Sepsis and bacterial suppurative meningitis-meningoencephalitis in critically ill neonatal Piedmontese calves: Clinical approach and laboratory findings

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Summary

Sepsis (S) and bacterial suppurative meningitis-meningoencephalitis (M-ME) are common causes of death in bovine neonates. The aim of this prospective study was to evaluate the prevalence of S and M-ME in critically ill neonatal Piedmontese calves. Critically ill animals up to 15 days old referred by practitioners were registered according to their status and subsequently assigned to clinical standardized score. Calves with a clinical score ≥ 5 were further assessed under a clinical and clinical-pathological protocol to strengthen the suspicion of S and M-ME. Critically ill neonatal calves sent for necropsy were included in the study as well. Fifty-nine calves were investigated, 26 of which referred alive and 33 dead. Ten out of the 26 clinically evaluated calves were classified as suspicious of S on the basis of the clinical and clinical-pathological protocols. S was confirmed by positive bacteriologic culture in 7 cases and in 3 cases on the basis of necroptic lesions. Concomitant suppurative M-ME suspected in 6 of these 10 calves was subsequently confirmed by CSF analysis or histological findings. Of the 33 calves examined only post-mortem, 20 showed pathognomonic findings of S and 14 signs of M-ME. The prevalence of S and M-ME was 46 and 36 %, respectively. Clinical signs of S were confirmed to be vague and overlapping with other diseases. The developed protocol was highly accurate in predicting S in these neonatal calves.

Sepsis und bakterielle eitrige Meningitis-Meningoencephalitis bei kritisch erkrankten neugeborenen Kälbern der Piemonteser Rasse: Klinische Studie und Ergebnisse der Laboruntersuchungen

Sepsis (S) und bakterielle eitrige Meningitis-Meningoencephalitis (M-ME) sind häufige Ursachen, die zum Tod von neugeborenen Kälbern führen. Ziel der Untersuchung war es, die Prävalenz von S und M-ME bei kritisch erkrankten neugeborenen Kälbern der Piemonteser Rasse zu evaluieren. Zu diesem Zweck wurden kritisch erkrankte Tiere im Alter von bis zu 15 Tagen von Tierärzten untersucht und anschliessend auf der Grundlage eines standardisierten klinischen Protokolls ausgewertet. Kälber mit einem klinischen Wert ≥ 5 wurden einem klinischen und klinisch-pathologischem Protokoll unterzogen. Mitberücksichtigt wurden auch kritisch erkrankte neugeborene Kälber, die einer Nekroskopie unterzogen wurden. Insgesamt wurden 59 Kälber untersucht, darunter 26 lebende und 33 tote Tiere. 10 der 26 klinisch erkrankten Kälber wurden anhand der klinischen und klinisch-pathologischen Protokolle als S-verdächtig eingestuft. S wurde in 7 Fällen anhand von positiven bakteriologischen Kulturen und in 3 Fällen anhand von typischen Läsionen diagnostiziert. Gleichzeitig auftretende eitrige M-ME, wurde bei 6 dieser 10 Kälber durch Liquor-Analysen oder histologische Befunde im Nachhinein bestätigt. Von 33 Kälbern, die ausschliesslich post-mortem untersucht wurden, zeigten 20 charakteristische Befunde von S und 14 Anzeichen von M-ME. Die Prävalenz von S betrug 46 %; die von M-ME lag bei 36 %. Klinische Anzeichen von S waren nicht eindeutig und überlappten sich mit anderen Krankheiten. Das erstellte Protokoll war zur

Keywords: bovine, neonatal diseases, sepsis, meningitis-meningoencephalitis, clinical signs

Identifikation von S bei den neugeborenen Kälbern ausgesprochen präzise.

Schlüsselwörter: Kalb, Krankheiten bei Neugeborenen, Sepsis, Meningitis-Meningoencephalitis, klinische Symptome

Introduction

Sepsis (S), a major health problem in calves less than 2 weeks of age associated with a high mortality rate (Aldridge et al., 1993; Fecteau et al., 2009), is described as the systemic inflammatory response syndrome (SIRS) to an active infectious process (Bone et al., 1992; Levy et al., 2003; Goldstein et al., 2005). Decreased or failed passive transfer (FPT) of immunity and exposure to virulent pathogens are important risk factors for the development of S. The most frequent etiological agent is *Escherichia coli* (Lofstedt et al., 1999; House and Gunn, 2009; Fecteau et al., 2009), followed by *Salmonella* spp., *Campylobacter* spp., *Klebsiella* spp., *Staphylococcus* spp., and *Streptococcus* spp. (Seimiya et al., 1992; Fecteau et al., 2009). S usually involves multiple organs and most commonly affects the respiratory and gastrointestinal systems with rapid and often fatal progression of disease. Early clinical signs are vague and nonspecific (Fecteau et al., 2009; Vaala et al., 2009) and laboratory values may be altered (Lofstedt et al., 1999; Irmak et al., 2006). Since blood culture has a low sensitivity and results are available only after 24 – 72 h, scoring schemes have been developed for several species to help identify septic subjects early in the course of the disease and to improve favorable outcome of therapy (Brewer and Koterba, 1988; Fecteau et al., 1997a; Lofstedt et al., 1999; Moore et al., 2009).
Neonatal bacterial suppurative meningitis-meningoencephalitis (M-ME) is commonly a result of S (Fecteau and George, 2004; House and Gunn, 2009). Bacteria can spread hematogenously to the leptomeninges, although the mechanism of pathogen entry is not clearly understood (Kim, 2003; Zachary, 2007; Fecteau et al., 2009). The definitive diagnosis relies on abnormal findings of cerebrospinal fluid (CSF) which, in acute stages, shows elevated neutrophils and increased protein content and/or intracellular bacteria (Green and Smith, 1992; Scott and Penny, 1993; Fecteau et al., 2009; Mayhew, 2009a; Stokol et al., 2009). The aim of this prospective study was to evaluate and refine a protocol for diagnosis of S and bacterial M-ME in young Piedmontese calves. The proportion of S and bacterial M-ME was also investigated in the referred population.

Animals, Material and Methods

Animals

A total of 59 critically ill Piedmontese calves up to 15 days referred live or dead to the Teaching Hospital of the Faculty of Veterinary Medicine of Turin, between February 2008 and March 2010, were used for our study.
All animals underwent complete physical and neurologic examination. A total clinical score, based on the classification scheme of Fecteau et al. (1997a), made up of five individual scores (attitude, hydration, feces, navel and sclera vessels), was assigned to each animal. Detailed anamnestic information of the dam and the calf were collected.

Definition of diseases

Calves were classified as suspicious of S if the clinical score was ≥ 5 and met at least one of the following criteria: presence of a focal infection site other than enteritis; serum glucose < 3.3 mmol/L; blood smear suggestive of S (left shift of neutrophils and signs of toxicity in neutrophils). S was confirmed by positive blood culture (*in vivo*) and/or bacterial isolation from tissue samples (*postmortem*), along with evidence of macro- and microscopic lesions strongly suggestive of S.
Calves were considered suspicious of M-ME if the neurologic examination was suggestive of an intracranial localization and CSF total cell count showed pleocytosis (> 10 cells/μL). M-ME was confirmed when the CSF analysis showed a neutrophilic pleocytosis and increased proteins (> 40 mg/dL) and/or when histological findings were indicative of the disease (Zachary, 2007).
Calves with a clinical score < 5 were managed with specific investigations to diagnose the underlying disease. Calves with a clinical score ≥ 5 were included in the protocol described below. For critically ill Piedmontese calves up to 15 days of age sent for necropsy, a standardized data collection form was compiled by interviewing the referring veterinarian. A control group of 20 healthy Piedmontese calves, 1 – 15 days old, was included in the study. All animals were examined simultaneously by a board certified person in neurology and bovine health management (ADA).
The study was performed according to ethical recommendations, animal welfare considerations and regula-

tions and approved by the Health Direction Section of Piedmont Region (reference number 466/2008).

Protocol for assessment of calves with a score ≥ 5

Blood for hematological and serum analyses was collected in EDTA-containing and plain tubes, respectively. Samples for blood gas analysis (pH, bicarbonate, Be$_{ecf}$), electrolytes (Na$^+$, Cl$^-$, iCa^{2+}, K$^+$), glucose, hemoglobin (Hb) concentrations and hematocrit (Hct) were anaerobically collected in a 2.5-mL heparinized plastic syringe (Preza-Pak II, Terumo) and immediately determined (i-STAT1 Analyzer, i-STAT Corporation). Blood samples for Celite Activate Clotting Time measurement (^{CELITE}ACT, Abbot Point of Care Inc) were drawn into a plastic syringe and immediately dispensed into the sample well of a cartridge. For blood culture, a volume of 10 mL of blood was aseptically collected, added to a proper broth (Signal Blood Culture System, Oxoid Limited) and submitted for bacteria culturing and isolation.

A CSF sample was collected from the lumbosacral site from all calves showing neurologic signs, and stored at 4 °C. Total nucleated cell count was assessed in the field using a Nageotte hemacytometer. For CSF differential cell count and total microprotein concentration, samples were analyzed within 1 h of collection in the lab. Blood culture and organism identification were performed using standard procedures.

Hematology (ADVIA120 Hematology System, Siemens Diagnostics), blood chemistry analysis including ALP, AST, CK, BUN, CREA, total bilirubin, P, and Mg (ILab 300 Plus, Instrumentation Laboratories), and standard clotting profile composed of aPTT, PT and fibrinogen (Start 4 Hemostasis Analyzer, Diagnostica Stago) were performed in the lab. For left shift of neutrophils and toxicity, a blood smear was stained with May-Grünwald Giemsa and examined. Passive transfer of immunity was indirectly estimated in total serum solids by a temperature compensated hand-held refractometer (Leica VET 360 Total Solids Refractometer, Leica Inc.).

Post-mortem examination and bacteriological isolation

Calves that did not survive or were humanely euthanized were sent for post-mortem examination.

Complete necropsy was performed. The internal organs and brain of all animals were collected and routinely processed for histopathological examination. Aerobic and anaerobic culture of tissue samples was performed.

Statistical analysis

Statistical analysis was performed using a freeware statistical software package (R 1.12.2; http://www.r-project.org/). Normally distributed data were tested using the Shapiro-Wilk Normality Test. The analytical parameters

(5th – 95th percentiles) of the control group were used as internal comparison data. The analytical parameters of the septic calves (median values) out of the 5th – 95th percentiles obtained from the control group were tested using the Wilcoxon signed-rank test. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the clinical and clinical-pathological methods used to suspect S were calculated. Results were considered significant if the *P*-value was less than 0.05.

Results

Fifty-nine critically ill calves (29 males and 30 females; mean age, 9.6 ± 5.4 days) were investigated, 26 (44 %) of which were clinically evaluated and 33 (56 %) were examined only post-mortem.

Critically ill calves

S was suspected in 10/26 (38 %) calves with a score ≥ 5 and confirmed in 7/10 animals by positive blood culture and/or tissue isolation. In the 3 remaining animals, macro- and microscopic lesions were strongly suggestive of S (Tab. 1). Analytical parameters between the septic and control groups are listed in Table 2. In 5 animals a failure of passive transfer of immunity was determined on the basis of decreased total serum solids (≤ 50 g/L). Table 3 shows the accuracy of clinical and clinical-pathological methods used to suspect S.

M-ME was clinically suspected in 6 but confirmed in 7/10 calves with a score ≥ 5 (Tab. 1). The median number of CSF nucleated cells in suspicious M-ME calves was 92.3 cells/µL (39.7 – 1840.0); the mean CSF protein concentration was 151.4 ± 122.8 mg/dL.

The 16 nonseptic animals (score < 5) included calves with neonatal diarrhea (n = 7), otitis media/interna (n = 3), spinal trauma (n = 2), acute perinatal asphyxia (n = 2), arthritis (n = 1) and abomasal ulceration (n = 1).

Critically ill reported dead calves

Twenty (61 %) out of the 33 dead calves were confirmed as septic on the basis of tissue bacterial isolation; 3 additional animals showed macro- and microscopic lesions strongly suggestive of S. Fourteen out of the 20 (70 %) septic calves showed concurrent histological features of M-ME. The frequency of the most common signs in the septic and M-ME animals is reported in Figure 1; some of the calves showed more than one sign.

Neonatal diarrhea (n = 8) and bronchopneumonia (n = 1) were diagnosed in 9 critically ill calves with negative tissue bacterial isolation but without gross and histopathological findings of S and/or M-ME. The cause of death in one other case was undetermined, but S and M-ME were excluded. *E. coli* accounted for 19 isolates (one of which yielded *E. coli* and *Streptococcus bovis*).

Table 1: Parameters for classifying critically ill septic calves with suspected S.

Case	Total score	Focal infection	Serum glucose < 3.3 mmol/L	Blood smear	Neurologic evaluation	CSF	Blood culture	Outcome	Necropsy/Tissue Isolation
		S suspicion criteria							
a	5	CNS[a]	No	+[b]	Altered mental status, wide base stance, proprioceptive deficits, trismus, seizures	npl[c]	E coli	Deceased or euthanized	S[d]/M-ME[e] E. coli
b*	5	Broncho pneumonia	No	+	Normal	X[f]	Coagulase-negative Staphylococci	Survived	
c*	6	CNS	No	-[g]	Altered mental status, opisthotonus, tetraparesis, proprioceptive deficits, ventral strabismus, positional nystagmus	npl	Negative	Deceased or euthanized	S/M-ME E. coli
d	5	Not detected	No	+	Normal	X	E. coli	Deceased or euthanized	Not performed
e*	6	Broncho pneumonia	No	+	Altered mental status, wide base stance, proprioceptive deficits	Normal	Negative	Deceased or euthanized	S/M-ME E. coli
f*	5	CNS	No	+	Altered mental status, wide base stance, opisthotonus, proprioceptive deficits, trismus, positional nystagmus	npl	Negative	Deceased or Euthanized	S/M-ME
g*	7	Uveitis and CNS	Yes	-	Altered mental status, circling, head pressing, wide base stance, tetraparesis, proprioceptive deficits, trismus	npl	E. coli	Deceased or Euthanized	S/M-ME E. coli
h	11	Uveitis and CNS	Yes	-	Altered mental status, tetraparesis, proprioceptive deficits, nystagmus	npl	Negative	Deceased or Euthanized	S/M-ME
i	5	Not detected	No	+	Normal	X	E. coli	Survived	
l	8	CNS	Yes	+	Altered mental status, tetraparesis, proprioceptive deficits	npl	Negative	Deceased or Euthanized	S/M-ME

*Calves pre-treated with antibiotics, [a] central nervous system, [b] blood smear suggestive of S, [c] neutrophilic pleocytosis, [d] pathological findings strongly suggestive of S, [e] pathological findings of M-ME, [f] not performed, [g] blood smear not suggestive of S.

Streptococcus pneumoniae was isolated in only one subject. The prevalence of S and M-ME in the critically ill calves was 46 % and 36 %, respectively.

Discussion

The reason to choose the Piedmontese breed was motivated by its economic relevance in Italy. Unweaned calves are very valuable and early diagnosis and treatment of neonatal infectious diseases should be mandatory for a favorable outcome and cost reduction. Among infectious diseases, S accounts for considerable morbidity and mortality in bovine neonates (Fecteau et al., 2009; Vaala et al., 2009). To the best of our knowledge, this is the first report to evaluate the proportion and clinical features of S and M-ME in Italy and in the Piedmontese Breed.

The high prevalence of S (46 %) in this study is similar to that (51 %) reported by Mosher et al. (1968) but higher than that found by other authors (Fecteau et al., 1997b; Lofstedt et al. 1999). Aldridge et al. (1993) reported an incidence of 26 % in dairy and beef critically ill calves considering criteria similar to those we applied in our study. Clinical signs of S in this study were nonspecific, confirming that early diagnosis remains a challenge for the veterinarian and that clinical signs are likely to be attributed to other diseases (Fecteau et al., 2009; Vaala et al., 2009).

Hematological and biochemical changes, as described in our study, are both a consequence of systemic inflammation and evidence of organ dysfunction; our observations are in line with previous data (Aldridge et al., 1993; Gerros et al., 1995; Lofstedt et al., 1999; Irmak et al., 2006). The CELITEACT test

Table 2: Analytical parameters of septic calves outside of reference data and significantly different from control animals.

Analytes	Units	Septic Calves		Reference data from control group (5th–95th percentiles)	P-value
		Median	Minimum-Maximum		
Red blood cells (RBC)	x 10^{12} /L	8.93	6.77–10.65	5.45–8.80	0.033
Total serum protein	g/L	45	40–72	50–71	0.003
Serum glucose	mmol/L	3.8	1.8–6.5	4.4–6.5	0.009
Urea	mmol/L	60.0	37.1–184.2	3.2–11.4	< 0.001
Creatinine	µmol/L	350	173–681	107–190	0.04
Alkaline phosphatase (ALP)	UI	157	84–314	184–458	0.005
Aspartate amino transferase (AST)	UI	84	27–182	19–65	0.003
Creatine kinase (CK)	UI	840	43–3460	41–284	< 0.001
Sodium	mmol/L	142	129–153	133–138	0.01
Platelets	x 103/µL	326	40–1000	485–1218	< 0.003
Activated clotting time (ACT)	s	237	114–304	112–190	0.002
Fibrinogen	g/L	7.8	3.9–16.0	2.7–5.4	< 0.01
Prothrombin time (PT)	s	38	26–48	18–25	< 0.001

Table 3: Accuracy of clinical and clinical-pathological methods used to suspect S (N = 26).

Criteria		95 % CI [a]
Sensitivity	100 %	59 %–100 %
Specificity	84.2 %	60.4 %–96.6 %
PPV [b]	70 %	34.8 %–93.3 %
NPV [c]	100 %	79.4 %–100 %

[a] Denotes 95 % confidence interval, [b] positive predictive value, [c] negative predictive value.
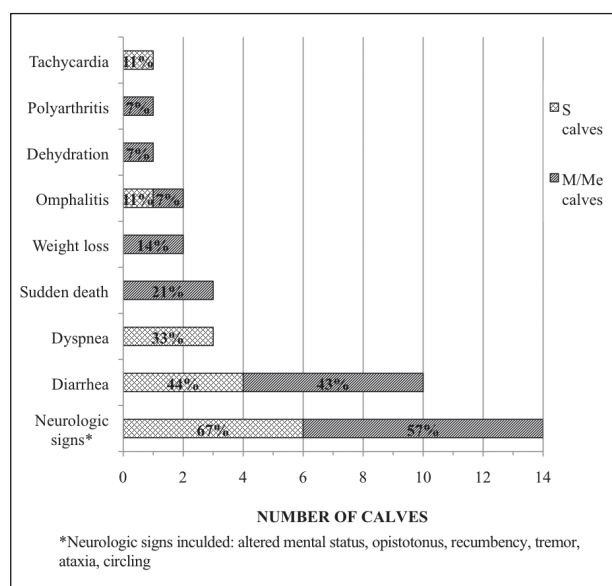

Figure 1: Frequency of the most common signs in the referred dead calves with S and M-ME.

measures the time required for complete activation of the coagulation cascade (Hattersley, 1966). The CELITEACT reference values of the present study were comparable to those of a previous one (Riley and Lassen, 1979). In the present report, the CELITEACT was used assuming that the bleeding disorders resulting from S could be identified in the field. Significant differences between septic and healthy animals were found; however, more extensive research of critically ill calves is required to validate the clinical reliability of this test.

Calves with FTP have a high risk for developing neonatal S and subsequent M-ME (Fecteau et al., 2009). In the present study, 5 septic live animals showed a FPT on the basis of low total serum protein concentration, which may have been underestimated since dehydration may have contributed to an individual increase in total protein (Constable et al., 1998). On the other hand, as it is customary for the Piedmontese breed to leave calves uncontrolled with the dam and allow them to suckle naturally, erratic nursing attempts in contaminated areas (tail, hock) may have exposed the calves to a massive pathogen load, regardless of their immune status.

Escherichia coli was confirmed to be the most frequent agent isolated from blood and tissue cultures from septic calves (Raska et al., 1978; Hariharan et al., 1992; Aldridge et al., 1993; Fecteau et al., 1997b; Lofstedt et al., 1999). *Streptococcus* spp. and *Staphylococcus* spp. cultured in 2 calves and one calf, respectively, have rarely been reported as a cause of S and M-ME (Seimiya et al., 1992; Fecteau et al., 1997b; Lofstedt et al., 1999). In 6 critically ill calves, although S was strongly suspected at necropsy, bacteriological culture of tissue specimens was negative. This could be related to prior antibiotic therapy, number of bacteria, and course of the disease.

The clinical and clinical-pathological protocols developed in this study showed a sensitivity and specificity superior to those previously described (Fecteau et al., 1997a; Lofstedt et al., 1999). Since a score of 5 or more can be achieved with diseases other than S (i.e. severe acidosis and dehydration), the score was modified by also using the presence of hypoglycemia and/or alterations in neutrophils. With the use of these protocols we were able to identify septic calves earlier than before and to exclude the disease in a large number of animals thus limiting costly antimicrobial and supportive therapies. Furthermore, the sensitivity value of the protocols (lower 95% confidence interval: 59%) was probably underestimated due to the 3 calves in which bacteriological isolation was negative. The fact that all 3 had M-ME also reinforces the suspicion that they could actually have had S. A limitation to this study is the small sample size on which we were able to apply the clinical-laboratory protocols. Their use on a larger population of critically ill calves is needed to confirm the promising results.

M-ME is commonly secondary to S (Green and Smith, 1992; Fecteau et al., 2009). The overall prevalence of M-ME in the present study (36%) is similar to that (43%) reported by Mosher et al. (1968) in 103 necropsied calves. In other studies (Green and Smith, 1992; Stokol et al. 2009)), the prevalence was 2% and 18%, respectively. All animals with M-ME had concomitant S. The clinical signs in the 7 calves that underwent a complete neurological examination were only partly similar to those previously described. No hyperesthesia or exaggerated spinal reflexes were observed (Green and Smith, 1992; Scott and Penny, 1993; Fecteau and George, 2004; Fecteau et al., 2009). Trismus – detected in 3 animals – has rarely been reported (Jamison and Prescott, 1987), while gait abnormalities, proprioceptive and cranial nerves deficits are a confirmation of neuroparenchymal involvement. Neurologic signs, reported by the referring veterinarian in septic calves without M-ME lesions could be related to extreme terminal weakness, electrolyte and acid-base disturbances, hypoglycemia, hypothermia and pain (Scott and Penny, 1993; House and Gunn, 2009; Moore, 2009). A neutrophilic pleocytosis was detected in all except one calf with clinical signs of M-ME in which CNS involvement was confirmed at necropsy. A previous steroid therapy could explain the normal CSF in this case.

In conclusion, failure of passive antibody transfer remains an important risk factor for neonatal S and subsequent M-ME. Clinical signs of M-ME were mostly indicative of brain parenchymal involvement. Clinical signs of S were confirmed to be vague and overlapping with other diseases. The in field application of the clinical and clinical-pathological protocols was found to be highly sensitive and specific in predicting S in these neonate calves.

Acknowledgements

Septicémie et méningite/méningo-encéphalite bactérienne purulente chez des veaux nouveau-nés gravement malades de race piémontaise : étude clinique et résultats des examens de laboratoires

La septicémie (S) et la méningite/méningo-encéphalite bactérienne purulente (M-ME) sont des causes fréquentes de morts chez les veaux nouveau-nés. Le but du présent travail était d'évaluer la prévalence de S et de M-ME chez des veaux nouveau-nés de la race piémontaise gravement malades. Pour cela, les animaux gravement malades âgés jusqu'à 15 jours ont été examiné par les vétérinaires puis évalués sur la base d'un protocole clinique standardisé. Les veaux avec une valeur clinique ≥ que 5 ont été soumis à un protocole clinique et clinico-pathologique. On a également tenu compte des veaux gravement atteints qui ont été autopsié. Au total, ce sont 59 veaux qui ont été examinés donc 26 vivants et 33 morts. 10 des 26 animaux cliniquement atteints, ont été, sur la base du protocole, considérés comme suspect de septicémie.

Sepsi e meningite-meningoencefalite batterica purulenta nei vitelli neonati della razza piemontese gravemente malati: studio clinico e risultati delle prove di laboratorio

Sepsi (S) e meningite-meningoencefalite (M-ME) batterica purulenta, sono le cause più frequenti che conducono i vitelli appena nati al decesso. Lo scopo di questo studio è di valutare la prevalenza di S e M-ME nei vitelli appena nati in condizioni critiche della razza piemontese. A questo scopo sono stati esaminati da veterinari, animali gravemente malati di età fino ai 15 giorni e infine valutati sulla base di un protocollo clinico standardizzato. I vitelli che ottenevano un punteggio clinico ≥ 5 sono stati sottoposti ad un protocollo clinico e clinico-patologico. Sono stati pure presi in considerazione vitelli appena nati, malati gravemente, riferiti morti. In totale sono stati esaminati 59 vitelli di cui 26 vivi e 33 morti. Sulla base del protocollo clinico e clinico-patologico sono stati classificati come sospetti di S, 10 dei 26 vitelli malati gravemente. Sono stati diagnosticati come S, 7 casi che presenta-

Une septicémie a été diagnostiquée chez 7 d'entre eux sur la base de culture biologique positives et chez 3 sur la base de lésions typiques. L'apparition simultanée d'une M-ME a été confirmée chez 10 veaux par analyse du LCR ou par histologie par la suite. Des 33 veaux qui ont été examinés uniquement post-mortem, 20 présentaient des signes caractéristiques de septicémie et 14 des signes de M-ME. La prévalence des septicémie s'élevait à 46 %, celle des M-ME à 36 %. Les signes clinique de septicémie n'étaient pas évidents et se confondaient avec d'autres affections. Le protocole créé était extrêmement précis pour identifié une septicémie chez les veaunouveau-nés.

vano culture batteriologicamente positive e 3 casi sulla base di lesioni tipiche. Contemporaneamente in 6 su10 vitelli è stata confermata, da un'analisi del liquor o da reperto istologico a posteriori, una M-ME purulenta. Dei 33 vitelli, esaminati solamente in post-mortem, 20 presentavano risultati caratteristici di S e 14 particolarità di M-ME. La prevalenza di S era del 46 % mentre quella per M-ME era del 36 %. Segni clinici di S non erano specifici oppure patognomonici e si sovrapponevano ad altre malattie. Il protocollo creato era rigorosamente preciso per identificare una S nei vitelli appena nati.

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