillus species	20	75
	Actinobacillus species	4	67
	Bordetella species	4	50
	Pseudomonas species	3	60
	Rhodococcus equi	3	100
		86	61
Total		**141**	**100**

Table 2. Bacterial species isolated from cart horses with respiratory diseases, Central Ethiopia.

Altitude	Isolated species	Number	Percentage
Highland	*Streptococcus* species	6	27
	Micrococcus specie	5	33
	Staphylococcus species	2	9
	Pasteurella species	6	31
	E. coli	7	41
	Bacillus species	12	44
	Bordetella species	3	60
	Pseudomonas species	1	33
		42	**32.6**
Lowland	*Streptococcus* species	16	73
	Micrococcus specie	10	67
	Staphylococcus species	19	91
	Pasteurella species	13	69
	E. coli	10	59
	Bacillus species	15	56
	Bordetella species	2	40
	Pseudomonas species	2	67
		87	**67.4**
Total		**129**	**100**

The bacteria are commensal in the upper respiratory tract of animals being frequently attributed to broncho-pneumonia in guinea pigs and other rodents, in swine and lower primates (Graves,1970) Hence, the isolation of the bacteria from HRTD might indicate its role in the upper respiratory tract diseases of the horses. *Actinobacillus*

Table 3. Overall bacterial species isolated from both apparently healthy and cart horses with respiratory diseases, Central Ethiopia.

	Isolated species	Number	Percentage
Gram positive	*Streptococcus* species	46	17
	Micrococcus species	30	11.1
	Staphylococcus species	45	16.7
	Bacillus species	54	20
	Rhodococcus equi	3	1.1
		178	**65.9**
Gram negative	*Pasteurella* species	31	11.5
	E. coli	34	12.6
	Actinobacillus species	6	2.2
	Bordetella species	13	4.8
	Pseudomonas species	8	3
		92	**34.1**
Total		**270**	**100**

species were recovered only from APHH at a rate of 2.2%. The finding was relatively in agreement with the findings of Teklesillasie (2005) and Desissa et al., (2009) who reported 1.4% and 2.56% of the bacteria from the upper respiratory tract of goats and donkeys, respectively. *Pseudomonas* species were the third least isolate as compared to the isolates of this study and isolated at a rate of 3% (3% from apparently health cart horses, 2% from cart horses with respiratory syndrome). Studies done by Cabbasi *et al.,* (1975) indicated as *Pseudomonas* species are transient flora of the nasal mucosa of horses. *Rhodococcus equi* was encountered as the least dominant among the isolates. It was isolated only from APHH in the lowland areas at a rate of 1.1% of the total isolates. The bacterium is an opportunistic pathogen and common soil inhabitant (Quinn et al., 2002). In agreement to this finding *Rhodococcus equi* was found as one of the common isolates of equine respiratory tract (Racklyeft and Love, 2000).

Conclusion

In conclusion, the study showed that wide variety of aerobic bacterial species inhabiting the upper respiratory tract of horses with similar distribution of the bacteria both in APHH and HRTD with the exception of *Actinobacillus species* and *Rhodococcus equi* which were only isolated from APHH. Isolation of most bacteria from both groups of horses might suggest that the bacteria which reside in the URT of healthy animals have a chance to cause opportunistic infections following various stress factors. Gram positives were more often recovered than Gram negatives. The authors recommended that clinician should consider the dominance of Gram positives primarily as a cause of upper respiratory tract diseases in selection of antibiotics when dealing with horses suffering from respiratory cases. Moreover, further comprehensive study ought to be conducted to ascertain the specific pathogenic role of anaerobic bacteria, fungal species and viruses and antibiogram of all pathogens involved in the respiratory tract diseases of horses which would help clinicians to select appropriate drugs for the management of the clinical cases.

Conflict of Interest

The author(s) have not declared any conflict of interests

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Full Length Research Paper

Decreased level of serum paraoxonase (PON) activity in dogs with dilated cardiomyopathy (DCM)

Mahadesh Prasad AJ [1], Monika Krueger[2], Maxi Krueger[2]

[1]Department of Microbiology, Comprehensive Cancer Center, University of Pennsylvania Medical School 201E Johnson Pavilion, 3610 Hamilton Walk Philadelphia.
[2]Institute of Bacteriology and Mycology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany.

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Serum Paraoxonase (PON1) is a high-density lipoprotein associated esterase capable of hydrolyzing numerous organophosphates and protects low-density lipoprotein against peroxidation. PON1 is believed to play a crucial role in the prevention of cardiovascular disease in animals and PON1 activity has been shown to be low after myocardial infarction, liver disease and during oxidative stress. Here in this article we demonstrated that PON1 level is significantly lowered during the dilated cardiomyopathy (DCM) in dogs. This investigation was carried out on 208 canine serum samples. The serum PON/arylestrase activity was measured in 84 healthy dogs and 124 dogs with dilated cardiomyopathy (DCM) of varying severity. Since heart failure is characterized by oxidative stress, inflammation, deficiency in metabolic substrates and lack of blood supply to heart. Decreased PON activity was significantly observed in advanced stages of DCM.

Key words: DCM, Paraoxonase, Phenyl acetate, High-density lipoprotein, NYHA-classification.

INTRODUCTION

Canine dilated cardiomyopathy (DCM) is a primary cardiac disease characterized by chamber dilatation, systolic and diastolic dysfunction mostly affecting the left side of the heart (Kittleson 1998), besides myxomatous valvular disease it is the most common heart disease in dogs, affecting mainly large and medium breed dogs (Fox 1989). Certain breeds, Doberman Pinscher, Great Dane, Irish Wolfhound, Newfoundland, and Cocker Spaniel known to be more affected (Monnet et al. 1995, Sisson et al. 2000) (Fox and Moise 1999). It has become more evident that inflammation is an important factor in the pathophysiology of heart failure, even though cytokine are not considered being the primary reason for heart failure, their enhanced release and production contributes to remodelling and the failing heart (Anker et al.1997, Sisson et al. 1995). Previous reports suggested that distinct enhancement of Reactive oxygen species (ROS) generation was noticed in failing myocardium(Burton et al. 1984). Surprisingly, antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidases (GSHPx) were not affected during the ROS generation, (Tsutsui et al. 2008). ROS subsequently lead to cellular growth, hypertrophy, remodelling, lipid oxidation, inflammation, and cardiomyocyte apoptosis (Byrne et al. 2003). Sorescu and Griendling (2002) reported the role of ROS in end-stage heart failure due to remodelling

*Corresponding author E-mail: prasadjavarappa@hotmail.com.

remodelling of the failing myocardium by fibrosis, collagen deposition and metalloproteinase activation.

Serum Paraoxonase 1 (PON 1) is mainly synthesized in liver and tightly associated with High-density lipo-proteins (HDL) (Hasset et al., 1991, Förstermann 2010). PON1 is calcium dependent enzyme and exhibit three distinct activities; PON, arylesterase and diazoxonase (Gan et al, 1991; Canales and Sanchez-Muniz, 2003; Bionaz et al, 2007). Besides the ability of PON1 to hydrolyze a variety of organophosphates and oxidized lipids and thus plays a major role in the prevention of cardiovascular disease in animals (Genest et al, 1992) (Aviram et al, 1995, 2000; Oda et al, 2001). The association between low PON1 levels in serum and increased risk of coronary artery disease (CAD) has been well established from last three decades in cardio-vascular research (Mackness et al. 1999, Mackness et al. 2001, Azarsiz et al. 2003, Granér et al. 2006). Besides in cardiovascular diseases reduced serum PON activity has been observed in numerous disease conditions and states of increased ROS formation as altered lipid metabolism, inflammation, diabetes, liver failure, chronic renal failure, and neoplasias (Katsuramaki et al, 2002). Our results from this present investigation showed that the levels of PON1 in serum specimen of dogs with DCM were drastically decreased compared to control healthy samples. Interestingly as disease prolongs (NYHA I to IV) the level of PON1 keeps on diminishing, suggesting the possible influence of cardiac disease on anti oxidative capacity in association with severity of heart disease.

MATERIALS AND METHODS

Study design and criteria of selection

We selected serum samples from healthy dogs (control) and dogs with DCM. DCM – dogs were classified according to New York Heart Association (1994) scheme (NYHA I to IV) depending on the severity of heart disease. PON1 levels in all these samples were measured by using the synthetic compound phenyl acetate as a substrate for Paraoxonase (PON1). 124 client-owned dogs with DCM were enrolled into this prospective multicenter study. Between May, 2005 and November, 2007, 41 dogs were presented to the Small Animal Hospital, University of Leipzig, 38 to the Small Animal Hospital Kaiserberg (Duisburg), 12 to the Small Animal Hospital Huettig (Reutlingen) and 33 to the Small Animal Hospital Vollmar (Wissen/Bonn) (Tab. 1) were included in this study. DCM was diagnosed with echocardiography and measurements were made in right parasternal views. Dogs were excluded if other underlying systemic diseases such as infectious, gastro-intestinal, endocrine (except hypothyroidism), neoplastic, auto-immune diseases or fever of unknown origin were present. All experimental dogs were monitored with thorough medical history and physical examination, followed by echocardiography and a standard 6 - lead electrocardiogram to assess heart rate and diagnose cardiac arrhythmias. For echocardiography, the unsedated patients were either in right lateral recumbency or in standing position, with simultaneous ECG-recording, using accepted techniques (Thomas et al. 1993, Chetboul et al. 2005). Measurements of left ventricular (LV) end-systolic diameter, end-diastolic diameter, LV free wall and septal thickness in diastole and systole were done on 2D-guided M-mode recordings. LV % fractional shortening (FS) and ejection fraction (EF) were calculated by using Teichholz' equation. E – Point septal separation (EPSS) was obtained in M – mode. Left atrial (LA) and aortic diameter were measured in short axis 2D mode and the LA/Ao ratio was calculated (Bonagura 1983, Thomas 1984). In order to determine DCM, criteria stated by the ESVC Taskforce for Canine Dilated Cardiomyopathy were used (2003). The degree of heart disease was classified according to NYHA recommendations (Criteria Committee, 1994). The control group consisted of 72 medium- to large- sized blood donor dogs of the University of Leipzig and 12 Irish Wolfhounds who underwent DCM screening (Table 1). All dogs received a thorough physical examination**,** prior to taking the blood samples. The Irish Wolfhounds were found normal through a cardiologic examination (echocardiography, ECG).

Blood sampling

All dog's blood samples were allowed to clot for 45 minutes at room temperature and centrifuged at 3000 rpm for 15 minutes and the isolated serum was stored at -20 °C for further analysis.

Paraoxonase /arylestrase activity assay

Paraoxonase/arylestrase activity was measured by spectrophotometrically using phenyl acetate as a substrate. The assay reaction buffer was prepared using 2mM phenyl acetate dissolved in 20mM Tris-HCl buffer pH 8.0 containing 2mM calcium chloride, and 20 µl isopropyl alcohol was added for the total reaction volume to facilitate complete dissolution of phenyl acetate in the buffer. Total volume of 3ml reaction mixture was used for the assay. The reaction was initiated by adding 10µl of serum into 2990 µl of buffer substrate. The increased absorbance at 270nm was read for 3 minutes continuously. Blanks were included to correct the spontaneous hydrolysis of phenyl acetate. Enzyme activity was calculated using molar extinction coefficient 1310 M^{-1} cm^{-1}. The Paraoxonase/arylestrase enzyme activity was expressed in U/L (Prasad et al, 2009).

Statistical analysis

The statistic evaluation of the data was performed with the program SPSS. (SPSS Inc. Illinois 60606). The examination of normal distribution was done with Kolmogorov Smirnov test. The data was further analyzed for statistically significant differences between groups with the Mann -Whitney U test, with a level of significance set at $p< 0.05$ or higher.

RESULTS

From the statistical evaluation data of PON activity, a significant difference was accomplished due to the significant deviations from the normal distribution with the non-parametric Kruskal Valais test and U-test after Mann Whitney. The normal PON1 activities in control samples were between 65,000 U/L to 96,000 U/L. No reference values have been published in veterinary medicine except studies done by Turk et al. (2004, 2005, 2007) in dairy cows where normal PON activity was ranging between 60,000 to 80,000 U/L. Table 1 summarizes the baseline characteristics for study participants. The average

Table 1. Client-owned dogs with DCM enrolled.

Parameter	Mean ±SD				
	NYHA I (n-30)	**NYHA II (n-23)**	**NYHA III (n-30)**	**NYHA VI (n-41)**	**Control (n-84)**
Age (years)	6.3±0.5	7.6±0.6	7.3±0.4	7.6±0.4	4.3±2.5
Sex					
Female	11	7	9	9	44
Male	19	16	21	32	40
Weight (kg)	64.9 kg±3	46.4 kg±4.7	47.4 kg±3.9	51.3 kg±3	37.1 kg±15.1
Heart rate (beats/minute)	120±6	131±7	135±9	174±7	90±12
Breeds	Bouvier (n-1)	Afghan (n-1)	Austr. Shepherd (-1)	AC Spaniel (n-1)	Barsoi (n-1)
	Dalmatian (n-1)	Am. C. Spaniel (n-1)	B. Collie (n-1)	Bobtail (n-2)	B. Collie (n-2)
	Dobermann (n-2)	Bouvier (n-1)	C. Spaniel (n-1)	Bullterrier (n-1)	Dalmation (n-1)
	GSH (n-1)	Briard (n-1)	Deerhound (n-1)	Dalmatian (n-1)	Dobermann (n-8)
	Great Dane (n-2)	Bullmastiff (n-1)	Dobermann (n-4)	Dobermann (n-10)	Dogue de Bordeaux (n-3)
	IW (n-20)	Dalmatian (n-1)	Great Dane (n-2)	Great Dane (n-11)	G. Shorthair (n-1)
	Labr. Retriver (n-1)	Great dane (n-1)	Engl. Setter (n-3)	G. Retriever (n-1)	G. Retriver (n-7)
	NFL (n-1)	GSH (n-1)	G. Retriver (n-1)	Hovawart (n-1)	Great Dane (n-2)
	Rottweiler (n-1)	I. Terrier (n-1)	Hovawart (n-1)	I. Setter 9 (n-1)	Greyhound (n-1)
	-	IW (n-9)	IW (n-10)	IW (n-2)	GSH (n-10)
	-	Labr. retriver (n-2)	Labr. Retriever (n-1)	Mongrel (n-2)	Hovawart (n-1)
	-	Mongrel (n-1)	Mongrel (n-1)	NFL (n-2)	IW (12)
	-	NFL (n-1)	NFL (n-1)	G. Schnauzer (n-2)	Labr. Retrievr (n-14)
	-	W. Shephered (n-1)	Port. Water Dog (n-1)	Rottweiler (n-1)	Malinois (n-5)
	-	-	Min. pinscher (n-1)	St. Bernard (n-3)	Mongrel (n-3)
	-	-	-	-	Rhod. Ridgback (n-2)
	-	-	-	-	Rottweiler (n-3)
	-	-	-	-	Munsterlander (n-1)
	-	-	-	-	St. Bernard (n-2)
	-	-	-	-	Vizsla (n-1)
	-	-	-	-	Weimaraner (n-1)

AC Spainel- American Cocker Spaniel, B. Collie- Border Collie, C. Spaniel-Cocker Spaniel, I.Setter- Irish Setter, I.Terrier- Irish Terrier, IW-Irish Wolfhound, G.

age, body weight and heart rate of the control dog group was 4.3 ± 2.5 years, 37.1 ± 15.1kg and heart rate 90 ± 12BPM, respectively and among these 52.4% were female. Dogs in NYHA I were 6.3 ± 0.5 years of age, 64.9 ± 3kg, heart rate 120 ± 6BPM, and 36.7% were female. Dogs in NYHA II were 7.6 ± 0.6 years, 46.4-±4.7kg body weight, and heart rate 131 ± 7BPM at an average with 30.4%female amongst them. In dogs with NYHA III mean age, body weight and heart rate were 7.3 ± 0.4 years, body weight 47.4 ± 3.9kg, and heart rate 135 ± 9, and 30% female. NYHA IV-classified

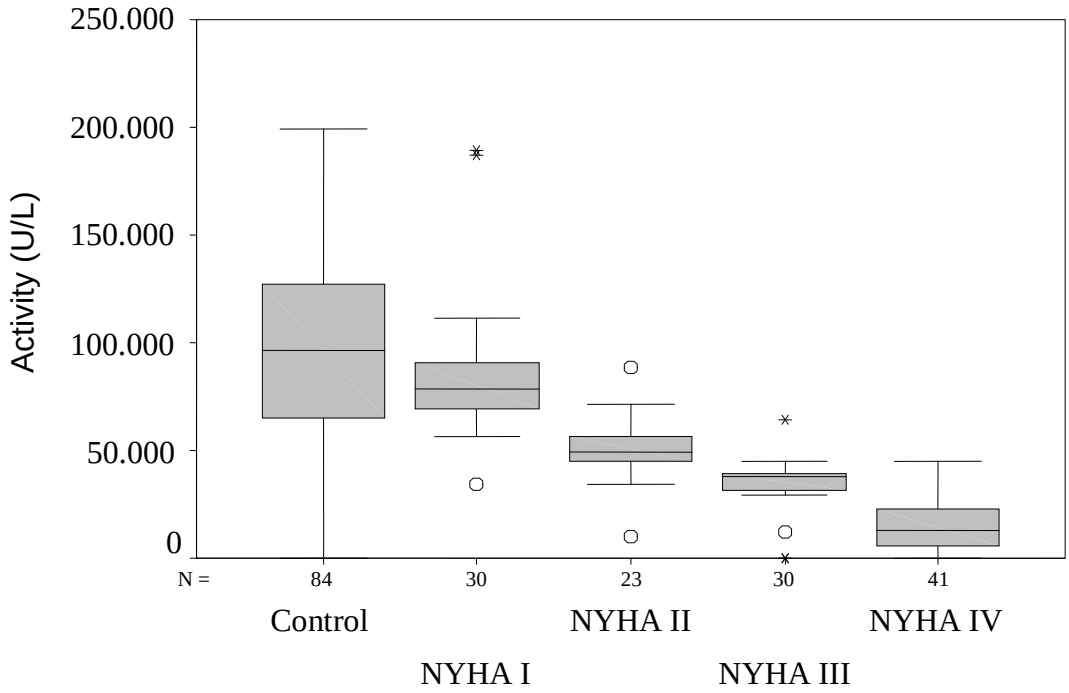

Figure 1. Paraoxonase 1 activity in association of severity of heart disease.

female. classified dogs were 7.6 ± 0.4 years of age, with a weight of 51.3 ± 3kg, heart rate 174 ± 7, and 22% females. The PON1 activity of the serum samples from dogs of NYHA I – IV and control dogs are shown in Figure 1. Median PON levels in control dogs were 96663.5 U/L (65191.8, 127384.8 U/L), in NYHA I 78756 U/L (68689.5, 90850 U/L), NYHA II 49129 (44657, 56783 U/L), NYHA III 37505.5 U/L (31068.8, 39376 U/L), and NYHA IV 12901 U/L (5236, 23000.5 U/L). Median PON concentrations were significantly higher in control group and dogs classified with NYHA I compared with dogs in NYHA class II – IV ($p = 0.0001$).

DISCUSSION

The potential role of reactive oxygen species (ROS) has been discussed on several occasions in scientific literature. With progression of congestive heart failure (CHF) unrelated to etiology increases in ROS a reduction of antioxidant levels (Keith et al. 1998) takes place. Animal models of CHF did not only show excessive ROS generation only, but also impaired myocardial defense mechanisms (Dhalla et al. 1996, Hill et al. 1997). Over the time chronic oxidative injury augments impaired myocardial function and as a consequence also heart failure (Mak and Newton 2001). Considering oxidative stress, production of free radicals, inflammation and genetic factors in cardiomyopathy (Jarvik et al. 2002, Schrier et al 1988), our results from present investigation suggests that oxidative stress and production of free radicals in different stages of heart failure is directly related to the PON1 activity in dogs. However, there was no notable alteration in PON1 activity in dogs with NYHA I, may be due to lack of appearance of clinical symptoms during the initial stage. Hence we assume that Patho physiological mechanisms contributing to de-compensation and ROS formation are activated to a minor degree at NYHA I, whereas in NYHA II, III and IV gradual increase in disease conditions decreases PON activity gradually, suggesting the impor-tance of PON1 in heart disease. Minimal PON activity was shown in dogs classified as NYHA IV, which allows the conclusion of an association between severity of heart disease and anti oxidative capacity. Other stu-dies were able to establish this relationship as well (Keith 1998, Mallat 1998). Other investigators have reported previously that decrease in PON levels in cardiovascular disease and CHF (Watson et al 1995, Xie and Zhao 2002, Aviram et al. 2008) indicating the potential effect of oxidative stress and inflammation on PON1 activity. Hence we could speculate that the lowered level of PON1 is due to the tissue injury and inflammation during the disease.

Previously, Feingold et al. (1998) showed that rapid and sustained decrease of PON1 mRNA level in the liver after LPS administration and now its more evident that our findings also clearly show that PON1 decreases at protein level in the serum, Suggesting that PON1 level is decreased both in post translationally (at protein level) as well as at transcript level (m-RNA level). Hence the results of our study suggested that the anti oxidative ca-pacity of dogs with DCM is impaired with lowered PON1

levels.

Conclusion

Finally we conclude that there is a clear correlation of PON1 activity and severity of heart disease, even though PON1 has been reported so far mostly in regards to cardiovascular disease and protection of HDL and LDL which is not reported in respect to DCM, PON1 activity as a marker of the status of the anti oxidative capacity and heat disease in dogs is very much alluring. Further, studies should be conducted to understand PON1 structural changes during the diseases condition in dogs, also research has to be carried out to understand the mechanism of increasing PON levels in dogs by dietary supplement to cope up with the heart disease and normal physiological conditions. Thus, this novel report on DCM and its correlation with antioxidant enzyme PON1 during pathological condition will open up new era of Paraoxonase research in animals for their better health.

LIMITATIONS

Only 12 dogs out of 84 were used as controls did not receive an echocardiographic evaluation. Possible occult stages of DCM cannot be excluded.

Conflict of Interest

The author(s) have not declared any conflict of interests

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