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Clinical and haematological parameters for the early diagnosis of pneumonia in foals

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Summary: Pneumonia is a frequent and serious disorder in the growing foal. The aim of this study was to evaluate the clinical signs, temperature, white blood cell (WBC) count and ultrasonographic findings of the lung as screening methods for early detection of pneumonia in foals. In a prospective study, 101 foals were examined weekly, starting at the age of two weeks, over a period of 16 weeks. Once weekly, clinical signs of the respiratory tract were summarized in a clinical score, rectal temperatures were taken and WBC were counted. Furthermore, a sonographic examination of the lung was carried out and the diameters of pulmonary consolidations were added to obtain an abscess score that represents the extension of pulmonary damage. Clinical signs and haematological findings were then compared to the sonographic findings. Further evaluation of the data was performed on the day of the pneumonia diagnosis. A receiving operating characteristics curve was used to evaluate the diagnostic value of the WBC count in comparison to the ultrasonographic examination of the thorax. A one-way frequency table was used to show possible correlations between clinical signs and haematological findings on the day of diagnosis. Pneumonia was diagnosed in 86 foals by ultrasonography of the thorax. No correlation between the clinical score, temperature, WBC count and sonographic findings indicative of pneumonia were found on the day of ultrasonographic diagnosis. Neither hyperthermia, nor elevation of the clinical score nor leucocytosis were found on the day of the pneumonia diagnosis in 39 of the 86 foals. In one foal only, all parameters were elevated on the day of diagnosis. The WBC count cut-off value ≥13,000 cells/ L had a sensitivity of 42% and specificity of 72% for the diagnosis pneumonia. Neither clinical signs nor an elevated temperature nor the WBC count are efficient for an early diagnosis of pneumonia in foals. A screening method based on sonography of the lung additionally to clinical signs and hematology seems very helpful to detect subclinical pneumonia in foals on horse breeding farms.

Keywords: foal, pneumonia, early diagnosis, pulmonary abscess, clinical parameters, haematological parameters, diagnostic imaging

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Introduction

Pneumonia is a frequent and serious disorder in the growing foal. Especially pyogranulomatous bronchopneumonia causes high morbidity and mortality rate in foals. The most significant pathogens causing abscessation in the lung are *Streptococcus equi ssp. zooepidemicus* (Strep. zoo.) and *Rhodococcus equi* (R. equi) (*Lavoie* et al. 1994). Much more than Strep. zoo., R. equi has been investigated intensely in the last few decades because of its frequent occurrence on horse breeding farms in most countries worldwide. R. equi is a gram-positive, intracellular, soil saprophyte that causes severe pyogranulomatous bronchopneumonia in foals between one and six months of age (*Magnusson* 1923, *Zink* et al. 1986, *Giguère* and *Prescott* 1997). R. equi induced disease in other species only extremely rarely (*Barton* and *Hughes* 1984, *Prescott* 1991). In contrast to R. equi, Strep. zoo., which is also a gram-positive bacterium, is not species specific. It is a facultative anaerobe, mucous membrane saprophyte located in the upper airways and can cause bronchopneumonia in foals and in adult horses (*Evans* 1936, *Lavoie* et al. 1994, *Sweeney* et al. 2005).

Previous studies, performed on farms with an endemic history of R. equi pneumonia in foals, have shown that the morbidity rate in foals can range from 40 to 80% (*Higuchi* et al. 1997, *Giguère* et al. 2011). The mortality rate of R. equi pneumonia can be reduced by early recognition and adequate treatment (*Sweeney* et al. 1987).

Once a foal is infected by a pathogen causing pyogranulomatous pneumonia, the initial progression is usually subclinical, and becomes first noticeable for the owner, when the pulmonary lesions are severe and chronic (*Althaus* 2004, *Slovis* et al. 2005, *Venner* et al. 2012). This is due to the fact that foals are able to compensate for the loss of lung function quite well surprisingly, until extended lung damage and final stages of the disease are reached (*Giguère* and *Prescott* 1997). Because of this subclinical progression of pneumonia in foals, there is a need for reliable screening methods to reduce the mortality rate.

Several diagnostic tools are available to diagnose pneumonia in foals: clinical signs, haematological findings, diagnostic imaging, such as ultrasonography and radiography, and, finally, isolation of the pathogen. Although bacteriologic culture and PCR are frequently mentioned to provide solid evidence of the pathogens causing pneumonia (*Giguère* and *Prescott* 1997, *Giguère* et al. 2003), the interpretation of the results requires great caution. In fact, previous studies have shown that the isolation of R. equi as well as Strep. zoo. in the airway secretions of foals can be negative, although one or both pathogens were isolated in the affected lung tissue examined post mortem (*Lavoie* et al. 1994, *Weimar* 2006). Additionally, both pathogens have been isolated as well in healthy foals (*Venner* et al. 2007). In order to improve the early diagnosis of pneumonia in foals, diagnostic imaging tools such as the sonography have gained more importance, due to a

convincing sensitivity in detecting pulmonary consolidations (*Ramirez* et al. 2004, *Reef* 2004, *Venner* et al. 2014).

The aim of the following study was to evaluate the commonly used screening methods to diagnose pneumonia in foals. For this, foals born on a farm with a history of endemic pneumonia were monitored closely from birth up to four months of age.

Material and Methods

Each foal was examined once weekly starting at the age of two weeks and over a period of 16 weeks. The mares and foals were left the foaling area one week postpartum and were then kept together in small groups in big pens with straw bedding adjacent to an outside area with a concrete floor. Approximately four weeks later, the mares and foals were sent to large fields in groups of 25 mares. All foals grew up with the same hygiene and medical management. The diagnostic methods tested as a screening program in the present study included a physical examination to determine the clinical score, measurement of the body temperature, white blood cell (WBC) count and a sonographic examination of the thorax.
Each foal was examined once weekly and the findings on size and texture of the mandibular lymph nodes, nasal discharge classified as either serous, mucous or purulent, and auscultatory findings of the trachea and lung were recorded. These findings were gradedand, finally, added to the clinical score (*Gravert* 2006). Clinically healthy foals had a score from 0–2, mildly sick foals a score of 3–4, moderately sick foals a score of 5–6 and severely sick foals had a score greater than 7. As the rectal temperature is not included in the clinical score, further analysis was performed separately. Values above 39.0°C were considered as elevated, based on the study performed by *Prescott* et al. (1989).

A venous blood sample was taken in a tube containing ethylene-diamine-tetra-acetic acid (EDTA) right after the clinical examination and processed immediately with the automated cell counter "SYSMEX KX 21N", which provided the WBC concentration.

The ultrasonographic examination of the thorax was performed with the portable ultrasound machine SONOVET 2000 (Osteosys Co, Seoul, Korea) with a 7.5 MHz linear transducer (LV5-9AD, SonoAce, Osteosys Co, Seoul, Korea). Isopropyl alcohol (2-Propanol, 99.5%, Rebo-Pharm, Bochholt, Germany) was sprayed on both sides of the thorax to obtain a sufficient ultrasonic coupling. The ultrasonographic examination was then performed from the 15th to the 3th intercostal space, dorsally to ventrally, to detect pulmonary abnormalities adjacent to the pleura. A lung sheet was used for the precise documentation. Each side of the thorax was drawn and divided into intercostal spaces with three areas: dorsal, medial, ventral (A, B and C, respectively) (Fig. 1). The pulmonary findings, which were marked in each square, were classified as follows: 0 = no tissue damage, A1 = consolidation with a maximum diameter of 1cm, A2 = consolidation with a maximum diameter of 2 cm and so on. The diagnosis pneumonia was based on the presence of a consolidation only. At the end of each ultrasonographic examination, all diameters of the consolidations found were added, giving the so-called abscess score, which represents the severity of pulmonary tissue damage.

Foals suffering from health issues that needed medical treatment during the study period, for example, leucocytosis above 25,000 cells/ L or colic, were excluded from the study.

Statistical analyses

The statistical analyses were performed using the Statistical Analysis System for Windows SAS®, version 9.4, by using the SAS® Enterprise Guide® version 7.1 Client to analyse the data collected. Error probabilities of $p < 0.05$ were considered significant for all analyses performed.

General frequencies of occurrence, age on the day of diagnosis, WBC count, temperature, clinical score and abscess score were tested for normal distribution.

Calculating a logistic regression model, the receiving operating characteristic (ROC) curve was used to assess the diagnostic performance of the WBC count for early diagnosis of pneumonia where ultrasonographic examination (with the determination of the abscess score as a binary variable) is the reference method. The area under the curve relates to the ROC curve as a quality feature of the overall diagnostic performance. The best value for a test would be an area under the curve of 1. In that case, the sensitivity and the specificity are 100%. The Youden Index for the best WBC count cut-off value can be derived from the ROC curve. For this index, the sum of sensitivity and specificity is a maximum.

A one-way frequency analysis table was used to find any possible correlation between clinical score, temperature, WBC count and abscess score.

Results

A total of 86 from 101 foals developed pneumonia in our study in 2016. Eight foals did not develop pulmonary lesions during the whole examination period and seven foals had to

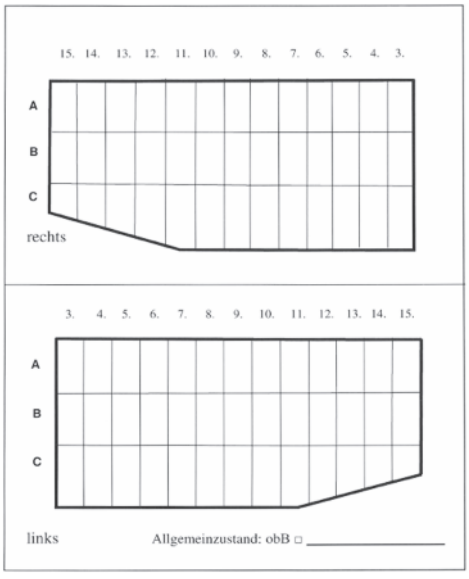

Fig. 1 Template of both sides of a lung from the 15th to the 3rd intercostal space | *Vorlage beider Lungenhälften von dem 15. bis zum 3. Intercostalraum*

be excluded from the study due to disorders unrelated to pulmonary disease.

The eight foals, which remained healthy during the investigation, showed a median clinical score (median; 25th–75th percentile) of 2 (1–2) with a minimum of 1 and a maximum of 2. The body temperature ranged from 38.4°C to 38.8°C with a median of 38.6°C (25th–75th: 38.5–38.7°C). Additionally, the WBC count of the eight healthy foals ranged from 8,550 cells/ L to 14,200 cells/ L (median: 12,675 cells/ L; 25th–75th: 10,313–13,213 cells/ L) over the study period of 16 weeks.

From here on, the data of the 86 sick foals was analysed on the day of pneumonia diagnosis. The age, at which pneumonia was detected first by sonographic examination (day of diagnosis), is shown in Figure 2. It varied between 4 and 18 weeks with a median of 11 weeks (25% percentile: 8 weeks; 75% percentile: 14 weeks) (Fig. 2).

The body temperature of the foals on the day of pneumonia diagnosis ranged from 37.9 to 40.9°C. The median was 38.6°C (25% percentile: 38.4°C; 75% percentile: 38.8°C). Only 13 sick foals had an elevated body temperature (39.0–40.9°C) on the day of pneumonia diagnosis (Fig. 3).

The clinical respiratory findings were physiologic in the majority (78/86) of the foals (clinical score: 0–2) on the day of pneumonia diagnosis (Fig. 4). Seven foals showed mild (clinical score: 3–4) and one foal moderate clinical signs (clinical score: 5–6) (Fig. 4).

The WBC count of sick foals on the day of pneumonia diagnosis had its minimum at 5,600 cells/ L and its maximum at 21,100 cells/ L. A total of 50 foals out of the 86 examined had a WBC count below 13,000 cells/ L on the day of diagnosis (Fig. 5). A leucocytosis on the day of pneumonia diagnosis, however, was shown in 36 foals with WBC concentrations above 13,000 cells/ L.

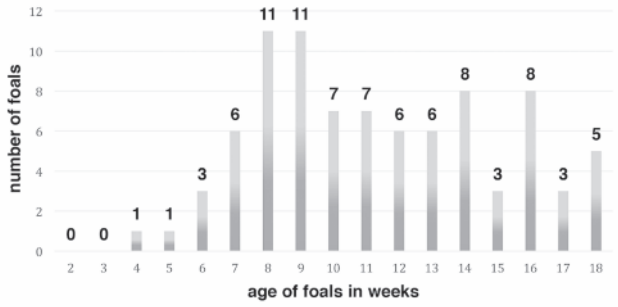

Fig. 2 Age of foals on the day of pneumonia diagnosis in weeks (n = 86) | *Alter der Fohlen in Wochen am Tag der Diagnose „Pneumonie" (n = 86)*

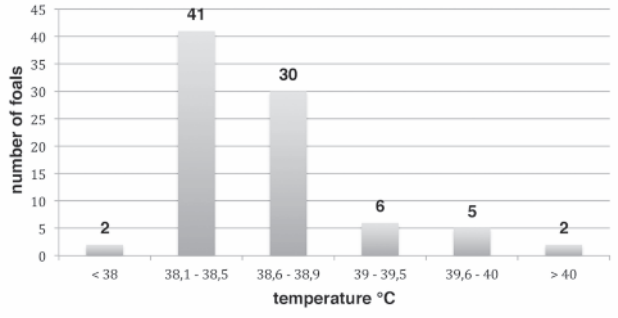

Fig. 3 Body temperature of foals on the day of pneumonia diagnosis (n = 86) | *Körperinnentemperatur der Fohlen am Tag der Diagnose „Pneumonie" (n = 86)*

Healthy and sick foals (n = 94) were included in the further analysis to evaluate the sensitivity and specificity of different WBC count cut-off values. The ROC curve (Fig. 9) plots the sensitivities and specificities for different WBC count cut-off' values for the early detection of pneumonia (Tab. 1). With an increase in the cut-off value, the specificity increases and the sensitivity decreases. The Youden Index (Fig. 6), which is described as the optimal WBC count cut-off value on the day of diagnosis, is at 10,200 cells/ L with a sensitivity of 76% and a specificity of 45% (Tab. 1).

The abscess score ranged from 1 to 23 cm on the day of first detection of pulmonary lesions (Fig. 7). The median was 2 cm (25% percentile: 2 cm; 75% percentile: 3 cm).

The results of the correlation between the clinical score, temperature and WBC count in association with the first sonographically visible pneumonia in the lung of foals are described in the following paragraph (Tab. 2). Thirty-nine foals showed neither clinical nor haematological findings on the day of pneumonia diagnosis. Only one foal had clinical signs, hyperthermia and haematological findings on the day of diagnosis (clinical score: ≥ 3, temperature ≥ 39°C, WBC

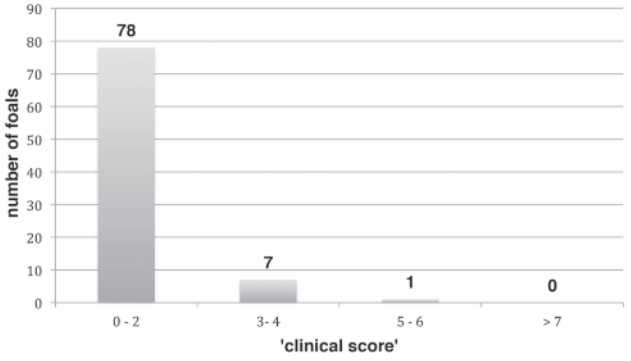

Fig. 4 Clinical score of foals on the day of pneumonia diagnosis (n = 86) | *„Klinischer Score" der Fohlen am Tag der Diagnose „Pneumonie" (n = 86)*

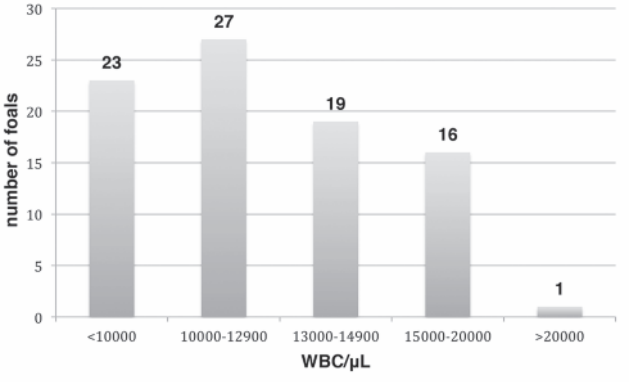

Fig. 5 White blood cell (WBC) count of the foals on the day of pneumonia diagnosis (n = 86) | *Anzahl an Blutleukozyten der Fohlen am Tag der Diagnose „Pneumonie" (n = 86)*

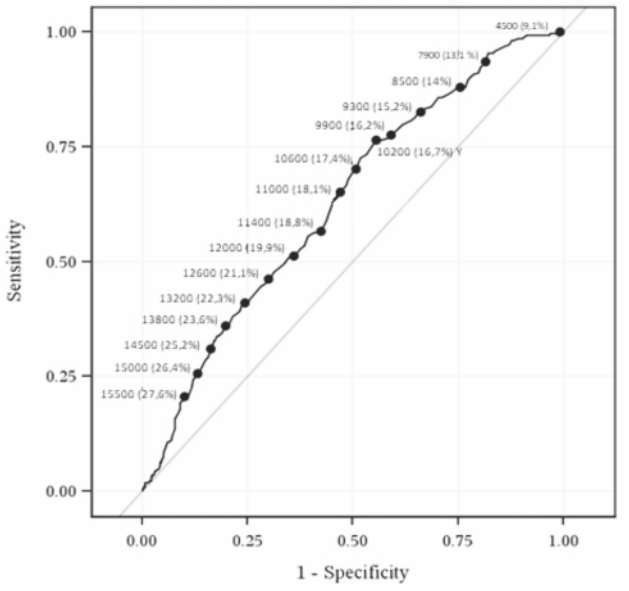

Fig. 6 Receiving operating characteristics (ROC) curve labelled with various WBC count cut-off values for the early diagnostic of pneumonia in foals. The value in brackets states the probability of the individual WBC count cut-off value to diagnose pulmonary abscesses | *Receiving operating characteristics (ROC) Kurve gekennzeichnet mit verschiedenen Grenzwerten der Anzahl an Blutleukozyten, zum Zweck der Diagnose „pulmonaler Abszesse" in Fohlen. Der Wert in Klammern gibt die Wahrscheinlichkeit des Vorliegens von Abszessen für die individuellen Grenzwerte der Anzahl an Blutleukozyten an*

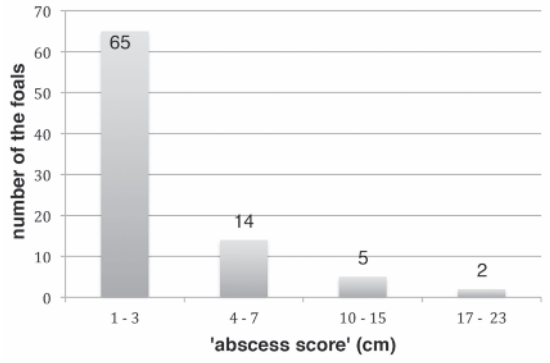

Fig. 7 Abscess score of the foals on the day of pneumonia diagnosis in cm (n = 86) | *„Abszess-Score" der Fohlen am Tag der Diagnose „Pneumonie" in cm (n = 86)*

≥13,000 cells/ L). Twenty-seven foals showed leucocytosis (WBC≥13,000 cells/ L) as the only finding on the day of diagnosis (Tab. 2).

A total of 20 of the 86 sick foals were defined as clinically sick based the clinical signs and/or hyperthermia (Tab. 2). The sensitivity of clinically sick foals was only 45% and the specificity was 59%. The WBC count cut-off value ≥13,000 cells/ L has a sensitivity of 45% and a specificity of 59% for the diagnosis of clinically apparent pneumonia in foals.

Discussion

The aim of the study was to monitor foals in order to evaluate a screening program set up for an early diagnosis of pneumonia in foals on a stud with a history of endemic pneumonia.

The screening methods used most frequently include clinical examinations, WBC count measurements, haematology and finally, diagnostic imaging (*Giguère* et al. 2003, *Chaffin* et al. 2013). Although the isolation of Strep. zoo. or R. equi in airway samples of a patient would support the diagnosis of Strep. zoo. or R. equi pneumonia, neither bacterial culture nor PCR of airway samples from patients can be considered as a 'golden standard' for verifying Strep. zoo. or R. equi, as earlier studies have shown (*Lavoie* et al. 1994, *Weimar* 2006, *Venner* et

Tab. 1 Sensitivity, specificity and probability of selected WBC count cut-off values for the early detection of pneumonia in foals | *Sensitivität, Spezifität und die Wahrscheinlichkeit von ausgewählten Grenzwerten der Anzahl an Blutleukozyten zur Diagnose der Pneumonie beim Fohlen*

Cut-off value (WBC/µL)	Sensitivity (%)	Specificity (%)	Probability (%)
5000	99.6	1.49	9.64
7000	98.0	11.5	12.0
9000	85.7	29.8	14.8
10200	76.3	44.5	16.7
13000	42.2	73.9	21.9
15000	25.6	86.9	26.4
17000	9.30	94.6	31.3
21000	1.94	98.6	42.8

Tab. 2 One-way frequency table showing correlations between clinical score, WBC count, temperature and abscess score on the day of pneumonia diagnosis. The screening parameters are coded with '0' and '1', where '0' stands for 'No' and '1' stands for 'Yes'. | *Die „one-way-frequency-Tabelle" zeigt Korrelationen zwischen dem „klinischen Score", der WBC Konzentration, der Körpertemperatur und dem „Abszess Score" am Tag der Diagnose „Pneumonie". Die Parameter sind codiert mit den Zahlen 0 und 1, wobei 0 „trifft nicht zu" und 1 „trifft zu" angibt.*

clinical score ≥ 3	WBC ≥ 13,000 cells/□L	Temperature ≥ 39.0 °C	abscess score > 0 cm	Frequency n = 86
0	0	0	1	39
0	1	0	1	27
1	0	0	1	4
1	1	0	1	3
0	0	1	1	7
0	1	1	1	5
1	1	1	1	1

al. 2007). R. equi was only identified in the airway secretions of one from four foals with pneumonia diagnosed post-mortem and R. equi growth in the infected lung lesion (*Weimar* 2006). Additionally, Strep. zoo. was isolated in the airway secretions of only two from five foals with a post mortem diagnosed pneumonia and Strep. zoo. growth in the infected lung parenchyma (*Weimar* 2006). Consequently, the authors decided not to include the Strep. zoo. or R. equi isolation of airway secretion in infected foals as a screening method in the present investigation, because of its low sensitivity.

Radiography was validated as a diagnostic tool for the identification of consolidations in the lung of foals, over 30 years ago (*Falcon* et al. 1985). Subsequently, a comparative study between radiography and sonography showed that sonography is even more sensitive than radiography, as 90 abscesses were diagnosed by sonographic examination in 42 foals and only 27 abscesses were found on the chest radiographs in 20 of the 42 sick foals (*Venner* et al. 2014). The higher sensitivity of the ultrasonographic examination is also shown in the results of Ramirez et al. (2004), where 16 out of 17 foals presented consolidations at sonography and pulmonary abscesses were diagnosed in only 13 out of 17 foals.

As ultrasonography only detects consolidations adjacent to the pleura (*Reef* 2004), abscesses in the deeper lung tissue cannot be diagnosed with this method, but might be by radiology. A further comparative investigation, however, showed that the areas overlying the diaphragm and the heart silhouette on chest radiographs are difficult to evaluate due to overlaps (*Venner* et al. 2014). In conclusion, the ultrasonographic examination seemed to identify more pulmonary consolidations than radiology. This is the reason why the ultrasonographic examination of the thorax was chosen to identify pneumonia in foals in the present study. With this diagnostic tool, many foals (86/101) that developed pneumonia between the 2nd and the 18th week of life were detected in the present investigation.

Clinical signs such as coughing, increased respiratory rate, rattles or wheezes on the lung are commonly described as clinical signs of pneumonia (*Zink* et al. 1986, *Prescott* et al. 1989). But these typical clinical signs appear late in the course of pneumonia in foals (*Giguère* and *Prescott* 1997, *Althaus* 2004, *McCracken* and *Slovis* 2009, *Venner* 2009). This is as well supported by our results, which show that only 8 out of 86 foals with pneumonia at sonography were clinically sick (clinical score above 2). Therefore, clinical signs alone are not reliable for the early detection of foals with pneumonia.

Considering the value of hyperthermia as a diagnostic tool, it was mentioned to correlate with pneumonia in foals, especially caused by R. equi (*Zink* et al. 1986). In the present study, however, only 13 out of the 86 foals with pneumonia showed hyperthermia (≥39 °C). These results show no correlation between temperature and first pulmonary consolidations. Daily measurements should be evaluated and might show to improve the sensitivity of the temperature as a screening parameter.

Another important and well-evaluated screening parameter is the monitoring of the WBC count. In the present study, the weekly WBC measurements allowed the early detection of variations in WBC concentrations. More than half of the sick foals (50/86) had a WBC concentration below 13,000 cells/μL, when showing first pulmonary consolidations on the ultrasound. Consequently, the diagnostic performance of the WBC count cut-off value ≥13,000 cells/μL for the early diagnostic of pneumonia in foals is low (sensitivity: 42%, specificity: 74%) in the present study. However, *Giguère* et al. (2003) described a high diagnostic value of a WBC count cut-off value greater than 13,000 cells/μL (sensitivity: 95%, specificity: 61%) for the diagnosis of severe pneumonia. These results suggest that the WBC concentration is clearly helpful for the detection of pneumonia in clinically sick foals. Compared to the present study, in which the sonographic examination of the lung was used as evidence for pneumonia, *Giguère* et al. (2003) only focused on clinical signs, such as cough, nasal discharge, tachypnea, fever and auscultatory findings as diagnostic for pneumonia. Therefore, the foals in this study were more severely sick than those in our study. This explains, why sensitivity and specificity of WBC concentrations in *Giguère* et al. (2003) were higher than in the present investigation. If we consider the results of the present study, only 9 foals showed a leucocytosis in combination with clinical signs and hyperthermia and further 27 foals had a leucocytosis as the only finding on the day of diagnosis pneumonia. Consequently, the WBC count cut-off value ≥13,000 cells/μL for diagnosing clinically sick foals has a sensitivity of 45% and a specificity of 59% in our study, which indicates a low diagnostic value of leucocytosis in detecting foals with clinically apparent pneumonia in the early course of the disease. The results show that most of the foals developed subclinical pneumonia, which is characterised by pulmonary consolidations at sonography of the thorax, without any clinical or haematological abnormalities in the early stage of the disease.

Conclusion

Parameters such as clinical signs, body temperature or the WBC count do not seem to provide reliable results for an early detection of pneumonia in foals. Furthermore, even the combination of these screening methods was not sensitive enough to detect the early stages of pneumonia in foals. The ultrasonographic examination, however, enabled the examiners to detect the early stages of pulmonary disorders in foals suffering from pneumonia. Although the sensitivity of the ultrasonographic examination has been very high in previous and the present investigation, there are still limitations to this method, such as the lack of ability to detect consolidations in the deeper lung tissue. However, deep lung lesions can either regress by self-healing or increase in volume and appear at the lung periphery in the following weeks. The lesions can therefore still be diagnosed in an early stage of pneumonia, far before a severe stage of the disease (data on kinetic of sonographical findings not published yet). Therefore, the authors believe that a monitoring on a weekly basis by means of sonography provides the highest sensitivity for detecting pneumonia in foals.

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