**Statistical analysis**

Prevalence data were presented with 95% mid-P exact confidence intervals calculated using available freeware (OpenEpi: Open Source Epidemiologic Statistics for Public Health, www.OpenEpi.com). Apparent prevalences determined by the filtration and sedimentation methods were compared using McNemar’s chi-square tests and agreement between tests was assessed using kappa statistics. The normality assumption of quantitative data was assessed by calculating descriptive statistics, plotting histograms, and performing the Anderson-Darling test in commercial software (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA). Quantitative data were compared based on faecal detection groups (gastrodiscus detected, fasciolosis detected, and neither detected) using one-way ANOVA or Kruskal-Wallis tests for normally distributed and apparently non-normal data, respectively. Horses were classified as gastrodiscus or fasciolsis positive when either or both of the sedimentation and filtration methods were positive for the respective parasitic infestation. Univariate binary logistic regression was used to calculate odds ratios and 95% confidence intervals to evaluate the association between potential risk factors and gastrodiscuss and fasciolosis statuses. Variables with P < 0.2 based on Wald tests were selected for multivariable modelling. Selected variables were ranked smallest to largest based on the univariate Wald P values with the first variable selected to initiate the multivariable model. All other variables were assessed using a manual forward selection process based on P value ranking. Variables were manually added one-by-one to the multivariable model. Variables with P > 0.05 after entry were removed at each step and the next variable evaluated. Model building continued until all selected variables were evaluated. Unless stated otherwise, all statistical analyses were performed using commercial software (IBM SPSS Statistics Version 28, International Business Machines Corp., Armonk, New York, USA) with significance set as P < 0.05.

**Prevalence**

Presented as a percentage with a 95% confidence interval

Overall gastrodiscus via sediment: 23/207; 11.1 (7.4, 16.0)

Overall gastrodiscus via filtration: 17/207; 8.2 (5.0, 12.6)

Overall gastrodiscus via either test: 24/207; 11.6 (7.7, 16.5)

Clinical signs horses gastrodiscus via sediment: 10/123; 8.1 (4.2, 14.0)

Clinical signs horses gastrodiscus via filtration: 8/123; 6.5 (3.1, 12.0)

Clinical signs horses gastrodiscus via either test: 11/123; 8.9 (4.8, 15.0)

No clinical signs horses gastrodiscus via sediment: 13/84; 15.5 (8.9, 24.4)

No clinical signs horses gastrodiscus via filtration: 9/84; 10.7 (5.4, 18.8)

No clinical signs horses gastrodiscus via either test: 13/84; 15.5 (8.9, 24.4)

Overall fasciolosis via sediment: 7/207; 3.4 (1.5, 6.6)

Overall fasciolosis via filtration: 8/207; 3.9 (1.8, 7.2)

Overall fasciolosis via either test: 9/207; 4.3 (2.1, 7.8)

Clinical signs horses fasciolosis via sediment: 5/123; 4.1 (1.5, 8.8)

Clinical signs horses fasciolosis via filtration: 5/123; 4.1 (1.5, 8.8)

Clinical signs horses fasciolosis via either test: 6/123; 4.9 (2.0, 9.9)

No clinical signs horses fasciolosis via sediment: 2/84; 2.4 (0.4, 7.6)

No clinical signs horses fasciolosis via filtration: 3/84; 3.6 (0.9, 9.4)

No clinical signs horses fasciolosis via either test: 3/84; 3.6 (0.9, 9.4)

Comparison of prevalence by test

|  |  |  |  |
| --- | --- | --- | --- |
| **Count of Equid\_num** | **Sedimen\_gastrodiscus** |  |  |
| **Filtra\_gastrodiscus** | **1** | **0** | **Grand Total** |
| 1 | 16 | 1 | 17 |
| 0 | 7 | 183 | 190 |
| **Grand Total** | **23** | **184** | **207** |
|  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chi-Square Tests** | | | | |
|  | Value | Exact Sig. (2-sided) | Exact Sig. (1-sided) | Point Probability |
| McNemar Test |  | .070a | .035a | .031a |
| N of Valid Cases | 207 |  |  |  |
| a. Binomial distribution used. | | | | |

The sedimentation method detected more positive horses but the apparent difference in detection sensitivity was not significant (P = 0.07).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Symmetric Measures** | | | | | | |
|  | | Value | Asymptotic Standard Errora | Approximate Tb | Approximate Significance | Exact Significance |
| Measure of Agreement | Kappa | .779 | .075 | 11.367 | <.001 | <.001 |
| N of Valid Cases | | 207 |  |  |  |  |
| a. Not assuming the null hypothesis. | | | | | | |
| b. Using the asymptotic standard error assuming the null hypothesis. | | | | | | |

Kappa (95% CI): 0.779 (0.632, 0.926)

There was good agreement between the sedimentation and filtration methods for gastrodiscus detection.

|  |  |  |  |
| --- | --- | --- | --- |
| **Count of Equid\_num** | **Sedimen\_fasciola** |  |  |
| **Filtra\_fasciola** | **1** | **0** | **Grand Total** |
| 1 | 6 | 2 | 8 |
| 0 | 1 | 198 | 199 |
| **Grand Total** | **7** | **200** | **207** |
|  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chi-Square Tests** | | | | |
|  | Value | Exact Sig. (2-sided) | Exact Sig. (1-sided) | Point Probability |
| McNemar Test |  | 1.000a | .500a | .375a |
| N of Valid Cases | 207 |  |  |  |
| a. Binomial distribution used. | | | | |

The filtration method detected slight more horses as fasciolosis positive but the apparent sensitivity was not different between tests (P = 1.0)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Symmetric Measures** | | | | | | |
|  | | Value | Asymptotic Standard Errora | Approximate Tb | Approximate Significance | Exact Significance |
| Measure of Agreement | Kappa | .793 | .117 | 11.430 | <.001 | <.001 |
| N of Valid Cases | | 207 |  |  |  |  |
| a. Not assuming the null hypothesis. | | | | | | |
| b. Using the asymptotic standard error assuming the null hypothesis. | | | | | | |

Kappa (95% CI): 0.793 (0.564, 1.0)

There was good agreement between the sedimentation and filtration methods for fasciolosis detection.

**Table 1.** Comparison of quantitative data among outcome groups. Please revise as a descriptive table title if you retain.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Faecal negative** | |  | **Gastrodiscus positive** | |  | **Fasciola positive** | |  |  |
| **Variable** | **n** | **Descriptive data\*** | | **n** | **Descriptive data\*** | | **n** | **Descriptive data\*** | | **P value†** |
| Age (yr) | 174 | 12.6 (6.1) | | 23 | 14.0 (7.8) | | 8 | 13.6 (3.9) | | 0.549 |
| Residence duration (months) | 170 | 36 (12, 72) | | 22 | 36 (16, 75) | | 8 | 54 (38, 144) | | 0.218 |
| Time since most recent anthelmintic treatment (days) | 114 | 109 (97, 197) | | 15 | 110 (109, 197) | | 5 | 109 (107, 109) | | 0.578 |

\* Presented as mean (standard deviation) for normally distributed data and median (interquartile range) for data violating the normality assumption

† Based on 1-way ANOVA for normally distributed data and Kruskal-Wallis tests for data violating the normality assumption

**Table 2.** Univariate associations between gastrodiscus faecal detection and potential risk factors. Please revise as a descriptive table title.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Level** | **Parameter estimate ()** | **Odds ratio**  **(95% CI)** | **P value** |
| Age |  |  |  | 0.888 |
|  | < 10 years | -0.251 | 0.78 (0.27, 2.22) | 0.639 |
|  | 10 – 15 years | -0.051 | 0.95 (0.34, 2.63) | 0.922 |
|  | ≥ 16 years | Referent |  |  |
|  |  |  |  |  |
| Sex | Female | 0.182 | 1.20 (0.50, 2.90) | 0.685 |
|  | Male | Referent |  |  |
|  |  |  |  |  |
| Breed |  |  |  | 0.132 |
|  | Arabian | 0.029 | 1.03 (0.27, 3.90) | 0.966 |
|  | BP | -0.050 | 0.95 (0.28, 3.27) | 0.937 |
|  | TB | -1.503 | 0.22 (0.06, 0.89) | 0.034 |
|  | WB | -1.030 | 0.36 (0.10, 1.28) | 0.114 |
|  | Other breed | Referent |  |  |
|  |  |  |  |  |
| Clinical signs | Yes | -0.623 | 0.54 (0.23, 1.26) | 0.154 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Diarrhoea | Yes | -1.025 | 0.36 (0.08, 1.60) | 0.178 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Lucerne feeding | Yes | -1.107 | 0.33 (0.11, 1.01) | 0.051 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Hay covered | All or some | -0.782 | 0.46 (0.17, 1.20) | 0.112 |
|  | None | Referent |  |  |
|  |  |  |  |  |
| Dam present | Yes | 1.008 | 2.74 (1.08, 6.92) | 0.033 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Marshy ground | Present | 0.770 | 2.16 (0.88, 5.29) | 0.093 |
|  | Absent | Referent |  |  |
|  |  |  |  |  |
| Other equids with gastrodiscus | Yes | 0.701 | 2.02 (0.85, 4.78) | 0.111 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Fowl present | Yes | 0.646 | 1.91 (0.81, 4.49) | 0.139 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Livestock present | Yes | 2.806 | 16.5 (1.44, 190) | 0.024 |
|  | No | Referent |  |  |

CI = confidence interval. Add other acronyms

**Table 3.** Multivariable associations between gastrodiscus faecal detection and potential risk factors. Please revise as a descriptive table title.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Level** | **Parameter estimate ()** | **Odds ratio**  **(95% CI)** | **P value** |
| Dam present | Yes | 0.994 | 2.70 (1.05, 6.95) | 0.039 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Livestock present | Yes | 2.763 | 15.9 (1.29, 194) | 0.031 |
|  | No | Referent |  |  |

CI = confidence interval.

The model is a very poor fit to the data so I do not have much confidence in the findings. There were only 3 horses with reported exposure to livestock but two of these horses were gastrodiscus positive.

**Table 4.** Univariate associations between fasciola faecal detection and potential risk factors. Please revise as a descriptive table title.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Level** | **Parameter estimate ()** | **Odds ratio**  **(95% CI)** | **P value** |
| Age |  |  |  | 0.403 |
|  | < 10 years | -1.401 | 0.25 (0.03, 2.26) | 0.215 |
|  | 10 – 15 years | 0.078 | 1.08 (0.26, 4.51) | 0.915 |
|  | ≥ 16 years | Referent |  |  |
|  |  |  |  |  |
| Sex | Female | -0.605 | 0.55 (0.11, 2.70) | 0.458 |
|  | Male | Referent |  |  |
|  |  |  |  |  |
| Breed |  |  |  | 0.490 |
|  | Arabian | 0.742 | 2.10 (0.13, 35.3) | 0.606 |
|  | BP | 1.617 | 5.04 (0.50, 51.1) | 0.171 |
|  | TB | 0.759 | 2.14 (0.22, 21.2) | 0.517 |
|  | WB | -0.214 | 0.81 (0.05, 13.3) | 0.881 |
|  | Other breed | Referent |  |  |
|  |  |  |  |  |
| Harmonie yard | Yes | 1.797 | 6.03 (1.54, 23.6) | 0.010 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Lunaventus yard | Yes | 1.856 | 6.40 (1.60, 25.6) | 0.009 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Wormer w/ gastrodiscus activity | Yes | 1.423 | 4.15 (1.06, 16.3) | 0.041 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Previous gastrodiscus treatment | Yes | 1.833 | 6.25 (1.59, 24.5) | 0.009 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Eragrostis feeding | Yes | -1.335 | 0.26 (0.07, 1.02) | 0.053 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Pasture access (veld) | Yes | 1.628 | 5.10 (1.31, 19.9) | 0.019 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| River present | Yes | 1.230 | 3.42 (0.89, 13.2) | 0.075 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Other equids with gastrodiscus | Yes | 2.447 | 11.6 (1.42, 94.2) | 0.022 |
|  | No | Referent |  |  |
|  |  |  |  |  |
| Fowl present | Yes | 2.574 | 13.1 (1.61, 107) | 0.016 |
|  | No | Referent |  |  |

CI = confidence interval. Add other acronyms.

No multivariable model fit these data.

**Normality**





