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Prevalence of Taylorella equigenitalis in Icelandic mares and geldings in Southern Germany and Austria

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ABSTRACT

Contagious Equine Metritis (CEM) caused by the bacterium *Taylorella equigenitalis (T. equigenitalis)*, is a venereal infection of equids which is of international concern to the equine breeding industry. A recent study showed a high prevalence of *T. equigenitalis* in Icelandic stallions when compared to stallions of other breeds also using for natural breeding. Consequently, the objectives of the present study were to investigate the prevalence of *T. equigenitalis* in Icelandic mares and geldings and to determine factors associated with a *T. equigenitalis*-positive qPCR result. In total, 361 Icelandic horses located in Southern Germany and Austria were tested for *T. equigenitalis* using a qPCR assay. An overall prevalence of 14.4 % was detected. Positive qPCR results were found in 2.2 % (3/134) of brood mares, 9.0 % (11/122) of maiden mares and in 36.2 % (38/105) of geldings. The odds for a *T. equigenitalis*-positive qPCR result were significantly lower in both brood (OR = 40.1, 95 % CI: 8.38-192, $P < 0.001$) and maiden mares (OR = 9.51, 95 % CI: 3.26-25.7, $P < 0.001$) when compared to geldings. Advancing age was not associated with higher odds for a *T. equigenitalis*-positive qPCR result (OR = 0.98, 95 % CI: 0.94-1.03, $P = 0.51$). However, horses of the younger age group showed significantly lower C_t values compared to horses of the older age group ($P = 0.04$). Furthermore, geldings showed significantly lower C_t values than brood ($P < 0.03$) and maiden mares ($P < 0.001$). This study showed a significantly higher prevalence of *T. equigenitalis* in Icelandic geldings compared to Icelandic mares. Icelandic geldings might therefore represent a reservoir for *T. equigenitalis*.

1. Introduction

Taylorella equigenitalis (T. equigenitalis), the causative agent of Contagious Equine Metritis (CEM), is a mainly venereally transmitted bacterial disease that is of concern to the international equine breeding industry [1]. It is a World Organization for Animal Health (WOAH)--listed disease and has been identified in various equine breeds and in many countries worldwide [2–5]. After its initial identification following major outbreaks in Thoroughbreds in Newmarket (United Kingdom, UK) and Ireland in 1977 [6] and in Kentucky (United States, US) in 1978 [7,8], international breeding regulations were established to control the spread of the disease. The Horserace Betting Levy Board's (HBLB) Code of Practice for Breeders [9], implemented after the initial outbreak in 1977, focused mainly on biosecurity and identification of carrier animals and proved remarkably effective in preventing recurrence of *T. equigenitalis* outbreaks in the UK and Ireland [10,11].

In mares, clinical signs include vaginal, cervical and endometrial inflammation leading to temporary infertility [3,12]. In most cases, infected mares will clear themselves of infection but may go on to become carrier animals. Stallions show no clinical signs and become subclinical carriers, remaining undetected for years [13]. Traditionally, transmission of *T. equigenitalis* occurred via natural breeding, however, in recent outbreaks [4,13], fomites and contaminated fresh and frozen semen used for artificial insemination (AI) have posed the most risk. Correspondingly, due to increased worldwide trade in semen used for AI in the equine breeding industry, biosecurity and import/export regulations have gained importance [4]. *Taylorella equigenitalis* has also been detected in placental tissues, aborted fetuses, newborn foals as well as

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colts and fillies. Therefore, intrauterine or perinatal infection due to horizontal transmission was assumed [14,15].

In Germany, some horse breeds are still using natural breeding, especially Icelandic, Draft and Haflinger horses. There are currently no legal requirements for *T. equigenitalis* testing prior to natural breeding. In a recent study from Germany [16], Icelandic stallions had a significantly higher prevalence of *T. equigenitalis*-positive qPCR results compared to Draft horse and Haflinger stallions also used for natural breeding. Interestingly, this study also showed that Icelandic stallions never used for breeding were significantly more likely to have a *T. equigenitalis*-positive qPCR result than active Icelandic breeding stallions. In these cases, environmental contamination or direct transmission from animals in the same herd were discussed. Therefore, non-breeding Icelandic horses were also assumed to be *T. equigenitalis* carriers.

The objectives of the present study were to investigate the prevalence of *T. equigenitalis* in Icelandic mares (brood and maiden) and geldings. Furthermore, any factors associated with a *T. equigenitalis*-positive qPCR result would be identified. We hypothesized that brood mares would have a higher prevalence of *T. equigenitalis* compared to maiden mares and geldings due to potential venereal transmission following the routine use of natural breeding in this breed. We also hypothesized that Icelandic maiden mares and geldings may also have positive qPCR results, since it had recently been shown that stallions without breeding history had tested positive for *T. equigenitalis* [16].

2. Material and methods

2.1. Ethical approval

This study was conducted in accordance with national laws for animal use and approved by the Ethics Committee of the Veterinary Department of the Ludwig-Maximilians-Universität (LMU) in Munich (Germany; Approval number, 294-28-12-2022; Approval date, February 2022). The owners of all sampled animals gave informed consent to testing.

2.2. Animals

In total, 361 Icelandic horses, including 134 brood mares, 122 maiden mares and 105 geldings were included in the study. Study participants were recruited by contacting Icelandic horse owners from the patient pool of the Equine Clinic (LMU Munich, Germany) and through the resulting contacts, to larger Icelandic studs. Therefore, convenience samples were taken on 11 different studs [A-K] located in Southern Germany (Bavaria and Baden-Wuerttemberg) and Austria (Tyrol). All studs were geographically and logistically separated from each other. According to the owner's information, there had been no direct connection between the studs nor any known active exchange of animals. However, due to a close Icelandic horse community in Germany, interbreeding of some horses could not be ruled out with certainty. Only studs with >10 horses in total were included in the study. The responsible caretaker at each stud, who was not involved in the study, selected the horses for sampling. Apart from seven individually stabled Icelandic horses, all horses were kept in herds, which varied in group composition regarding size and reproductive status. For each horse, a short questionnaire regarding breed, age, reproductive status, type of husbandry and previous breeding history was completed before sampling. Only mares that, according to the owner, had never been in contact with a stallion or been bred before, were included in the group of maiden mares. All horses included were further divided into two age groups: younger horses from 1 to 11 years and older horses from 12 to 32 years. A second, retrospective questionnaire was sent to all owners of a horse with a *T. equigenitalis*-positive qPCR result. The detailed information on both questionnaires can be found in supplementary data (Appendix A: Supplementary material).

2.3. Sampling procedure

Samples were taken between April and August 2022. In all mares, sampling was performed without sedation from standardized locations, internationally recommended by the WOAH i.e. the clitoral fossa and clitoral sinuses [9,17]. In geldings, sampling was carried out after oral sedation with 'acepromazine (0.3 mg/kg, Relaquine 35 mg/ml, Dechra Veterinary Products Deutschland GmbH, Aulendorf, Germany)' for penile let down. On three studs, oral sedation was not accepted by the owner, therefore, sampling was only performed on one of these studs on seven individual cooperative geldings. Samples were taken at the same standardized locations as recommended for stallions, i.e. urethral fossa, urethra and penile sheath [9]. In 3/105 (2.9 %) geldings, no sample could be taken from the penile sheath due to incomplete penile let down despite sedation. Nevertheless, the pooled sample of one gelding without sample from the penile sheath revealed a *T. equigenitalis*-positive qPCR result. Samples from each location were taken separately with a 'dry polyester swab (Dry Swabs 159 C, Copan Diagnostics, CA, USA)' and immediately placed in a 'coded reaction tube (Safe-Lock Tubes, 1.5 mL, Eppendorf SE, Hamburg, Germany)' filled with 400 µL 'isotonic saline (NaCl 0.9 %, 1000 ml, B. Braun, Melsungen Germany)'. Samples were then stored on ice, transported to the laboratory within three hours and frozen at -80 °C until further examination. At the time of sampling, all horses appeared healthy without any clinical signs of CEM (i.e. vaginal discharge).

2.4. DNA extraction and qPCR

Isolation of *T. equigenitalis* DNA was performed from all samples using the DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instruction. For DNA isolation all swabs from one animal were pooled according to Mawhinney et al. [18].

For all qPCRs, the SensiFAST Probe Lo-ROX Kit (Meridian Bioscience, Ohio, US) was used. Oligonucleotide primers (Tay377for 5′-CCGCGTGTGCGATTGA, Tay448rev 5′-TTTGCCGGTGCTTATTCTTCA; 400nM) and probe (TequiFAM, 5′-FAM-AAAGGTTTGTGTTAA-TACCATGGACTGCTGACGG-BHQ1; 100nM), as previously described by Wakeley et al. [19], were used for *T. equigenitalis* detection. Briefly, the thermal profile of the qPCR was set at 95 °C for 5 min, 42 cycles of 94 °C for 15 s and 60 °C for 60 s. The AriaMx real-time PCR system (Agilent, California, USA) was used together with the corresponding Aria 1.8 software to perform and analyze the qPCRs. Negative and positive controls were added to every run. All positive samples with a C_t value $\geq$ 30 were repeated. Double positive results were considered truly positive. For C_t values from $\geq$ 30 to 35 in the first run, and a negative result in the second run, a third crucial run was performed. For C_t values from > 35 to 40 in the first run and a negative result in the second run, the sample was scored negative.

2.5. Statistical analyses

The statistical analysis was performed using R version 4.3.1. (2023-10-19, R Foundation for Statistical Computing, Vienna, Austria). Multivariable mixed effects logistic regression (MELR) with 'stud' as random effect was conducted to estimate probabilities of a positive qPCR result. Reproductive status and age of Icelandic horses were used as predictors with 'studs' as random effect. Odds Ratio (OR) with 95 % confidence interval (95 % CI) were calculated for all models. All contrasts (differences between reproductive status) were assessed after model-fitting by the estimated least-squares marginal means ("emmeans" package). A Kruskal-Wallis test was performed to compare C_t values between brood mares, maiden mares and geldings, due to a non-normal and highly heteroskedastic distribution of data. Results with a *P*-value <0.05 were considered statistically significant. *P*-values were corrected for multiple comparisons with the Tukey method for MELR and with Holm method for Kruskal-Wallis analysis.

3. Results

3.1. Animals

In total, 361 Icelandic horses (134 brood mares, 122 maiden mares, 105 geldings) were included with a mean age of 12.14 ± 6.52 years. Samples were collected from horses on 11 studs in Germany and Austria. Seven out of eleven (63.6 %) studs located in Bavaria (Germany), 3/11 (27.3 %) in Baden-Wuerttemberg (Germany) and 1/11 (9.1 %) in Tyrol (Austria). One stud (D) kept only 11 horses, all other studs kept 70 or more horses in total. Since the owners refused sedation, on 2/11 (18.2 %) studs (D, J) only mares were sampled although both studs also housed geldings. On all other studs, samples from all status groups were taken. No stallion was present on stud D, while on all other studs, Icelandic stallions were present, housed separately or together with geldings, but theoretically could also have had contact with mares (see Table 1).

The second questionnaire was only sent to owners of *T. equigenitalis*-positive horses and was answered by 43/52 (82.7 %) of them, including 3/3 (100.0 %) owners for *T. equigenitalis*-positive brood mares, 9/11 (81.8 %) for *T. equigenitalis*-positive maiden mares and 31/38 (81.6 %) for *T. equigenitalis*-positive geldings. All *T. equigenitalis*-positive brood mares (3/134, 2.2 %) had not been bred in the year of sampling, but 2/3 (66.7 %) had tested negative before the last natural breeding attempt. All three *T. equigenitalis*-positive brood mares were stabled on studs with only Icelandic horses, were kept together with maiden mares and geldings and had had no known contact with any *T. equigenitalis*-positive horses on the same or other studs according to the owner's information. Based on the nine responses from the second survey, all *T. equigenitalis*-positive maiden mares had contact with brood mares and 6/9 (66.7 %) were kept in a herd together with brood mares. No *T. equigenitalis*-positive maiden mare had contact with known *T. equigenitalis*-positive horses before sampling according to the owner. All maiden mares were stabled on studs with only Icelandic horses. In *T. equigenitalis*-positive geldings, previous breeding history was reported in 3/31 (9.7 %) geldings. However, one *T. equigenitalis*-positive gelding was observed attempting to cover mares and geldings in the same herd. The group composition for geldings varied, with 23/31 (74.2 %) living in a herd with other geldings only, 3/31 (9.7 %) living together with mares and 5/31 (16.1 %) living in a group with stallions. The majority of geldings (27/31; 87.1 %) were stabled on studs with Icelandic horses only. Detailed information of the second questionnaire can be found in supplementary data (Appendix A). The results from all *T. equigenitalis*-positive horses from the second questionnaire were insufficient and therefore not statistically investigated further.

3.2. qPCR results

3.2.1. qPCR results regarding reproductive status

An overall prevalence for a *T. equigenitalis*-positive qPCR result in all sampled Icelandic horses of 14.4 % (52/361) could be detected: 3/134 (2.2 %) of brood mares, 11/122 (9.0 %) of maiden mares and 38/105 (36.2 %) of geldings. Fig. 1 shows the odds for a *T. equigenitalis*-positive qPCR result in the three different status groups. Odds for a *T. equigenitalis*-positive qPCR result were significantly lower in brood mares (OR = 40.1, 95 % CI: 8.38-192, $P < 0.001$) and in maiden mares (OR = 9.51, 95 % CI: 3.26-25.7, $P < 0.001$) compared to geldings. No statistically significant difference in odds for a *T. equigenitalis*-positive qPCR result could be detected between maiden mares and brood mares (OR = 9.15, 95 % CI: 3.26-25.7, $P = 0.074$).

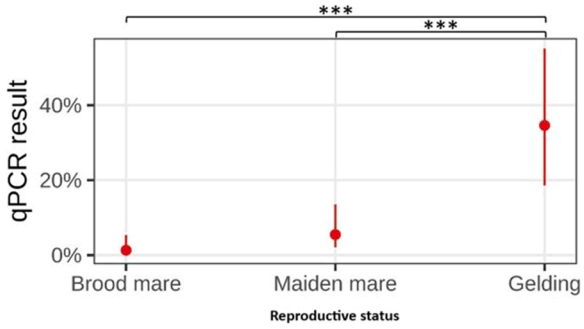

Fig. 1. Probabilities for qPCR results of brood mares, maiden mares and geldings evaluated with multivariable mixed effects logistic regression.

Table 1
Number of *T. equigenitalis*-positive mares and geldings present on 11 studs (A-K) in South Germany and Austria.

Stud	Number of horses present on stud	Stallion present on stud (Y/N[a])	Total number of horses sampled	Total number of *T. equigenitalis*-positive horses	Number of *T. equigenitalis*-positive brood mares	Number of *T. equigenitalis*-positive maiden mares	Number of *T. equigenitalis*-positive geldings
A	70	Y	46	2/46 (4 %)	0/18 (0 %)	1/12 (8 %)	1/16 (6 %)
B	> 200	Y	39	5/39 (13 %)	0/16 (0 %)	0/15 (0 %)	5/8 (63 %)
C	> 100	Y	49	6/49 (12 %)	2/20 (10 %)	0/14 (0 %)	4/15 (27 %)
D	11	N	10	0/10 (0 %)	0/7 (0 %)	0/3 (0 %)	0 (0 %)
E	80-100	Y	46	2/46 (4 %)	0/11 (0 %)	0/16 (0 %)	2/19 (11 %)
F	80	Y	24	5/24 (21 %)	0/8 (0 %)	1/9 (11 %)	4/7 (57 %)
G	> 100	Y	32	8/32 (25 %)	0/5 (0 %)	2/13 (15 %)	6/14 (43 %)
H	> 100	Y	51	17/51 (33 %)	1/20 (5 %)	7/20 (35 %)	9/11 (82 %)
I	> 100	Y	22	6/22 (27 %)	0/5 (0 %)	0/5 (0 %)	6/12 (50 %)
J	> 100	Y	31	0/31 (0 %)	0/18 (0 %)	0/13 (0 %)	0 (0 %)
K	> 100	Y	11	1/11 (9 %)	0/6 (0 %)	0/2 (0 %)	1/3 (33 %)

[a] Y = Yes; N = No

3.2.2. qPCR results regarding age

Fig. 2a shows the odds for a *T. equigenitalis*-positive qPCR result in younger horses (1-11 years) and older horses (12-32 years). There was no statistically significant difference in odds for a *T. equigenitalis*-positive qPCR result between both age groups (OR = 0.99, 95 % CI: 0.49-2.01, P > 0.9). The odds for a *T. equigenitalis*-positive qPCR result were also calculated for individual horses regarding age. Advancing age was not associated with higher odds for a *T. equigenitalis*-positive qPCR result (OR = 0.98, 95 % CI: 0.94-1.03, $P = 0.51$). However, horses of the younger age group showed significantly lower C_t values compared to horses of the older age group ($P = 0.04$) independent of their reproductive status (see Fig. 2b).

3.2.3. qPCR results regarding studs

In Table 1, all sampled animals from each stud are listed in detail. Horses to be sampled were selected individually by an independent caretaker on each stud, therefore, the number of sampled horses per stud differed, with only 10 horses sampled on smaller studs and up to 51 horses sampled on larger studs. Mixed effects logistic regressions with 'stud' as random effect obtained identical results in terms of significance between mares and geldings compared to univariable logistic regression without random effect, which indicates relative homogeneity of results between studs. Moreover, a logistic regression with qPCR results as a response and studs as a predictor also showed no significant differences among studs. In brief, only two studs (one small stud: D, 10 sampled horses and one big stud: J, 31sampled horses) had no horses with a *T. equigenitalis*-positive qPCR result. On both studs, geldings were present, but were not sampled, while on all other studs both mares and geldings were tested. Four studs [F-I] showed a high overall prevalence (>20 % of tested horses) for *T. equigenitalis*-positive qPCR results. Each of these studs housed >80 horses with all reproductive status groups present.

3.2.4. C_t values

After the first qPCR run, 73/361 (20.2 %) samples tested positive, of which 18/73 (24.7 %) had a C_t between 30 and 35, and 19/73 (26.0 %) had a $C_t > 35$. After the second qPCR run, 11/18 (61.1 %) samples with a C_t value of 30 to 35 in the first run and 5/19 (26.3 %) of samples with a $C_t > 35$ in the first run were double positive and therefore regarded positive in total. The seven samples with a C_t of 30 to 35 in the first run and a negative result in the second run, tested double negative in the second and third qPCR run. As a result, 52/73 (71.2 %; corresponding to 14.4 % of all the 361 samples analyzed) of the original positive samples after the first qPCR run were considered positive after a second and a third qPCR run.

The median C_t value of all horses with a *T. equigenitalis*-positive qPCR result was 21.45 (range 15.1-36.1) independent of age and reproductive status (Fig. A, supplementary data). The median C_t value of brood mares was 33.7 (range: 33.0 -34.3), of maiden mares, 32.8 (range: 23.1-35.7) and of geldings, 20.0 (range: 15.1-36.1). As shown in Fig. 3, the C_t values of geldings were significantly lower compared to C_t values of brood mares ($P < 0.03$) and maiden mares ($P < 0.001$).

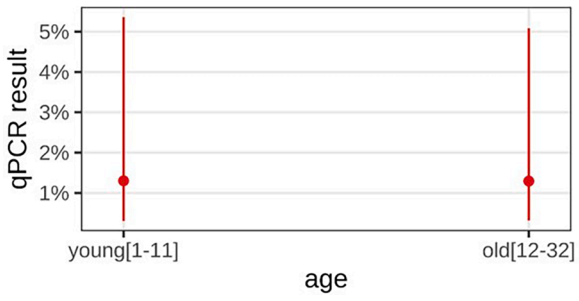

Fig. 2a. Comparison of both age groups regarding positive qPCR results in Icelandic brood mares, maiden mares, and geldings. There was no statistically significant difference in odds for a *T. equigenitalis*-positive qPCR result between both age groups (OR = 0.99, 95 % CI: 0.49-2.0167-2.19, $P > 0.9$).

4. Discussion

The Icelandic horse represents a special breed due to the extensive husbandry in mixed groups and the use of mainly natural breeding. This study showed that *T. equigenitalis* is present in the Icelandic mare population and contrary to our expectation, is most prevalent in the Icelandic gelding population (38/105, 36.2 %, $P < 0.001$). In addition, *T. equigenitalis*-positive qPCR samples from geldings showed significantly lower C_t values than mares. This is in accordance with Grabatin et al. [16] who detected higher odds for *T. equigenitalis*-positive qPCR results in Icelandic stallions without breeding use compared to Icelandic stallions actively used for breeding. The assumption, based on the reported high prevalence of *T. equigenitalis* in non-breeding Icelandic stallions [16], that horses not used for breeding (maiden mares and geldings) might also have *T. equigenitalis*-positive qPCR results was therefore confirmed. To the authors' knowledge, this is the first study to investigate the prevalence of *T. equigenitalis* in Icelandic mares and geldings. Our study indicated that geldings may serve as a reservoir of infection of *T. equigenitalis*, maintaining the infection even after castration. Horizontal transmission in bachelor herds or via infected fomites (bedding, tack, grooming equipment) was the most likely route, as has previously been suggested in both, horses [3,4,13,20,21] and donkeys [22,23]. Although described, perinatal transmission routes [14,15] are unlikely as the *T. equigenitalis* infection would have had to persist in the genital tract for years or even decades as the oldest gelding with a *T. equigenitalis*-positive qPCR result in the present study was 28 years old. The classic route of venereal transmission also seemed to play a subordinate role since only 9.7 % of *T. equigenitalis*-positive geldings had previously been used for breeding.

Due to a lack of official regulations regarding natural breeding under European law, routine testing for venereal diseases is not obligatory in the Icelandic breed. Although the difference between brood and maiden mares was not statistically significant, a trend towards more *T. equigenitalis*-positive qPCR results in maiden mares (9.0 %) compared to brood mares (2.2 %) was obvious ($P = 0.074$). However, brood mares were the only active breeding group of the study population that might have become infected via venereal transmission [14]. We assume that brood mares with sub- or infertility would have been noticed in the context of breeding management and would have been subsequently examined and treated in case of a *T. equigenitalis*-positive test result. Since no brood mares in our study were bred in the year of sample collection, the infection must have existed for a longer time without causing obvious clinical signs. In maiden mares, regular testing for venereal diseases is not routinely performed due to their non-breeding status. Thus, maiden mares with a *T. equigenitalis*-positive qPCR result might not be detected and treated. According to the owners of maiden mares included in the present study, none had ever been used for breeding nor had any contact with stallions. Therefore, infection must have occurred either perinatally [14,15] or via horizontal transmission from other carrier animals living in the same herd.

The results of our study are also in accordance with Parlevliet et al. [24] and indicate that *T. equigenitalis* may be endemic in the Icelandic horse population without causing any clinical problems. Parlevliet et al. [24] demonstrated the presence of *T. equigenitalis* in 14/36 (38.9 %) mares (brood mares, maiden mares, and mares with unknown reproductive status) housed in the Netherlands (12/36) or immediately after importation from Iceland to the Netherlands (24/36), although *T. equigenitalis* had never been reported in Iceland before. In Germany, only a few CEM cases have been officially reported in recent years, although a trend towards more positive CEM cases was recorded from 2020 to 2022 (2020: 43 cases, 2021: 46 cases, 2022: 61 cases) [25]. Compared to these official reported cases, results of this study lead to the

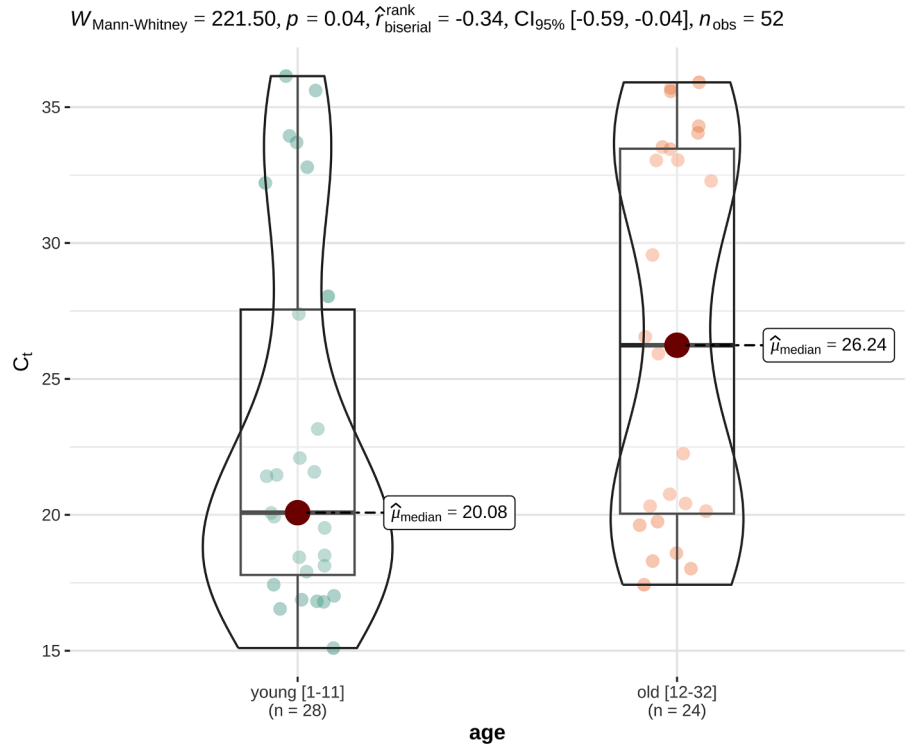

Fig. 2b. Comparison of both age groups regarding Ct values. Younger horses had significantly lower Ct values compared to older horses ($P = 0.04$).

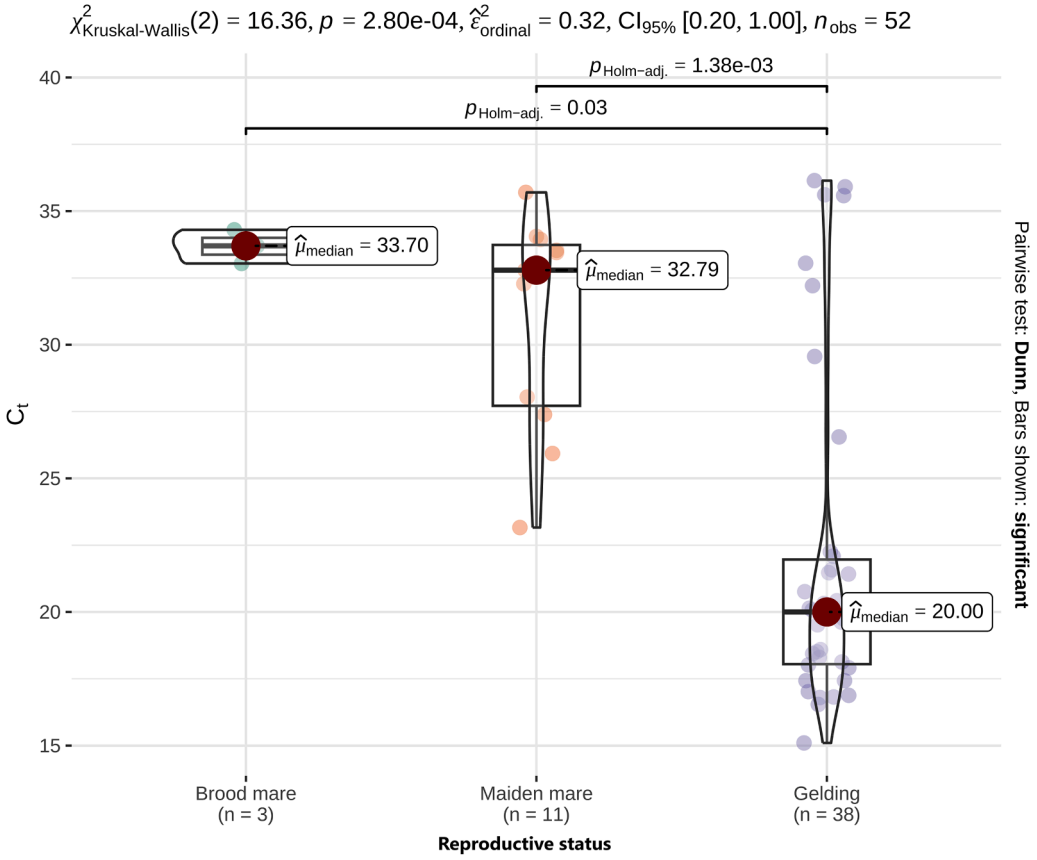

Fig. 3. Comparison of Ct values of brood mares, maiden mares and geldings. The Ct values of geldings are significantly lower compared to the Ct values of brood mares ($P < 0.03$) and maiden mares ($P < 0.001$).

assumption that the official prevalence of *T. equigenitalis* in Germany is probably extremely underestimated. The officially reported CEM cases are mainly in breeding animals, with clinical signs or signs of sub- or infertility observed by the owners. The other main reason for *T. equigenitalis* testing includes animals for export or stallions in artificial insemination programs. Furthermore, the officially reported CEM numbers do not state any breeding affiliation.

Limitations of the present study include the convenience sampling strategy, the small study population and that not all animals (especially no stallions) on the studs were sampled, preventing epidemiological tracing. The absence of tested stallions in this study does not allow comparisons of all status groups (i.e. stallions, geldings, mares), but since Grabatin et al. [16] already demonstrated a higher prevalence in Icelandic stallions compared to other horse breeds, we intended to focus on Icelandic mares and geldings only. Furthermore, the sampled horses were only located in Southern Germany and Austria, where, according to the International Federation of Icelandic Horse Associations (FEIF) [26] around 80.000 Icelandic horses reside. Therefore, the results only reflect a small portion of the Icelandic horse population in continental Europe where around 300.000 Icelandic horses were registered in 2023 [26]. Additional data such as previous breeding use, contact with *T. equigenitalis*-positive horses, contact with breeding animals and group composition were collected with the second retrospective questionnaire and unfortunately, due to lack of information or uncertain statements by the owners regarding these questions, the results were insufficient for further statistical investigation.

In our study, a routine qPCR was used to detect *T. equigenitalis* [19]. Since qPCR has a very high sensitivity, it is possible that only a small amount of *T. equigenitalis* DNA left in the reproductive tract is sufficient for a *T. equigenitalis*-positive qPCR result. However, all weakly positive samples (with a C_t value above 30) were tested repeatedly via qPCR and only double positive results were regarded truly positive. If, according to literature, all C_t values < 36 had been regarded as positive [27], the overall prevalence would have been slightly higher (18.6 %) compared to our test procedure with an overall prevalence of 14.4 %. Additional bacteriological culture was not performed due to the higher sensitivity of the qPCR [19] and the risk of false negative results with culture [28]. The objective of this study was to determine the prevalence of *T. equigenitalis* in Icelandic mares and geldings, and therefore, no further strain differentiation has been completed so far. Strain differentiation should be performed to ensure tracing of the *T. equigenitalis* strains and to gain further epidemiological insight, especially in comparison to other breeds.

In summary, this study shows a high prevalence of *T. equigenitalis* in Icelandic horses, especially in non-breeding animals. The present study was not intended to prove an impact of infection with *T. equigenitalis* on fertility. However, although all tested animals with a *T. equigenitalis*-positive qPCR result showed no clinical signs, testing for *T. equigenitalis* is strongly recommended before natural breeding to optimize breeding management and achieve higher pregnancy rates. Geldings showed the highest prevalence for *T. equigenitalis* in this study, so testing of non-breeding horses in close contact with breeding horses would be highly recommended, especially in mixed herds.

5. Conclusion

An overall prevalence of *T. equigenitalis* of 14.4 % in Icelandic mares and geldings was detected in this study, indicating that *T. equigenitalis* is widespread in the Icelandic horse population and is particularly common in Icelandic geldings (36.2 %) when compared to Icelandic mares (5.5 %). Therefore, Icelandic geldings might represent a reservoir of *T. equigenitalis* in this breed. Since both, breeding and non-breeding horses, were tested positive for *T. equigenitalis*, horses intended for breeding should be kept separately from other horses and should be tested frequently. This would help to avoid further transmission of *T. equigenitalis*.

Funding information

The study received no external funding.

Ethical animal research

The study was approved by the ethics committee of the Center of Clinical Veterinary Medicine, Faculty of Veterinary Medicine of the Ludwig-Maximilians University München, Germany (Reference number 294-28-12-2022; Approval date, February 2022).

Informed consent

The consent for the investigations was given by the horse owners or those with the owners' authority. The horses and farms as well as horse owners have been anonymized.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Veronika Solbach: Writing – original draft, Investigation, Data curation, Conceptualization. **Markus Grabatin:** Writing – review & editing, Investigation. **Yury Zablotski:** Writing – review & editing, Visualization, Validation, Software, Formal analysis. **Robert Fux:** Writing – review & editing, Validation, Methodology, Formal analysis. **Holm Zerbe:** Writing – review & editing, Supervision, Conceptualization. **Tanja Semira Witte:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing interests related to this report.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2024.105247.

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