Exercise-associated sudden death in Finnish standardbred and coldblooded trotters : - a case series with pedigree analysis

Trachsel, Dagmar S.

2021-09

Trachsel , D S , Calloe , K , Mykkänen , A K , Raistakka , P , Anttila , M , Fredholm , M , Tala , M , Lamminpää , K , Klaerke , D A & Buhl , R 2021 , ' Exercise-associated sudden death in Finnish standardbred and coldblooded trotters : - a case series with pedigree analysis ' , Journal of Equine Veterinary Science , vol. 104 , 103694 . https://doi.org/10.1016/j.jevs.2021.103694

http://hdl.handle.net/10138/345333
https://doi.org/10.1016/j.jevs.2021.103694

Exercise-associated sudden death in Finnish standardbred and coldblooded trotters - a case series with pedigree analysis

Dagmar S. Trachsel , Kirstine Calloe , Anna K. Mykkänen , Pia Raistakka , Marjukka Anttila , Merete Fredholm , Martti Tala , Katariina Lamminpää , Dan A. Klaerke , Rikke Buhl

PII: S0737-0806(21)00324-5
DOI: https://doi.org/10.1016/j.jevs.2021.103694
Reference: YJEVS 103694

To appear in: *Journal of Equine Veterinary Science*

Received date: 16 December 2020
Revised date: 30 April 2021
Accepted date: 19 June 2021

Please cite this article as: Dagmar S. Trachsel , Kirstine Calloe , Anna K. Mykkänen , Pia Raistakka , Marjukka Anttila , Merete Fredholm , Martti Tala , Katariina Lamminpää , Dan A. Klaerke , Rikke Buhl , Exercise-associated sudden death in Finnish standardbred and coldblooded trotters - a case series with pedigree analysis, *Journal of Equine Veterinary Science* (2021), doi: https://doi.org/10.1016/j.jevs.2021.103694

Exercise-associated sudden death in Finnish standardbred and coldblooded trotters - a case series with pedigree analysis

Dagmar S. Trachsel[a,b,*], Kirstine Calloe[a], Anna K. Mykkänen[c], Pia Raistakka[c], Marjukka Anttila[d], Merete Fredholm[a], Martti Tala[e], Katariina Lamminpää[e], Dan A. Klaerke[a], Rikke Buhl[b]

[a]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlaegevej 100, 1870 Frederiksberg C, Denmark

[b]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Højbakkegaard Alle 5, 2630 Taastrup, Denmark

[c]Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Koetilankuja 2, 00790 Helsinki, Finland

[d]Veterinary Bacteriology and Pathology Research Unit, Finnish Food Authority, Mustialankatu 3, 00790 Helsinki

[e]Suomen Hippos, the Finnish Trotting and Breeding Association

[*]Corresponding author. Dagmar S. Trachsel, Equine Clinic, Surgery and Radiology, Freie University Berlin, Oertzenweg 19B, 14163 Berlin, e-mail: dagmarsenta.trachsel@fu-berlin.de

Highlights

- The incidence for sudden death in Finnish standardbred was 3.9/100 000 starts
- The incidence for sudden death in Finnish coldblooded trotters was 2.9/100 000 starts
- The incidences for sudden death were higher than for catastrophic musculoskeletal injuries in both breeds
- SD was mainly related to vessel rupture or unexplained reasons (presumed cardiac arrest)
- The study could not show clear indices for genetic cause in sudden cardiac death

Abstract

Exercise-associated sudden deaths (EASDs) are deaths occurring unexpectedly during or immediately after exercise. Sudden cardiac death (SCD) is one cause of EASD. Cardiac arrhythmias caused by genetic variants have been linked to SCD in humans. We hypothesize that genetic variants may be associated with SCD in animals, including horses. Genetic variants are transmitted to offspring and

their frequency might increase within a family. Therefore, the frequency of such variants might increase with the inbreeding factor. Higher inbreeding could have a negative impact on racing performance.

Pedigree data and career earnings from racehorses diagnosed with SCD between 2002 and 2017 were compared using non-parametric tests with 1) control horses that died due to catastrophic musculoskeletal injuries and 2) horses that raced during the same period without reported problems. Diagnosis of SCD was based on necropsy reports, including macroscopic and microscopic examinations.

Death was registered in the study period for 61 horses. Eleven of these horses were excluded due to missing autopsy reports. In 25 cases, the diagnosis remained unknown and death was possibly caused by cardiac arrhythmia, in 2 cases cardiac disease was identified, in 7 cases a rupture of a major vessel had occurred. In addition, 16 horses died or were euthanized due to severe musculoskeletal injuries. No significant differences in inbreeding coefficients or in career earnings were found between the groups or between horses with EASD compared with other horses racing during the same period. The study provides no evidence for increased inbreeding factor in Finnish racehorses with SCD.

Keywords

Inbreeding coefficient; coldblooded trotter; standardbred trotter; post-mortem examination; exercise, sudden cardiac death

1. Introduction

Fatal incidents, often reported as exercise-associated sudden deaths (EASDs), occur relatively often during equine sporting events, but are only sporadically reported, and many countries have no official register [1]. While musculoskeletal injures have recently been reported to have an incidence of 117/100 000 starts [2], EASD of unknown origin has been reported to have an incidence of 8-30/100 000 starts in Standardbreds (STB) or Thoroughbreds (THB) depending on the racing discipline [3-5]. Recently, in Scandinavia, more attention has been paid to EASD in equine athletes, and in Finland necropsy has been mandatory for all horses with a fatal incident on a racetrack since 2002. Earlier publications have reported that 20-69% of these EASD cases do not have structural lesions sufficient to explain the death on necropsy [1, 6-8]. Such deaths are frequently attributed to a cardiac origin. In humans, several cardiac disorders have been linked to sudden cardiac death (SCD) in athletes [9-11], including channelopathies such as the repolarization disorder long QT syndrome (LQTS). LQTS presents on the surface ECG as an increased interval between the Q and T wave and is associated with an increased risk of *Torsade de pointes* arrhythmias and SCD. Inherited LQTS has been associated with mutations in genes encoding ion channels, particularly voltage-gated K^+ channels [12], including the genes *KCNQ1* and *KCNH2* encoding the voltage-gated channels $K_v7.1$ and $K_v11.1$, respectively. Mutations in *KCNQ1* are associated with arrhythmias during high sympathetic stimulation and SCD during exercise [13, 14]. Sympathetic drive is therefore important in the inherited form of LQTS, and, as in humans, horses might be at risk of severe arrhythmias due to repolarization disorders in relation to sympathetic activation during exercise or in stressful situations. Equine $K_v7.1$ and $K_v11.1$ have been sequenced and shown to have similar importance as is humans in repolarization [15, 16]. However, no data are available on genetic variants of these K^+ channels or on their potential involvement in the occurrence of SCD in horses. In humans, the most common forms of LQTS are typically inherited in an autosomal dominant manner, whereas some of the less frequent forms have a recessive inheritance or an incomplete penetrance [17, 18]. In dogs, several inherited cardiac disorders are known, including arrhythmogenic disorders leading to SCD [19-21]. Pedigree analyses in this species have proposed autosomal dominant inheritance, autosomal dominant inheritance with incomplete penetration, and autosome recessive inheritance or polygenic inheritance [19, 20, 22]. An

autosomal dominant inheritance seems improbable in horses since SCD appears to have a low incidence and no family predisposition has been described even in closely monitored breeds. A genetic variant with low penetrance or a multigenetic deleterious combination that causes disease only when all or most of the involved genes are homozygously expressed is more probable. Also, it is well known that inbreeding increases the frequency of recessive traits and diseases due to an increasing probability for an offspring to be homozygous for a deleterious recessive gene. The associated reduction in fitness has been referred to as inbreeding depression [23]. Based on pedigree analyses in different horse breeds, inbreeding depression has been linked to reduced racing performance [24, 25], unfavourable conformation traits [26], and reduced fertility [27-30]. A recessive or multigenetic condition with low penetrance could therefore translate into reduced fitness in parallel with an increased inbreeding factor. However, the relationship between the inbreeding coefficient and fatal incidents, such as EASD, during races has never been investigated. Diminished racing performance could potentially be reflected in reduced performance parameters. However, few easily accessible and reliable parameters exist for racing performance. Career earnings, even if imprecise, would be an easy approach to obtain a rough overview of racing results.

We hypothesize that genetic variants or inbreeding may be associated with SCD in animals, including horses. The aim of this study was to analyse the frequency and causes of EASD in the Finnish racehorse population based on necropsy reports collected between 2002 and 2017. We then compared the inbreeding coefficients in horses with SCD with those of horses that died from other causes as well as with all horses racing during the study period. Lastly, we compared the mean career earnings of horses with SCD with those of horses that died from other causes and horses that raced during the study period.

2. Materials and Methods

2.1. Horses

A full necropsy has been performed on all horses dying on a racetrack in Finland since 2002 by the Veterinary Bacteriology and Pathology Research Unit of the Finnish Food Authority. All necropsies are performed under the supervision of an experienced board-certified pathologist. Reports from 2002

to 2017 were reviewed for analysis. The Finnish racehorse population included Finnish standardbred racehorses (FSTB) and Finnish coldblooded racehorses (FCB). These two breeds were analysed separately. Exercise-associated sudden death (EASD) was defined as an acute collapse and death during or immediately after racing (including the warm-up period of a maximum of 1 hour 30 min prior to the race and the cool-down period up to 15 min after the race) in a closely observed apparently healthy horse in the absence of clinical data indicative of a catastrophic orthopaedic injury [3]. SCD was defined as a case of EASD based on macroscopic and microscopic examinations judged by the pathologist to be certainly or presumptively related to a sudden cardiac arrest and in which there were no pre-existing lesions that would explain the acute death [1]. The necropsy included systematic macroscopic inspection of all organ systems, and histological samples were taken from all major organs and from all organs with macroscopic changes. The histological sections were stained with haematoxylin and eosin. The group of SCD was further divided into two subgroups: SCD with unknown origin (SCD-unknown, SCD_u) when the death was related to cardiac arrest in the absence of identifiable macroscopic or histological findings other than congestion of the lung or other organs and SCD with identified cardiac lesion (SCD-cardiac disease, SCD_{cd}).

Horses in which a rupture of a major vessel was identified with associated internal haemorrhage were classified in the group "vessel rupture" (VR). The group of horses classified as catastrophic musculoskeletal injuries (CMI) consisted of horses that were closely observed and previously healthy, but that had died or were euthanized during or immediately after racing (in the observation period from 1 hour 30 min prior to the race to 15 min after the race) due to catastrophic musculoskeletal injuries that cause immediate death or necessitate euthanasia due to the severity of the lesion and the associated poor prognosis. An overview of the classifications of the groups and the numbers of horses is provided in Figure 1. Horses born in the same years as horses in the SCD and CMI groups that are registered as having been racing in Finland were used as a reference population (group "raced"). Lastly, the money earned during the entire career by the horses that died at the racetrack was compared with the mean career earnings of racehorses of the same age as the deceased horses at the time of death. Incidence of fatality was calculated per 100 000 starts. The pedigree data for each horse, the number of races (official recorded race, excluding qualification races) and starts (a horse

participating in an official recorded race), and the career earnings were available from the Finnish Trotting and Breeding Association (Suomen Hippos).

2.2. Data analyses

The findings in the necropsy reports were reviewed for consistency with the diagnoses of EASD and SCD. Due to the low number of cases, the assumption of normal distribution was difficult to assess, and a non-parametric approach was chosen. The results are therefore reported as median, range, and 2.5-97.5 percentiles. In FCB horses (N=7), only descriptive results were reported. For FSTB horses, non-parametric analyses were performed. Homogeneous distribution of age, gender, and incidence of fatality in the groups was analysed with Fischer's Exact test. Incidence of fatality among groups was compared with a Mann-Whitney test. The inbreeding coefficients were calculated by taking into account all registered ancestors in the pedigree with the software CFC[1] and compared with a Kruskal-Wallis test and Dunn's test for correction of multiple comparisons. The career earnings of the horses in each group were compared with a Mann-Whitney test or a Kruskal-Wallis test and Dunn's test for correction of multiple comparisons when more than two groups were compared. Horses were further divided into groups based on whether they earned more or less than the mean career earnings of same-aged horses. This was done for all groups of horses (EASD, SCD, VR, and CMI). The results were analysed using Fischer's Exact test.

Statistical analyses were performed with commercially available software.[2,3.] A p-value of less than 0.05 was deemed significant.

[1] CFC, Contribution, Inbreeding (F), Coancestry, version 1.0, released 2006, downloaded at http://animalbiosciences.uoguelph.ca/~msargol/

[2] GraphPad Prism, version 7.00 (GraphPad Software, San Diego, CA, USA, www.graphpad.com)

[3] R-software®, version 3.1.0 (R Development Core Team, Vienna, Austria)

3. Results

Between 2002 and 2017, altogether 61 horses died or were euthanized during or immediately after racing, on average 4.1 horses/year. In this period, 127 949 races took place (average 7996 ± 858 (standard deviation (STD)) races/year), representing 1 086 259 starts (average 67 891 ± 6929 (STD) starts/year). The incidence of fatality over the study period was 5.9/100 000 starts. For 11 of these horses, the autopsy report had not been registered electronically, and therefore, was not available at the time of study (see Fig. 1). These horses were excluded from further analyses. For 34 racehorses (27 FSTB and 7 FCB), the diagnosis after necropsy examination was EASD, on average 2.3 horses/year (1.8 FSTB/year, 0.5 FCB/year). The incidence for EASD in FSTB was 3.9/100 000 starts and in FCB 2.9/100 000 starts. During the same period 16 horses (15 FSTB and 1 FCB) died or were euthanized for CMI (1.06 horses/year, 1.0 FSTB/year, 0.06 FCB/year). The incidence for CMI in FSTB was 2.2/100 000 starts and in FCB 0.3/100 000 starts. The incidence of EASD was higher than the incidence of CMI in both breeds (significant for FSTB, p=0.03). Demographic data are presented in Table 1. No significant difference in gender distribution (p=0.36) or age (p=0.94) emerged among the groups. The findings in necropsy reports are summarized in Table 2. In the FSTB group, 21 cases were diagnosed with SCD. In 2 cases, lesions that could have caused the SCD were identified in cardiac tissue (SCD-cardiac disease, SCD_{cd}). The diagnoses were mild lymphocytic myocarditis in association with a bronchointerstitial pneumonia consistent with the suspicion of viral infection and hamartoma. The remaining 19 cases were classified as SCD of unknown origin (SCD-unknown, SCD_u) based on the diagnosis peracute cardiac arrest with no changes other than acute congestion in the lungs and other organs. In the FCB group, no cardiac disease was diagnosed; all SCDs were classified as SCD_u. In 7 cases (6 FSTB, 1 FCB), death was caused by a ruptured vessel with related massive blood loss or cardiac tamponade due to a haemoperitoneum (group “vessel rupture”, VR). In

5 cases (4 FSTB, 1 FCB), the vessel could be identified. In 4 cases (3 FSTB, 1 FCB), the aorta was ruptured and in one FSTB case a major mesenteric artery. In 16 cases (15 FSTB, 1 FCB), death or euthanasia was explained by the presence of catastrophic musculoskeletal injury (group CMI). In 14 cases, the horses required euthanasia due to the severity of the injuries and the associated poor prognosis, and in 2 cases the horse died immediately after the injury before any medical treatment could be applied. There were 20 958 FSTB and 9986 FCB born in the same period as the SCD and CMI horses registered as having been or being raced (referred to in the paper as the racing population, or group "raced").

The calculated inbreeding coefficients are summarized in Table 3 and Figure 2. Comparison with a Kruskal-Wallis test revealed no significant difference between the inbreeding coefficients calculated for the groups "raced", CMI, SCD, or VR.

The career earnings in the groups are summarized in Figure 3. No significant difference emerged between the FSTB groups (comparison EASD vs. CMI, p=0.40; comparison SCD vs. VR or CMI, p=0.53). Further, the proportion of horses earning more or less than the mean career earnings of horses of the same age was not different between the groups (EASD vs. CMI, p=0.20; SCD vs. CMI, p=0.09; VR vs. CMI, p=1.0; Figure 4).

4. Discussion

This study reports the number of horses that died or were euthanized during or immediately after racing in Finland over a 15-year period. The overall incidence of fatality with 5.9/100 000 starts was lower in our study population than in data published for THB in flat races (44-76/100 000 starts [4, 31]) or for STB (64/100 000 starts [5]). The incidence for EASD in our study (FSTB 3.9/100 000 starts, FCB 2.9/100 000 starts) was also lower than in the data published in THB in flat races (8-10/100 000 starts [4, 31]) or for STB (10/100 000[5]). In contrast to former studies where CMI was the predominant cause of death [4, 5, 31], in the Finnish racehorse population more horses had died from EASD than from CMI. There is no obvious explanation for this finding, however, a recent meta-analysis reported also differences in incidence of CMI in different geographic regions and highlighted

that factors such as differences in management, race category, and jurisdiction might explain part of these discrepancies [2].

Most of the horses with EASD were classified as SCD based on acute congestion in the lungs and other organs in the absence of specific macroscopic or microscopic findings, as defined in the Materials and Methods section. In accordance with earlier studies, the horses that died from SCD on racetracks in Finland often showed pulmonary congestion, haemorrhage, and/or oedema [1, 7]. These findings are, however, unspecific, and therefore, most of the horses were finally classified as SCD_u. By using this approach, we accounted for the fact that in these cases the actual biochemical or functional cause of the acute cardiac arrest was only speculative, as discussed by Lyle et al. [1]. Possible causes for acute cardiac arrest in these cases include acute pain, electrolyte and/or acid-base imbalance, or hypoxia. However, also such disorders as functional abnormality in the conduction system, abnormal cardiomyocyte physiology, or latent metabolic disease would have been included in this group. To date, such causes have been virtually impossible to diagnose in veterinary medicine. Therefore, the proportion of cardiac death of unknown cause is reported to account for 20-69% of EASD cases at necropsy [1, 6-8]. Cardiac lesions are rarely described, despite some studies reporting up to 25% of cases with macroscopic or histological cardiac lesions [1, 32]. The described lesions were mostly related to changes in chamber size, myocardial inflammation, or fibrosis [1, 32]. However, the significance of these findings is still undergoing debate [6]. Viral infection can potentially lead to myocarditis in the horse [33] and may contribute to the death of horses with mild lymphatic myocarditis. In contrast, well-defined heart diseases, such as hypertrophic cardiomyopathy, coronary arterial diseases, or arrhythmogenic cardiopathy, are commonly identified in human athletes with SCD [9-11]. Further, pre-screening programmes exist to identify such anomalies in athletes in order to minimize the risk of SCD during exercise [34]. However, such examinations do not exist in equine medicine. The lack of diagnostic tools or procedures to predict EASD in horses is a real issue for driver and rider safety as well as for animal welfare. A uniform post-mortem examination protocol, as recently proposed [35], would contribute to better understanding of SCD in horses.

Rupture of large vessels in horses is regularly reported, accounting for up to 9% of deaths [1, 7, 32]. The diagnosis is supported by the large amount of blood found in body cavities and identification of

the side of rupture of the vessel. However, in up to 50% of cases, the site of rupture cannot be identified [7, 32]. Similarly, the anatomical location of the ruptures could not be specified in two cases of our population. In accordance with the findings of DeLay et al. [32], the aorta was more commonly affected in our population. However, in some previous studies ruptured intra-abdominal vessels were identified more often [1, 7]. Therefore, rupture of a main intrathoracic or intra-abdominal artery remains an important differential diagnosis for EASD on a racetrack. Spontaneous rupture due to acute hypertension during excitement or racing has been considered as the most probable cause of EASD in racehorses [36]. Histological changes identified in the aorta of the racehorse [37] have been speculated to weaken the aorta, facilitating the rupture. Whether these changes are related to locomotion-associated trauma, as postulated for capillary lesions in the lung, causing equine exercise-induced pulmonary haemorrhage [38], or to genetic-based altered metabolism of the extracellular matrix, as speculated for Frisian horses [39], remains to be elucidated.

The inbreeding coefficients calculated for the group "raced" in FSTB and FCB reflected closely the inbreeding coefficients reported in 2009 for the same breeds [27]. The inbreeding coefficient for the group "raced" was lower in FCB (0.039) than in FSTB (0.108). The inbreeding coefficient for FCB was similar or slightly lower than for other breeds with a relatively small population, e.g. Campolina horses (0.024) [26], Franches-Montagnes horses (0.06) [40], Norwegian-Swedish coldblooded trotters (0.062) [41], or Black Forest Draught horses (0.075-0.096) [29]. Regarding the FSTB, the inbreeding coefficient for our population was higher than that found in a French study (0.024) [42] and similar to that published in the USA (0.103) [30]. The inbreeding coefficient was lower than for THB (0.13) [43]. In several populations, the inbreeding coefficient tends to increase over the years, especially for smaller populations or for breeds with high selection pressure, despite efforts of breeding programmes [26, 29, 40, 41, 44]. Further, an inbreeding depression has been reported to start with an inbreeding coefficient around 0.12 [45]. The inbreeding coefficient for FSTB with EASD in our study population approached this value. As reported for performance parameters [24, 25] or fertility [27-30], the high inbreeding coefficient could point towards a reduced genetic fitness and a more fragile constitution, making our study population more vulnerable to fatal incidents. However, the horses with SCD were not more inbred than the horses in the group "raced" with vessel ruptures or with CMI. Further, the

overall incidence of fatality was low compared with results for other countries. Therefore, we found no clear evidence for increased inbreeding in FSTB or FCB with SCD, and, in turn, the study does not provide any compelling evidence for the impact of genetics on the development of SCD and vascular rupture in the examined Finnish racehorse population. This result is in accord with a recent genome-wide association study (GWAS), where none of the tested alleles seemed to be associated with SCD, TBH, or STB [6]. Furthermore, we could not show any evidence that the horses with SCD had greater or lower career earnings than their peers racing during the same period.

One of the main limitations of the study is the poor definition of SCD. There is certainly a consensus that a better definition is needed [35], but we used the most objective definition currently available, which is based on results of a full necropsy, including meticulous macroscopic and microscopic examinations [1, 3, 6]. To account for this weakness, we provide the group SCD_u. However, basing our diagnoses on the results of necropsies led to exclusion of 11 horses due to missing necropsy reports. We include these horses in the calculation of incidence of fatality in the study population, but we excluded them from the analyses of inbreeding coefficient or cause of death. Consequently, we had a low number of horses for analysis in this part of the study, comprising a further limitation. Because of our definition of EASD excluding horses with clinical data indicative of a catastrophic orthopaedic injury, we cannot fully rule out that some of these horses had orthopaedic injuries due to cardiac arrest. Further, some of the horses might have had unnoticed dynamic obstruction of the upper airways that could have contributed to EASD.

Comparing the inbreeding coefficient in case and control groups is an indirect way of proving/disproving the involvement of genetics. Still, it is a simple approach to merely gain an overview, although our results are consistent with recently published results for a GWAS [6]. It is, however, important to bear in mind that to draw conclusions on the involvement of genetics all individuals should be unambiguously clinically characterized. We also assessed the earnings of horses with EASD to determine the potential impact of inbreeding on racing performance. Career earnings is an imprecise parameter since many confounding parameters may influence the earnings. These factors are horse-related but also management-related, as the number of races that a horse runs will highly depend on the number of years in racing or the racing strategy of the trainer. This is why we

compared the career earnings of dead horses with the mean earnings of same-aged horses raced during the same period. By doing so, we attempted to reduce the effect of management factors.

Despite the negative results of this study, further efforts should be undertaken to increase knowledge of the equine genome to elucidate whether genetic variants play a role in SCD in horses, as in dogs [19, 20, 22]. The development of more accessible genetic analyses and the implementation of uniform necropsy protocols, making results from different countries more comparable, will also contribute to better understanding of SCD in horses, eventually reducing these deaths.

5. Conclusion

The study shows that Finnish racehorses (i.e. FSTB and FCB) have a lower incidence of EASD than racehorses in other countries. Further, the incidence of EASD was higher than the incidence of CMI in our population, contrary to reports in most other racehorse populations.

We conclude that no difference exists in the inbreeding factor between SCD, VR, or CMI horses and the overall horse racing population in Finland. Similarly, we could not show any difference in earnings in horses with EASD for any reason compared with the overall racing population. This suggests that the fitness for racing of horses with EASD is similar to that of the overall racing population in Finland.

Ethics approval

This study required no ethics approval under the Finnish legislation.

Authors' contributions

DST, KC, RB, DAK, AKM, PR, MA, MF, MT, and KL contributed to hypothesis generation and the interpretation and analysis of data. In addition, MA performed and reviewed the necropsy findings and MT and KL provided the pedigree and racing data. All authors contributed to the drafting of the manuscript and have read and approved the final manuscript.

Declaration of Competing interests

The authors have no conflicts of interest to declare.

Acknowledgements

Funding was received from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska Curie grant (agreement no. 656566).

References

[1] Lyle CH, Uzal FA, McGorum BC, Aida H, Blissitt KJ, Case JT, et al. Sudden death in racing Thoroughbred horses: an international multicentre study of post mortem findings. Equine Vet J 2011;43:324-31.

[2] Hitchens PL, Morrice-West AV, Stevenson MA, Whitton RC. Meta-analysis of risk factors for racehorse catastrophic musculoskeletal injury in flat racing. Vet J 2019;245:29-40.

[3] Lyle CH, Blissitt KJ, Kennedy RN, Mc Gorum BC, Newton JR, Parkin TD, et al. Risk factors for race-associated sudden death in Thoroughbred racehorses in the UK (2000-2007). Equine Vet J 2012;44:459-65.

[4] Boden LA, Anderson GA, Charles JA, Morgan KL, Morton JM, Parkin TD, et al. Risk of fatality and causes of death of Thoroughbred horses associated with racing in Victoria, Australia: 1989-2004. Equine Vet J 2006;38:312-8.

[5] Physick-Sheard PW, Avison A, Chappell E, MacIver M. Ontario Racehorse Death Registry, 2003-2015: Descriptive analysis and rates of mortality. Equine Vet J 2019;51:64-76.

[6] Molesan A, Wang M, Sun Q, Pierce V, Desideri R, Palmer S, et al. Cardiac Pathology and Genomics of Sudden Death in Racehorses From New York and Maryland Racetracks. Vet Pathol 2019;56:576-85.

[7] Boden LA, Charles JA, Slocombe RF, Sandy JR, Finnin PJ, Morton JM, et al. Sudden death in racing Thoroughbreds in Victoria, Australia. Equine Vet J 2005;37:269-71.

[8] Gelberg HB, Zachary JF, Everitt JI, Jensen RC, Smetzer DL. Sudden death in training and racing Thoroughbred horses. J Am Vet Med Assoc 1985;187:1354-6.

[9] Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. Circulation 2009;119:1085-92.

[10] Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? J Am Coll Cardiol 2003;42:1959-63.

[11] Harmon KG, Drezner JA, Maleszewski JJ, Lopez-Anderson M, Owens D, Prutkin JM, et al. Pathogeneses of sudden cardiac death in national collegiate athletic association athletes. Circ Arrhythm Electrophysiol 2014;7:198-204.

[12] Tester DJ, Ackerman MJ. Genetics of Long QT Syndrome. Methodist Debakey Cardiovascular Journal 2014;10:29-33.

[13] Crotti L, Celano G, Dagradi F, Schwartz PJ. Congenital long QT syndrome. Orphanet J Rare Dis 2008;3:18.

[14] Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 2001;103:89-95.

[15] Pedersen PJ, Thomsen KB, Flak JB, Tejada MA, Hauser F, Trachsel D, et al. Molecular cloning and functional expression of the K+ channel KV7.1 and the regulatory subunit KCNE1 from equine myocardium. Res Vet Sci 2017;113:79-86.

[16] Pedersen PJ, Thomsen KB, Olander ER, Hauser F, Tejada Mde L, Poulsen KL, et al. Molecular Cloning and Functional Expression of the Equine K+ Channel KV11.1 (Ether a Go-Go-Related/KCNH2 Gene) and the Regulatory Subunit KCNE2 from Equine Myocardium. PLoS One 2015;10:e0138320.

[17] Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. Circulation 1999;99:529-33.

[18] Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Europace 2011;13:1077-109.

[19] Ware WA, Reina-Doreste Y, Stern JA, Meurs KM. Sudden death associated with QT interval prolongation and KCNQ1 gene mutation in a family of English Springer Spaniels. J Vet Intern Med 2015;29:561-8.

[20] Meurs KM, Weidman JA, Rosenthal SL, Lahmers KK, Friedenberg SG. Ventricular arrhythmias in Rhodesian Ridgebacks with a family history of sudden death and results of a pedigree analysis for potential inheritance patterns. J Am Vet Med Assoc 2016;248:1135-8.

[21] Moise NS. Inherited arrhythmias in the dog: potential experimental models of cardiac disease. Cardiovasc Res 1999;44:37-46.

[22] Moise NS, Meyers-Wallen V, Flahive WJ, Valentine BA, Scarlett JM, Brown CA, et al. Inherited ventricular arrhythmias and sudden death in German shepherd dogs. J Am Coll Cardiol 1994;24:233-43.

[23] Snustad DP, Simmons MJ. Extensions of Mendelism. Principles of genetics. 6th ed: John Wiley & Sons, Inc.; 2012. p. 62-88.

[24] Klemetsdal G. The effect of inbreeding on racing performance in Norwegian cold-blooded trotters. Genet Sel Evol 1998;30:351-66.

[25] Todd ET, Ho SYW, Thomson PC, Ang RA, Velie BD, Hamilton NA. Founder-specific inbreeding depression affects racing performance in Thoroughbred horses. Sci Rep 2018;8:6167.

[26] Bussiman FO, Perez BC, Ventura RV, Peixoto M, Curi RA, Balieiro JCC. Pedigree analysis and inbreeding effects over morphological traits in Campolina horse population. Animal 2018:1-10.

[27] Sairanen J, Nivola K, Katila T, Virtala AM, Ojala M. Effects of inbreeding and other genetic components on equine fertility. Animal 2009;3:1662-72.

[28] Langlois B, Blouin C. Statistical analysis of some factors affecting the number of horse births in France. Reprod Nutr Dev 2004;44:583-95.

[29] Müller-Unterberg M, Wallmann S, Distl O. Effects of inbreeding and other systematic effects on fertility of Black Forest Draught horses in Germany. Acta veterinaria Scandinavica 2017;59:70.

[30] Cothran EG, MacCluer JW, Weitkamp LR, Pfennig DW, Boyce AJ. Inbreeding and reproductive performance in Standardbred horses. Journal of Heredity 1984;75:220-4.

[31] Rosanowski SM, Chang YM, Stirk AJ, Verheyen KL. Descriptive epidemiology of veterinary events in flat racing Thoroughbreds in Great Britain (2000 to 2013). Equine Vet J 2017;49:275-81.

[32] DeLay J. Postmortem findings in Ontario racehorses, 2003-2015. J Vet Diagn Invest 2017;29:457-64.

[33] Durando MM, Birks EK, Hussey SB, Lunn DP. Cardiac troponin I concentrations in ponies challenged with equine influenza virus. J Vet Intern Med 2011;25:339-44.

[34] Corrado D, Pelliccia A, Bjornstad HH, Vanhees L, Biffi A, Borjesson M, et al. Cardiovascular pre-participation screening of young competitive athletes for prevention of sudden death: proposal for a common European protocol. Consensus Statement of the Study Group of Sport Cardiology of the Working Group of Cardiac Rehabilitation and Exercise Physiology and the Working Group of Myocardial and Pericardial Diseases of the European Society of Cardiology. Eur Heart J 2005;26:516-24.

[35] Diab SS, Poppenga R, Uzal FA. Sudden death in racehorses: postmortem examination protocol. J Vet Diagn Invest 2017;29:442-9.

[36] Briceño AM, Mendez A, Brewer K, Hughes C, Tobin T. Sudden death, aortic rupture in horses, literature review, case studies reported and risk factors. Brazilian Journal of Veterinary Research and Animal Science 2015;52:298-309.

[37] Imaizumi K, Nakamura T, Kiryu K, Kanemaru T, Kaneko M. Morphological changes of the aorta and pulmonary artery in thoroughbred racehorses. J Comp Pathol 1989;101:1-9.

[38] Schroter RC, Marlin DJ, Denny E. Exercise-induced pulmonary haemorrhage (EIPH) in horses results from locomotory impact induced trauma--a novel, unifying concept. Equine Vet J 1998;30:186-92.

[39] Ploeg M, Grone A, van de Lest CHA, Saey V, Duchateau L, Wolsein P, et al. Differences in extracellular matrix proteins between Friesian horses with aortic rupture, unaffected Friesians and Warmblood horses. Equine Vet J 2017;49:609-13.

[40] Poncet PA, Pfister W, Muntwyler J, Glowatzki-Mullis ML, Gaillard C. Analysis of pedigree and conformation data to explain genetic variability of the horse breed Franches-Montagnes. J Anim Breed Genet 2006;123:114-21.

[41] Velie BD, Sole M, Fegraeus KJ, Rosengren MK, Roed KH, Ihler CF, et al. Genomic measures of inbreeding in the Norwegian-Swedish Coldblooded Trotter and their associations with known QTL for reproduction and health traits. Genet Sel Evol 2019;51:22.

[42] Pirault P, Danvy S, Verrier E, Leroy G. Genetic structure and gene flows within horses: a genealogical study at the french population scale. PLoS One 2013;8:e61544.

[43] Cunningham EP, Dooley JJ, Splan RK, Bradley DG. Microsatellite diversity, pedigree relatedness and the contributions of founder lineages to thoroughbred horses. Anim Genet 2001;32:360-4.

[44] Binns MM, Boehler DA, Bailey E, Lear TL, Cardwell JM, Lambert DH. Inbreeding in the Thoroughbred horse. Anim Genet 2012;43:340-2.

[45] Huisman J, Kruuk LE, Ellis PA, Clutton-Brock T, Pemberton JM. Inbreeding depression across the lifespan in a wild mammal population. Proc Natl Acad Sci U S A 2016;113:3585-90.

Table 1: Demographic data for horses with exercise-associated sudden death (EASD) and horses that died or were euthanized for an identified cause (group catastrophic musculoskeletal injures (CMI)) on the racetrack.

	Exercise-associated sudden death (EASD)			**Death due to catastrophic musculoskeletal injures (CMI)**		
	FSTB	**FCB**	**Total**	**FSTB**	**FCB**	**Total**
Number of stallions	9	0	9	3		3
Number of geldings	13	5	18	6		5
Number of mares	5	2	7	6	1	7
Median age in years	6.0	9	6.5	5.0		6.0
with 2.5-97.5 percentile	4-8	4-10	4-9	4-8		4-8
(range)	(3-11)	(4-11)	(3-11)	(4-10)	10	(4-10)

FCB, Finnish coldblooded racehorses; FSTB, Finnish standardbred racehorses

Table 2: Necroscopic findings in horses that died on racetracks in Finland between 2002 and 2017.

Diagnosis based on necropsy	**SCD-unknown (SCD_u)**		**SCD-cardiac disease (SCD_{cd})**		**Vessel rupture (VR)**		**Death due to catastrophic musculoskeletal injures (CMI)**	
Breed	**FSTB** (N=19)	**FCB** (N=6)	**FSTB** (N=2)	**FCB** (N=0)	**FSTB** (N=6)	**FCB** (N=1)	**FSTB** (N=15)	**FCB** (N=1)
Reported findings								
Lung								
-Congested and/or oedematous lung (macroscopic and/or microscopic findings)	19	6	2		6			
-Bleeding in the lung (macroscopic and/or microscopic findings)	9	3			1			
-Foamy liquid in the trachea	16	6	2		5	1		
Heart								
-Petechiae/haematomas		1			2	1		
-Haemopericardium					2	1		
-No contraction after death/large flaccid heart	8	5						
-Myocarditis (lymphocytic)			1					
-Hamartoma			1					
Other organs								
-Congestion of the liver (macroscopic and/or microscopic findings)	6	2			1			
-Congestion of the spleen (macroscopic and/or microscopic findings)	5	1			1			
-Congestion of the adrenal gland (macroscopic and/or microscopic findings)	1				1			
-Free blood in body cavity (5-20 litres)					6			
-Identification of ruptured vessel					4	1		
Cause for euthanasia								
Fracture/severe soft tissue trauma							15	1

FCB, Finnish coldblooded racehorses; SCD, sudden cardiac death; FSTB, Finnish standardbred racehorses

Table 3: Inbreeding coefficients for Finnish standardbred racehorses (FSTB) and Finnish coldblooded racehorses (FCB) expressed as median, 2.5-97.55 percentile, and range. No significant difference emerged between the groups of causes for death.

		Racing population ("raced")	SCD	SCD -unknown origin (SCD_u)	SCD -cardiac disease (SCD_{cd})	Vessel rupture (VR)	Death due to catastrophic musculoskeletal injures (CMI)
FSTB	median 2.5-97.5 percentile (range)	0.108 0.087-0.126 (0.002-0.312)	0.110 0.060-0.13 (0.027-0.157)	0.097 0.050-0.130 (0.027-0.157)	0.133 0.132-0.134 (0.132-0.134)	0.099 0.062-0136 (0.058-0.147)	0.120 0.055-0.139 (0006-0.142)
FCB	median 2.5-97.5 percentile (range)	0.039 0.031-0.047 (0.007-0.169)	0.036 0.027-0.046 (0.024-0.055)	0.036 0.027-0.046 (0.024-0.055)	-	0.066	0.045

FCB, Finnish coldblooded racehorses; SCD, sudden cardiac death; FSTB, Finnish standardbred racehorses

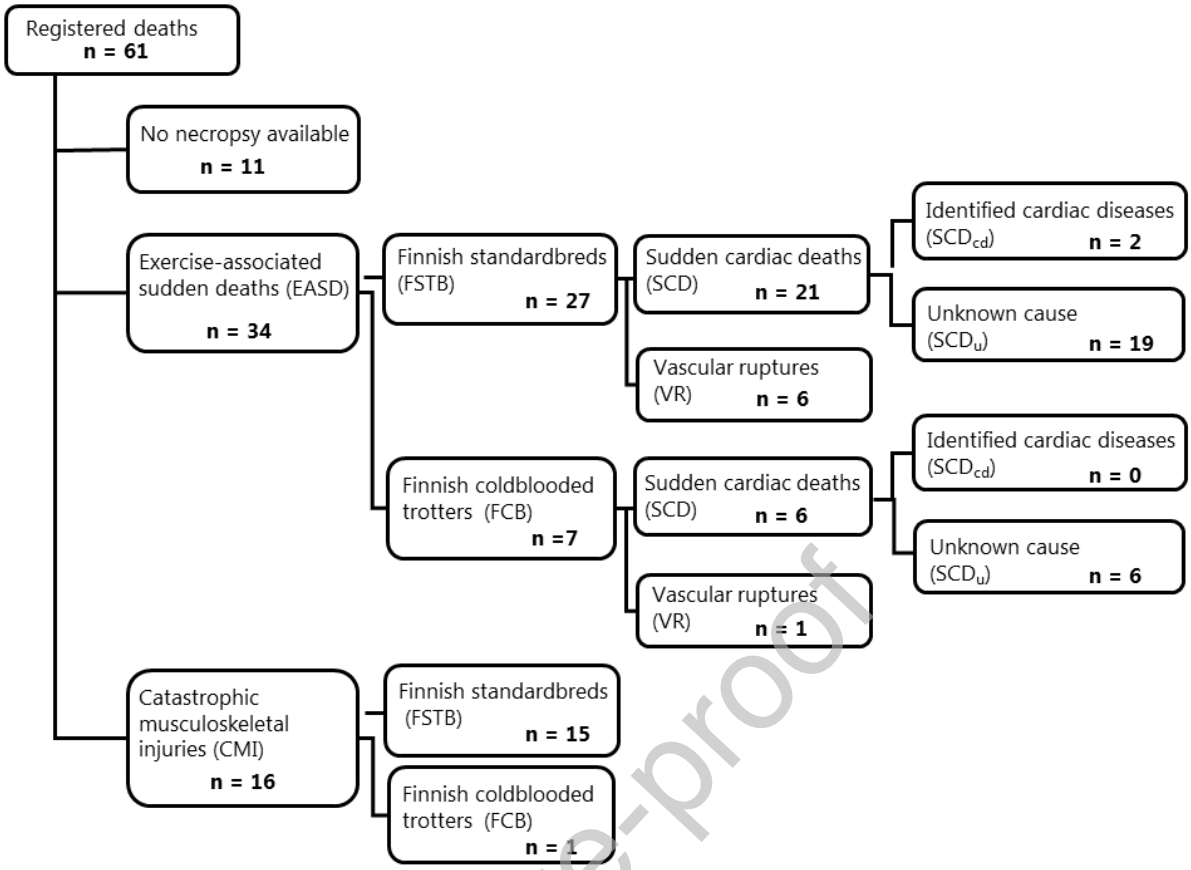

Figure 1: Flow chart showing the study population and classification of the different groups.

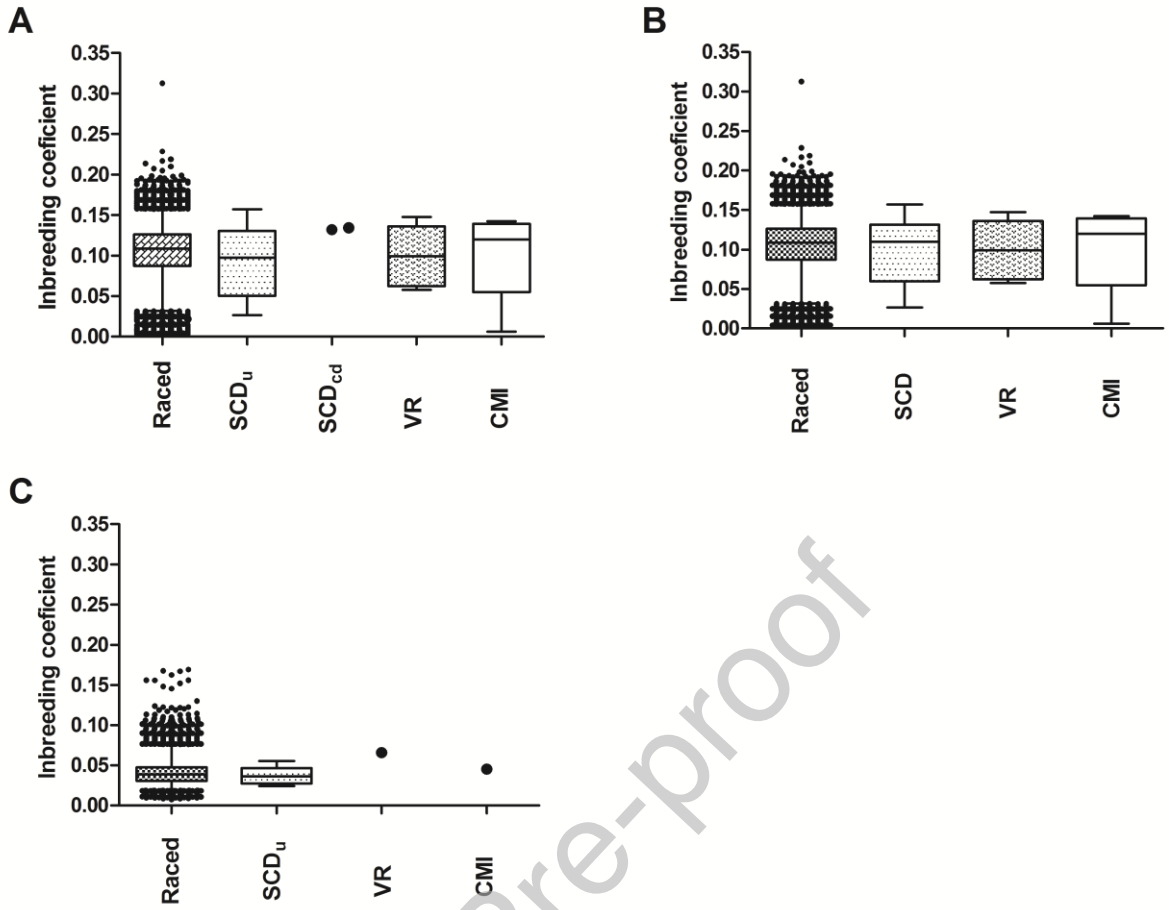

Figure 2: Comparison of inbreeding coefficients in horses that raced in the study period ("raced") or died on a race day for different reasons for Finnish standardbred racehorses (graphs A, B) and Finnish coldblooded racehorses (graph C). SCD, horses that died from sudden cardiac death; SCD_u, horses with SCD for unknown reason; SCD_{cd}, horses with SCD due to an identified cardiac disease; VR, horses that died due to a vascular rupture; CMI, horses that died due to a catastrophic musculoskeletal injury. Data are expressed as median and 2.5-97.5 percentiles.

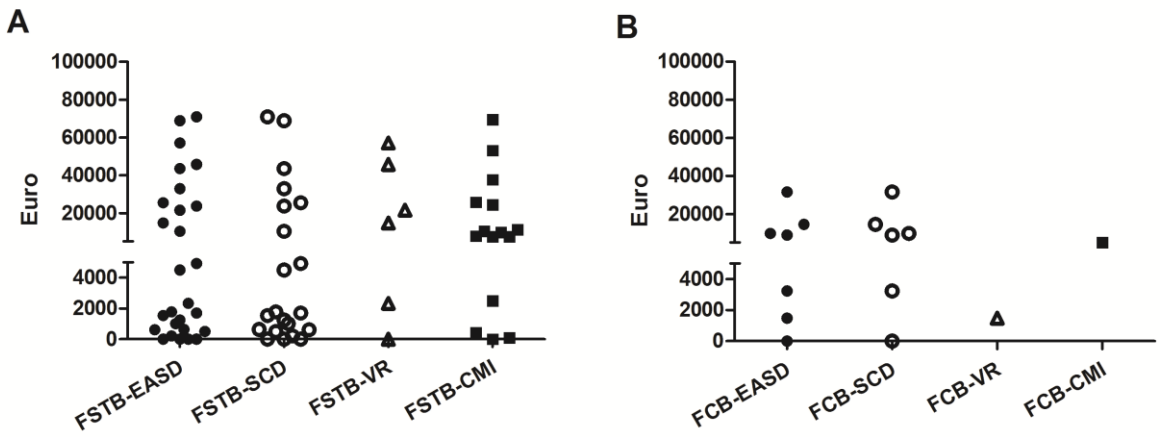

Figure 3: Career earnings (Euro) of Finnish standardbred racehorses (FSTB, graph A) and Finnish coldblooded racehorses (FCB, graph B). No significant differences existed between the groups. EASD, horses that died from exercise-associated sudden death; SCD, horses that died from sudden cardiac death; VR, horses that died due to a vascular rupture; CMI, horses that died due to a catastrophic musculoskeletal injury.

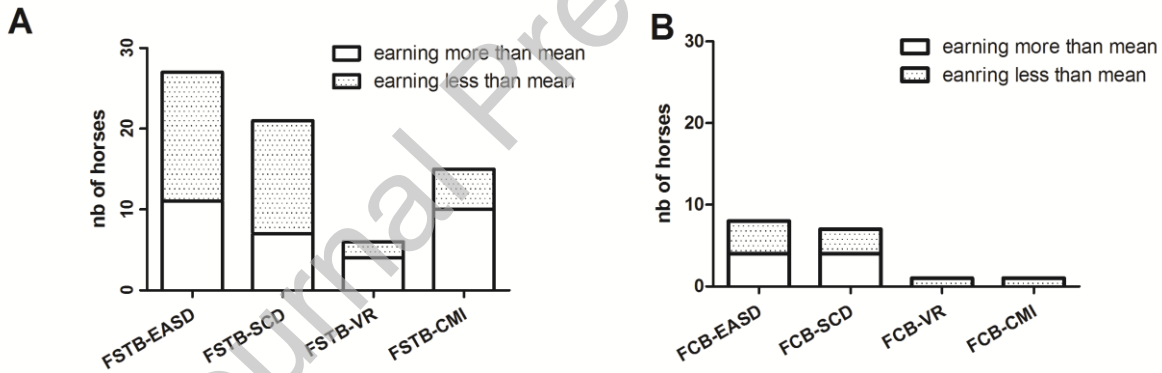

Figure 4: Number of Finnish standardbred racehorses (FSTB, graph A) and Finnish coldblooded racehorses (FCB, graph B) that earned more (white surface) or less (dotted surface) than the mean career earnings of same-aged horses. No significant differences existed between the groups. EASD, horses that died from exercise-associated sudden death; SCD, horses that died from sudden cardiac death; VR, horses that died due to a vascular rupture; CMI, horses that died due to a catastrophic musculoskeletal injury.