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Evaluation of the detection of antibodies against *Lawsonia intracellularis* and changes in serum biochemistry in foals with and without equine proliferative enteropathy

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Summary: Equine proliferative enteropathy (EPE) is a well-recognized disease in foals caused by *Lawsonia intracellularis*. The aim of this study was to analyse clinicopathologic parameters and antibody titres up to the diagnosis of EPE. During the 2019 season, all foals born on a large breeding farm in Germany were enrolled in a monitoring program for EPE from four to nine months of age, including evaluation of rectal temperature, white blood cell count as well as serum total protein and albumin. 42 foals developed EPE between September 2019 and January 2020. Data sets of these foals and 83 age matched control foals were included in this study. All affected foals showed hypoproteinaemia and 39/42 showed hypoalbuminaemia at the onset of EPE. Total protein and albumin decreased rapidly within 7.5 days on average before the diagnosis of EPE. Both total protein and albumin values were significantly lower in the EPE-group compared to the matching foals ($p < 0.0001$). Seroconversion was observed in all 42 affected foals and in 50/83 matching foals. At the time of diagnosis, affected foals had a significantly higher titre compared to the matching foals ($p < 0.0001$). Nevertheless, the titre increased significantly between the preceding sampling and the week of diagnosis in both groups ($p < 0.0001$). Faecal qPCR for *Lawsonia intracellularis* revealed positive results in 22/42 affected foals. Based on the data analysed here, a high IPMA-titre does neither indicate protection against EPE nor a definite diagnosis of EPE, as both healthy foals and those with clinical EPE show a positive antibody titre against *Lawsonia intracellularis*.

Keywords: foal, IPMA, serology, total protein, albumin, horse, EPE, *Lawsonia intracellularis*

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Introduction

Equine proliferative enteropathy (EPE) is an emerging disease in foals caused by *Lawsonia intracellularis*, an obligate intracellular, curved, gram-negative bacterium (*Lawson* and *Gebhart* 2000). The bacterium affects the intestinal crypt cells, resulting in proliferation and thickened walls of the small and sometimes large intestine (*Pusterla* and *Gebhart* 2013).

EPE was first reported by *Duhamel* and *Wheeldon* in 1982. Since then, cases of EPE have been described almost worldwide (*Lavoie* et al. 2000, *McClintock* and *Collins* 2004, *Cehak* et al. 2007, *Frazer* 2008, *Bohlin* et al. 2019). Affected horses are typically between four and seven months old. Common clinical signs are oedema, fever, diarrhoea, lethargy and colic. Thickened walls of the small intestines can often be visualised via transabdominal ultrasonography. Consistent clinicopathologic findings are severe hypoproteinaemia and hypoalbuminaemia (*Lavoie* et al. 2000, *Frazer* 2008). Two kinds of tests are available for ante mortem diagnosis: quantitative real-time polymerase chain reaction (qPCR) for pathogen detection in faeces and several serological screening assays, as the immunoperoxidase monolayer assay (IPMA) or the ELISA (*Guedes* et al. 2002, *Herbst* et al. 2003, *Gebhart* et al. 2012).

In addition to vaccination against *Lawsonia intracellularis*, mainly measures aiming at an early detection of the disease are described, for example monitoring of total protein, albumin and antibody titres (*Pusterla* et al. 2011). Various antimicrobials can be considered for the treatment of EPE, typically macrolides, tetracyclines or chloramphenicol are used (*Sampieri* et al. 2006, *Pusterla* and *Gebhart* 2013).

The aim of this study was to describe the period until diagnosis of naturally occurring EPE, with special attention to the course of common clinicopathologic values (serum total protein, albumin) and antibody titres.

Materials and Methods

Study population

All study foals were born during the 2019 breeding season on a large Warmblood breeding farm in Germany, where cases of presumptive EPE have already been diagnosed since 2017.

Monitoring

A close monitoring for EPE was first introduced on this farm in 2019, including all foals between four and nine months of age. Until weaning of the foals, body temperature, white blood cell (WBC) counts as well as total protein and albumin values were monitored every other week. Clinically healthy foals without any signs of EPE were weaned at 5.5 month of age.

After weaning, serum total protein and albumin was determined weekly. WBC count and body temperature continued to be checked every other week. Daily visual inspections of the general condition of each animal were carried out by trained staff.

Foals with serum total protein values < 50 g/l, albumin values < 25 g/l and/or clinical signs of EPE were subjected to transabdominal ultrasonography. In the case of suspicious ultrasonographic findings, such as thickened walls of the small intestine (normal < 3 mm), a rectal swab was examined for *Lawsonia intracellularis* by qPCR and therapy was initiated.

Criteria for inclusion in the study

Foals with decreased serum total protein and/or albumin values, sonographically thickened intestinal walls, a *Lawsonia intracellularis* positive qPCR result and/or a positive serum titre via IPMA were included in the study. Two matching foals were assigned to every affected foal to form the control group. Preference was given to foals of similar age and time of weaning, kept together with the affected foal and not suffering from EPE at the time of selection.

Sample processing, analysis and storage

A 4 ml serum sample was collected every other week from foals until weaning and subsequent weekly after weaning via jugular venipuncture. These samples were sent unprocessed to LABOKLIN GmbH & Co. KG (Bad Kissingen, Deutschland), where total protein and albumin values were determined the following day. Additionally, a 9 ml serum sample and a 2.7 ml EDTA sample were taken every other week from all foals included in the monitoring. The EDTA blood was used to determine the WBC count using a hematology device on site. The serum sample was centrifuged, and the separated serum was stored in individual 1.5 ml tubes at -20 °C. The stored serum samples of the affected foals were examined for antibodies against *Lawsonia intracellularis* via IPMA up to diagnosis. Samples of the matching foals were assessed up to the date of diagnosis of the assigned EPE affected foal. A titre of 60 was considered seropositive. 1:960 was the highest dilution performed in this study. A total of 949 samples were tested for antibodies via IPMA. 43 of these were analysed at the Equine Infectious Disease Research Laboratory (University of California, Davis, USA), the remaining 906 samples were analysed at Labor Dr. Böse GmbH (Harsum, Germany).

Rectal swabs of all affected foals and 19 matching foals were examined for *Lawsonia intracellularis* using qPCR (Labor Dr. Böse GmbH, Harsum, Deutschland). The rectal swabs of the matching foals were taken within three weeks after the diagnosis of the assigned affected foal, usually during the next regular examination.

Statistical analysis

The statistical analysis was done with SAS Software (Version 9.4, SAS Institute Inc.). Data was analysed by descriptive statistical analysis. Differences in serum total protein, albumin, WBC count and temperature between groups were analysed with mixed models. Those models included group and time of measurement as fixed main effects and the corresponding interaction. The individual random effects were modelled using an autoregressive correlation structure over time. Distributional assumptions of the residuals were checked visually, e.g. using QQ-plot. The nonparametric Wilcoxon rank-sum test was used to examine differences in baseline parameters and titres between the two groups. Logistic regression analysis was used to determine the effect of the month of weaning and age at seroconversion on the risk to develop clinical EPE. The difference in titre between the last sampling and one and two samplings prior were analysed with a signed rank test. P-values ≤ 0.05 were considered statistically significant.

Results

Population, signalment and clinical presentation

Forty-two foals born during the 2019 breeding season developed EPE between September 2019 and January 2020 and met the criteria for inclusion in the study. According to the study design, 84 matching foals were selected, but one had to be excluded later, because unintentionally some inclusion criteria were not met. As part of another study, two foals with EPE had been vaccinated intrarectally against *Lawsonia intracellularis* 71 days before the onset of the disease (Enterisol ileitis®, Boehringer Ingelheim). The baseline variables of both groups are shown in Table 1.

The foals affected by EPE were a median of 214 days (5^{th}–95^{th} percentiles: 192–269 d) old at diagnosis. Due to sample transport and laboratory effort, there is a difference of 2–3 days between the last sampling and the diagnosis in many cases.

Fourteen cases each occurred in November and January, ten foals developed EPE in December and two each in September and October. 40/42 foals were affected after weaning, only two were still with the mare when the diagnosis of EPE was made. All foals had been weaned between August 2019 and February 2020. Logistic regression analysis showed no effect of the weaning month on the occurrence of EPE ($p = 0.9$).

As required by inclusion criteria, ultrasonography of the abdomen revealed thickened walls of the small intestine in all affected foals. Clinical signs were fever (> 38.5 °C, 18/31), diarrhoea (7/42), peripheral oedema (4/42) and colic (3/42). Affected foals had a significantly higher rectal temperature (mean: 38.9 ± 0.9 °C, min. – max.: 37.0–40.4 °C) at the time of diagnosis compared to the matching foals (mean: 38.2 ± 0.4 °C, min. – max.: 37.3–39.4 °C; $p < 0.0001$).

Clinicopathologic values

Serum biochemistry revealed hypoproteinaemia (reference range 50–75 g/L) at the onset of disease in all affected foals (mean 36.7 ± 7.0 g/L, min. – max.: 22.1–48.0 g/L), 29/42 showed values below 40 g/L (Table 2).

Thirty-nine foals with clinical EPE were also hypoalbuminaemic (mean 19.0 ± 4.5 g/L, min. – max.: 7.1–28.1 g/L), three foals had albumin levels within the reference range (reference range 25–54 g/L; Table 2). In the matching foals, the mean serum total protein and albumin concentration at the last sampling was 60.2 ± 4.1 g/L (min. – max.: 45.2–67.5 g/L) and 31.8 ± 3.0 g/L (min. – max.: 22.9–37.8 g/l), respectively (Fig. 1). Both serum total protein and albumin values at the time of diagnosis were significantly lower in the affected foals than in the matching foals ($p < 0.0001$ for both).

In the week of diagnosis, one matching foal showed hypoproteinaemia, two showed hypoalbuminemia and one foal showed both, but none showed clinical or ultrasonographic signs of EPE.

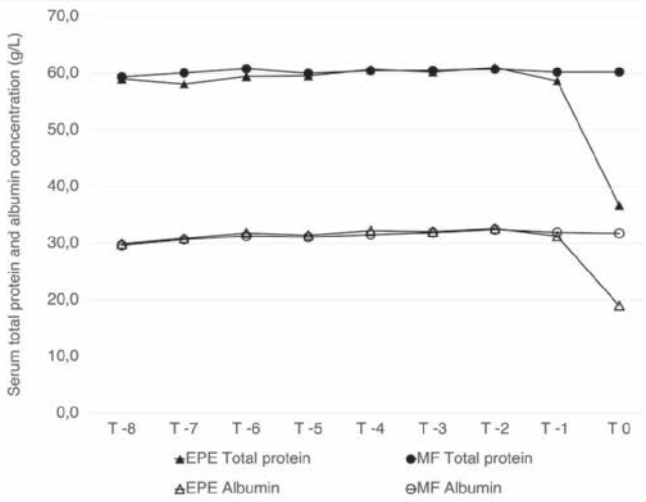

Fig. 1 Course of serum total protein and albumin in foals with clinical EPE (EPE) and healthy matching foals (MF) at different timepoints (T) up to the diagnosis of EPE (T 0). | *Der Verlauf von Totalprotein und Albumin im Serum von Fohlen mit klinischer EPE (EPE) und gesunden Vergleichsfohlen (MF) zu verschiedenen Zeitpunkten (T) bis zur Diagnose der EPE (T 0).*

Table 1 Baseline variables of affected and matching foals. | *Grundlegende Daten erkrankter Fohlen sowie gesunder Kontrollfohlen.*

	EPE	Matching Foals
Number of foals	42	83
Female (%)	27 (64.3%)	54 (65.1%)
Male (%)	15 (35.7%)	29 (34.9%)
Mean age at first sampling (d)	118 (± 14.1, 104–183)	116 (± 11.7, 106–168)
Median (5^{th} – 95^{th} percentiles) age at last sampling (d)	212 189–267)	223 (184–270)
Mean age at last sampling (d)	224 (± 28.9, 166–298)	225 (± 27.9, 151–295)
Median (5^{th} – 95^{th} percentiles) age at diagnosis	214 (192–269)	N/A
Mean age at diagnosis of EPE (d)	226 (± 29.4, 160–298)	N/A
Number of foals affected of EPE after weaning	40	N/A
Mean age at weaning (d)	179 (± 15.7, 154–221)	178 (± 15.5, 154–226)
Mean time between weaning and last sampling (d)	48 (± 18.6, 15–90)	50 (± 18.0, 15–90)

Table 2 Serum total protein and albumin values in 42 foals with clinical EPE at the time of diagnosis. Number of foals (%) with different degrees of hypoproteinaemia and hypoalbuminaemia. | *Totalprotein- und Albuminwerte im Serum der 42 Fohlen mit klinischer EPE zum Zeitpunkt der Diagnose sowie die Anzahl der Fohlen (%) mit verschiedenen Graden von Hypoproteinämie und Hypoalbuminämie.*

Total protein		Albumin	
mean	36,7 ± 7.0 (min – max: 22.1–48.0)	mean	19,0 ± 4.5 (min – max: 7.1–28.1)
≥ 50 g/L	0 foals (0%)	≥ 25 g/L	3 foals (7.1%)
< 50 g/L	13 foals (31.0%)	< 25 g/L	15 foals (35.7%)
< 40 g/L	22 foals (52.4%)	< 20 g/L	23 foals (54.8%)
< 30 g/L	7 foals (16.7%)	< 10 g/L	1 foal (2.4%)

In the weeks before the diagnosis, total protein and albumin levels below the reference ranges were observed in four affected foals and nine matching foals. Decreased values were detectable for one to three samplings. All foals were unremarkable in general clinical appearance and ultrasonography.

In 34 of the affected foals, the time between the last unremarkable sampling and the date of diagnosis could be calculated and was between 3 and 21 days (mean 7.5 d). Eight foals were not included in this calculation to avoid bias caused by the fact that individual samples could not be taken in the regular interval due to farm-specific processes. Both total protein and albumin values decreased significantly between the previous sampling and the diagnosis in the affected foals ($p < 0.0001$), with a mean difference of 22.9 ± 7.7 g/L (min.–max.: 6.0–33.9 g/L) in total protein and 12.2 ± 4.5 g/L, (min.–max.: 1.7–20.7 g/L) in albumin. No significant difference was detected in the matching foals ($p = 1.0$).

In 23 of the affected foals and 34 of the matching foals, the WBC count was determined in the week of diagnosis. The mean WBC count was $12.3 \pm 4.3 \times 10^3/\mu l$ (min.–max. $6.3–24.5 \times 10^3/\mu l$) for the sick foals and $14.4 \pm 3.0 \times 10^3/\mu l$ (min.–max. $9.0–21.5 \times 10^3/\mu l$) for the matching foals.

The difference between the WCB count in the week of diagnosis and the previous sampling was calculated for 21 affected and 34 matching foals. The sampling dates were between 7 and 14 days apart. This revealed a decrease in the WBC count in 81% of the affected foals, while 19% showed increased WBC counts. In the matching foals, only 41% showed a decrease in WBC count, while 47% of the values increased and 12% remained at the same level. The WBC count in the affected foals was significantly lower at the time of diagnosis compared to the previous sampling ($p = 0.04$), decreasing by $3.4 \pm 5.2 \times 10^3/\mu l$ (min.–max.: $-10.5–11.5 \times 10^3/\mu l$), but no difference was detected in the matching foals ($p = 0.6440$).

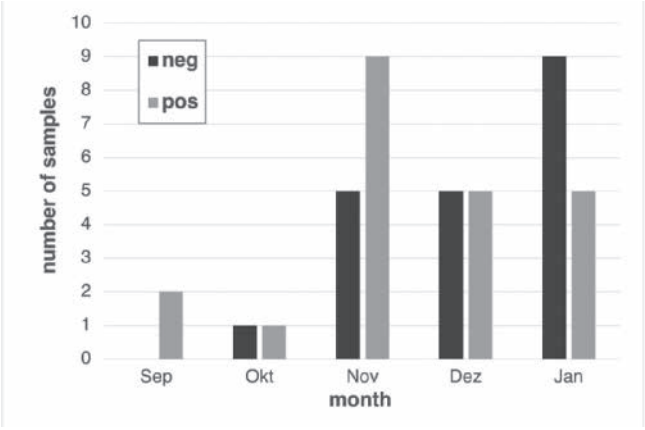

Fig. 2 Monthly results of fecal qPCR from foals with clinical EPE. *Die Ergebnisse der qPCR im Kot von Fohlen mit klinischer EPE je Monat.*

Molecular testing

qPCR for *Lawsonia intracellularis* in the faeces was performed in all 42 affected foals and in 19/83 matching foals.

22/42 foals with EPE had a positive PCR result. Among the sick foals, most positive PCR results were recorded in November (9/14; Fig. 2). In the matching foals, 18 samples had a negative PCR result and one sample was positive.

A course of antibody kinetics against *Lawsonia intracellularis* was determined for all study foals by analysing the stored serum samples via IPMA up to the time of diagnosis.

Seroconversion describes any increase in the antibody titre and was detected in all affected and 50 (60.2%) of the matching foals. Seroconversion was detected at the same age in both groups (Table 3), Logistic regression analysis showed no influence of the age at seroconversion on the risk to develop clinical EPE ($p = 0.6440$).

At the time of diagnosis all affected foals were seropositive: 35/42 had a titre of 960, the remaining seven had a titre of 480. 50/83 matching foals (60.2%) were also seropositive in the week of diagnosis, with titres between 60 to 960: 60 (5/83), 120 (7/83), 240 (9/83), 480 (12/83) and 960 (17/83). Thirty-three matching foals showed no response in antibody levels over the entire study period. The titre at diagnosis was significantly higher in the affected foals than in the matching foals ($p < 0.0001$). Furthermore, after dividing the matching foals into two groups, seropositive and seronegative, it could also be shown that the titre at diagnosis is

Table 3 Occurrence of seroconversion in the different groups. Seroconversion is considered as any increase in antibody titre. | *Beobachtung von Serokonversion in den verschiedenen Gruppen. Serokonversion wird definiert als jeder Anstieg des Antikörpertiters.*

	EPE	Matching foals	P value
Number of foals showing seroconversion	42 (100%)	50 (60,2%)	
Median (5^{th}–95^{th} percentiles) age at seroconversion (d)	211 (186–267)	223 (155–268)	0.3043
Mean (± SD, min. – max.) age at seroconversion (d)	220 (± 28.7, 156–286)	221 (± 32.1, 112–282)	
Number of foals showing seroconversion before weaning	2	3	
Median (5^{th}–95^{th} percentiles) time between weaning and seroconversion (d)	41 (23.5–83.5)	46 (27–78)	0.1512
Mean (± SD, min. – max.) time between weaning and seroconversion (d)	45 (± 18.6, 7–90)	48 (± 15.5, 20–78)	
Median (5^{th}–95^{th} percentiles) time between seroconversion and last sampling (d)	0 (0–12)	7 (0–70)	0.0258
Mean (± SD, min. – max.) time between seroconversion and last sampling (d)	4 (± 5.5, 0–26)	14 (± 22.2, 0–99)	

significantly higher in foals suffering from EPE compared to seropositive matching foals ($p < 0.0001$). In both seropositive control foals and EPE affected with EPE the titre in the week of diagnosis was significantly higher compared to the previous sampling ($p < 0.0001$ for both).

Seventeen affected foals were already seropositive in the week prior to EPE; in the remaining foals, an increase in titre was not detected until the diagnosis of EPE. Only a single foal with EPE showed a decrease in titre from 960 to 480 between the sampling before EPE and the diagnosis.

At the sampling prior to diagnosis of EPE, 57.5% of the affected foals and 68.7% of the matching foals were seronegative, while two samplings prior to diagnosis, it was 97.6% and 89.0% respectively (Fig. 3). Nonparametric analysis of variance revealed no significant differences between both groups in the titres one ($p = 0.3$) and two samplings ($p = 0.1$) prior to diagnosis.

Three matching foals showed seroconversion more than 4 weeks before the onset of the disease. In all cases antibodies could only be detected in a single sample. The measured titres were 60, 240 and 960. All three foals subsequently showed seroreversion below the cut off value (60) and seroconverted again in the week of diagnosis of their assigned EPE-foal.

Discussion

While EPE has played an increasingly important role in North America for several years now (*Lavoie* et al. 2000, *Frazer* 2008, *Pusterla* et al. 2008), there are only a few reports from isolated cases in Europe (*Wuersch* et al. 2006, *Cehak* et al. 2007, *Bohlin* et al. 2019).

The current study took place at a Warmblood breeding farm with occurrence of EPE for the past two years and provides a detailed characterisation of the course of commonly measured clinicopathologic values and antibody kinetics in foals up to the onset of clinical EPE, by analysing the data of 42 affected foals in comparison to 83 healthy matching foals.

All cases in this study occurred between September 2019 and January 2020 and mainly weanling foals five to ten months of age were affected. This is in accordance with previous literature describing a similar age and time for the occurrence of EPE (*Lavoie* et al. 2000, *Frazer* 2008, *Pusterla* and *Gebhart* 2013).

Weaning stress, changes in groups and cessation of milk intake are frequently discussed reasons for the age predilection and high occurrence of EPE in autumn and winter (*Lavoie* et al. 2000, *Page* et al. 2015a). However, an average of 50 days from weaning to the diagnosis of EPE allows us to question a close relation of these events. A large field study showed foals weaned before September to be at a lower risk of developing EPE compared to later weaned foals (*Page* et al. 2015b). In the current study there was no significant effect of the month of weaning on the occurrence of EPE.

The EPE monitoring at the farm was developed by modifying a program for herds with endemic status of EPE (*Pusterla* et al. 2011). In contrast to literature, no routine serological screening was carried out in the current study, but regular measurements of serum total protein and albumin. After two foals were not detected during biweekly monitoring, the frequency was increased to weekly controls in weaned foals. The rapid drop in total protein and albumin was already shown in a previous study of experimental infection with *Lawsonia intracellularis* (*Page* et al. 2011) and could be confirmed here with naturally occurring EPE. Even with close monitoring, a drop

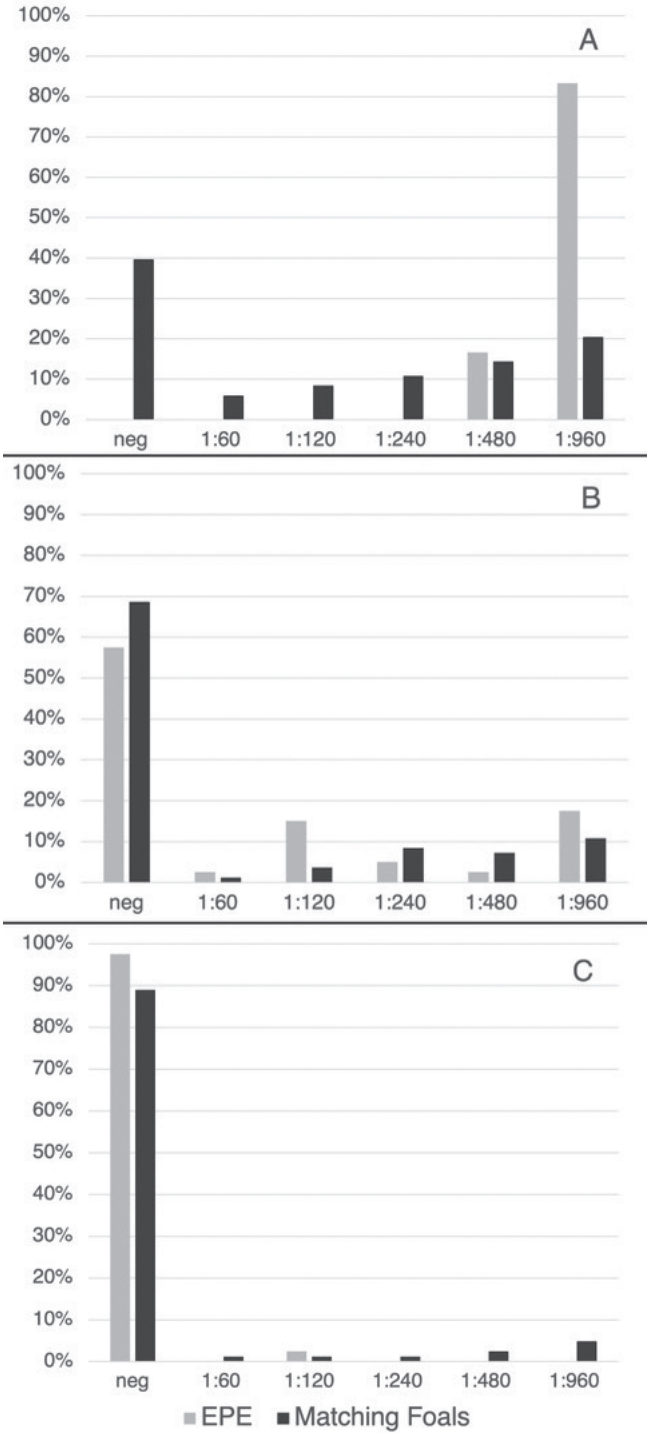

Fig. 3 Proportions of titres in both groups in the week of diagnosis (A), as well as one (B) and two (C) samplings before. | *Anteile der gemessenen Titer in den beiden Gruppen in der Woche der Diagnose (A), sowie eine (B) und zwei (C) Probenahmen zuvor.*

in total protein of more than 20 g/l was detected within seven to eight days in affected animals. Since all sick foals in the current study were hypoproteinaemic (total protein < 50 g/L), serum total protein appears to be a very reliable parameter and might be considered as the first diagnostic tool to detect suspicious foals during monitoring. Using refractometry could further reduce laboratory costs and the results are available quickly (*Pusterla* et al. 2011).

Faecal qPCR of the affected foals revealed positive results only in 22/42 cases. Previous literature already reported low sensitivity of faecal qPCR for *Lawsonia intracellularis*, for example because of antimicrobial treatment, intermittent shedding of pathogens or sampling in later stages of the disease (*Lavoie* et al. 2000, *Frazer* 2008, *Pusterla* and *Gebhart* 2013). One must keep in mind, that in the current study only rectal swaps were analysed, which are less reliable than faecal samples (*Pusterla* et al. 2010a). Furthermore, as only foals with thickened intestinal walls in ultrasonography were tested via qPCR for *Lawsonia intracellularis* in faeces subclinical cases, that might have shed *Lawsonia intracellularis*, might have been missed. Only one matching foal (1/19) had a positive qPCR without showing clinical signs compatible with EPE at any time. Similarly, in other studies pathogens could not or only occasionally be detected in the faeces of horses not suffering from clinical EPE, suggesting that healthy foals do not have major impact on the pathogen load in the environment (*Pusterla* et al. 2008, *Pusterla* et al. 2009).

The antibody titre of all study animals was determined by IPMA up to the time of diagnosis. Both groups in the current study seroconverted at about seven months of age. This differs to comparable studies that describe seroconversion in both younger and older foals (*Pusterla* et al. 2009, *Page* et al. 2015b). *Pusterla* et al. (2009) described a high pathogen exposure and seroconversion before weaning on breeding farms with endemic EPE, whereas in the current study all affected foals seroconverted after weaning, except for two foals that developed EPE before weaning. Only three of the matching foals showed seroconversion before weaning. Interestingly, the four matching foals assigned to the pre-weaning affected foals did not show seroconversion over the entire study period. Further, the mean time between weaning and seroconversion (50 days) confirms the presumed post-weaning exposure in the current study, as seroconversion was shown in foals after a maximum of 21 days after experimental infection (*Pusterla* et al. 2010b, *Page* et al. 2011). However, the age at seroconversion should be evaluated carefully, as the selection of EPE-affected foals and matching foals from the same group may bias these values and thus limits a reliable assertion about the time of seroconversion in healthy foals at the farm. A significant increase in antibodies against *Lawsonia intracellularis* was observed in both groups at the onset of disease and parallel to it. All affected foals had a titre of 480 or 960 at the diagnosis of EPE. Further, about 60% of the matching foals were also seropositive in the week of diagnosis of their assigned EPE-foal. Criteria for inclusion in the control group might explain the high exposure to the bacterium among the matching foals, since seroconversion in matching foals is mainly observed around the time of the disease in their assigned EPE-foal. After experimental infection seroconversion was also reported in a not-infected, sentinel foal kept together with an infected foal (*Pusterla* et al. 2010b). Similarly, in the field, seroconversion was observed in two healthy foals kept in the same group as a foal suffering from EPE (*Feary* et al. 2007).

It is not possible to say with certainty what induces the immune response in matching foals. Either it was a reaction to the pathogen introduced into the group from an external source or to the high number of pathogens shed by sick herd members (*Pusterla* et al. 2010b). As we observed concurrent seroconversion in both groups, it appears likely that the whole group was exposed to the pathogen at the same time, while only a few individuals got clinically affected. In case of increased exposure because of high pathogen shedding by clinically affected foals, seroconversion in matching foals might be delayed to the disease of the affected foals. But this study does not provide any information about the further course of antibodies in the almost 40% of matching foals, who did not seroconvert until the last sampling. And again, it is not possible to conclude from seroconversion in the study foals to the serological situation in groups without cases of clinical EPE. Further research is needed to find out why individual animals become clinically affected by EPE while others do not.

In general, it is recommended to perform both faecal qPCR and serological testing for in vivo diagnosis of presumptive clinical EPE (*Frazer* 2008). qPCR for *Lawsonia intracellularis* is known to have good specificity, but only variable sensitivity (*Pusterla* and *Gebhart* 2013). Based on the data evaluated here, the omission of PCR could be considered, as all clinically affected foals were seropositive at diagnosis. But previous studies have shown that antibodies can not necessarily be expected at the onset of the disease (*Dauvillier* et al. 2006, *Sampieri* et al. 2006, *Feary* et al. 2007) and as shown here, also healthy foals show measurable titres above the cut-off value. A diagnosis can be considered reliable in case of typical clinical and clinicopathologic signs, ultrasonographically thickened intestinal walls and a positive PCR result, positive antibody titre or both. Further other causes of gastrointestinal diseases and hypoproteinaemia should be ruled out, as one must keep in mind that a definite diagnosis of EPE can only be made by histological examination.

In conclusion, the value of serum total protein can be confirmed as an exceptionally reliable diagnostic and monitoring tool and should always be assessed for the diagnosis of EPE. Further it can be stated that also healthy foals seroconvert. In both study groups, the number of seronegative foals decreased rapidly towards the time of diagnosis, suggesting an association of seroconversion in healthy foals with the occurrence of clinical EPE in a group. Finally, a positive antibody titre against *Lawsonia intracellularis* cannot alone set the diagnosis of clinical EPE nor the protection against the disease. The question of what predisposes individual foals to develop clinical EPE remains largely unanswered and requires further studies.

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