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Factors Affecting the Success Rate of Treatment of Recumbent Dairy Cows Suffering from Hypocalcaemia

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With 10 tables

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Summary

We aimed to investigate the ratio of accompanying diseases in cows suffering from clinical hypocalcaemia and their influence on cure rate. In five veterinary practices in different regions of Germany, all recumbent cows around parturition were included in the study for a period of 1 year. After recording the case history a clinical examination was done and a serum sample was taken to measure the concentrations of calcium and phosphorus magnesium, β-hydroxybutyrate, total bilirubin, cholesterol, urea, and the activities of ASAT, CK, and GLDH. Only cows with hypocalcaemia entered the statistical analysis. Hypocalcaemia was the major cause of recumbency in cows of the second lactation or elder. Muscle damage was the second frequent diagnose in recumbent cows and the major concomitant disease in hypocalcaemic cows. The overall cure rate was between 89.4% and 94.8%. Calcium and phosphorus concentrations did not have an influence on cure rate. Non-cured cows had higher serum activities of CK ($p < 0.043$) and ASAT ($p < 0.006$). Nevertheless, the activities of CK and ASAT were no good predictors of treatment failure because of their low specificity and the high cure rate of the cows in the five practices.

Introduction

Hypocalcaemia is still the major cause of dairy cows becoming recumbent in the peripartal period (Shpigel et al., 2003; Gelfert et al., 2004, 2005). The onset of lactation increases the calcium requirement (Goff and Horst, 1997). If the mobilization of calcium from the bones and intestine fails to compensate for the loss of calcium in the milk, the plasma calcium concentrations will become too low to be able to support nerve and muscle function (Goff and Horst, 1997; Goff, 2004). It is still recommended to treat such animals with an intravenous infusion of organic calcium solutions (Staufenbiel, 1999; Martig, 2002). However, prior to the treatment of a recumbent cow, a detailed clinical examination must be made in order to exclude other possible causes of a recumbent cow. The main differential diagnoses vary from disorders of the musculoskeletal system (traumatic injuries, bone fracture), neural disorders (e.g. obturator paralysis) to metabolic diseases due to mineral deficiencies (e.g. hypomagnesaemia, hypocalcaemia), energy deficiency, or hepatic failure (Dirksen, 2002). Other possible differential diagnoses are toxic metritis, toxic mastitis, ileus, and shock due to the rupturing of the intestine, abomasal ulcer, or generalized peritonitis (Dirksen, 2002; Hunt and Blackwelder, 2002). Depending on the occurrence of a disturbed sensorium or the disturbed general health of the parturient cows, the possible differential diagnoses vary. In the field, the possibilities of a detailed clinical examination are often limited, in which the symptoms are either not clearly visible or non-specific (Bostedt et al., 1979; Stolla et al., 2000). Metzner and Klee (2005) showed that clinical signs are not specific for a particular mineral deficiency (e.g. calcium or phosphorus). They also found that the classical symptom of hypocalcaemia, the disturbed sensorium, was found less severe than in previous studies (Malz and Meyer, 1992). Therefore, a given uncertainty remains after the clinical examination, in which the examining veterinarian must choose the treatment with the highest probability of success.

Recent papers have reported a decreased success rate after the first treatment of the standard therapy (Meschke, 1997; Abele and Wolf, 2000; Roesch, 2000; Stolla et al., 2000). Some of them assume that the increased prevalence of recumbent cows suffering from hypophosphataemia may contribute to the impaired success rate (Abele and Wolf, 2000; Stolla et al., 2000). Stolla et al. (2000) also assumed that the high prevalence of recumbent cows with increased creatinkinase (CK) activity may contribute to the decreased success of the first therapy, but he did not prove it. Other authors found significant differences in hypocalcaemic cows with different treatment outcomes (Gelfert et al., 2004). Unsuccessfully treated cows had higher activities of CK and aspartate-amino-transferase (ASAT) than the cured ones.

It is still unknown whether such factors accompanying the hypocalcaemia have an impact on the curing rate of recumbent dairy cows suffering from hypocalcaemia.

Therefore, this multicentre study was initiated in order to analyse the curing rate of recumbent cows suffering from low calcium levels, and to find any possible factors constraining the healing process. The focus in the present study was on the peripartal period as the major period of recumbency and hypocalcaemia as the major cause of paresis at that time (Gelfert et al., 2005).

Materials and Methods

This multicentre study was realized in five veterinary practices in different regions of Germany. In the four practices in the northern parts of Germany (B–E) mostly Holstein–Frisian (HF)-cows were found, whereas in practice A in southern Germany, two-third of the cows were Simmental (FV), and one-third were HF-cows. In each practice, all dairy cows that became recumbent in the peripartal period (1 day a.p.–2 days p.p.) within 1 year were clinically examined (Dirksen, 2002; Baumgartner, 2005). If the case history and results of the clinical examinations resulted in the diagnosis 'hypocalcaemia', the cow entered the study and underwent the same procedure. The peripartal period was chosen to increase the probability of recumbency due to hypocalcaemia. After taking the case history, the animals were treated. Each cow was treated with an organic calcium solution (Table 1) as calciumborogluconate (Hunt and Blackwelder, 2002; Martig, 2002). Other drugs, such as glucose or dexamethason were added to this standardized therapy by the veterinarian due to his experience. It was ensured that these additional drugs were administered in the practice to all the cows included in our study. Additional calcium solutions were disallowed. Before each therapy, a serum sample was taken. In the serum, the concentrations of calcium, phosphorus, magnesium, β-hydroxybutyrate, total bilirubin, cholesterol, urea, and the activities of ASAT, CK, and glutamate-dehydrogenase (GLDH) were measured. Due to the study conditions, it was not possible to measure any indicator of acute inflammation. However, the careful clinical examination did not give any indication for the occurrence of the main acute inflammation, such as mastitis or endometritis. Therefore, the possible impact of any inflammation on the curing process could be neglected in our study. Based on the results of the laboratory analysis, the clinical diagnosis was confirmed and the absence or occurrence of other possible causes, such as myopathy, ketosis and hepatosis were proven using the threshold values shown in Table 2 (Rossow et al., 1987; Kraft and Dürr, 2005). The threshold value of calcium (2.00 mmol/l) was chosen to exclude cows with diminished calcium concentrations in a dimension that is typical for the peripartal period (Kraft and Dürr, 2005). The threshold value of CK was chosen to clearly exceed the area of slightly elevated CK activities found in cows with dystocia (Sitzenstock, 2003).

Table 1. Standardized therapy of recumbent cows with clinical diagnosis 'hypocalaemia' in the five practices

Practice	Therapy
A	500 ml Calciumborogluconate (=11.40 g Ca)[a]
B	500 ml Calciumborogluconate (=10.80 g Ca)[b], 15 ml Dexamethason
C	500 ml Calciumborogluconate (=11.4 g Ca)[a], 15 ml Dexamethason
D	500 ml Calciumborogluconate (=11.4 g Ca)[a]
E	500 ml Calciumborogluconate (10.75 g Ca)[c], 15 ml Dexamthason

[a]CalciTad®25, Animedica, Senden-Boesensell.
[b]C-B-Glukonat 24 plus 6®, Alvetra, Neumünster.
[c]C-B-Gluconat-Lösung N®, Belapharm, Vechta.

In all the statistical analyses using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) only the cows with hypocalcaemia, which was confirmed by the laboratory analysis were included. The concentrations of the minerals and enzyme activities were compared by a simple ANOVA. Aspartate-amino-transferase and CK values were log-transformed to obtain normal distributed values. The Fishers test was chosen for the analysis of the cure rates in the different groups. The threshold values for ASAT and CK were calculated by receiver operating characteristic (ROC)-analysis in order to support the decision as to whether to cure or to cull the recumbent cows (Greiner et al., 2000). From the curves, the cut off point was chosen by using the point on the curve being the closest to the upper left corner to obtain the threshold value having the best combination of both a good sensitivity and specificity. All analyses were made for each practice separately, for parameters, such as those examined in the present study are more different between the clusters than within (Cannon and Roe, 1982). Because the standard therapy did not change within one practice, no analyses were made concerning the different treatments used for the same reasons. The level of significance was fixed at $P = 0.05$ for all the tests.

Results

In the five practices, a total of 770 cows were included in the study (Table 3). In these cows, hypocalcaemia was the most

Table 2. Physiological ranges of the serum parameters of dairy cows ([a]Rossow et al., 1987; [b]Kraft and Dürr, 2005)

Parameter	Physiological range	Method
Calcium	>2.00 mmol/l[b]	Photometric determination, reagent Roche, modular analyzer
Phosphorus	>1.25 mmol/l[b]	
Magnesium	0.8–1.3 mmol/l[b]	
Aspartate aminotransferase (ASAT)	<50 mmol/l[a]	Kinetic enzymatic method, reagent Ranbut, modular analyzer, 37°C
Creatinkinase (CK)	>300 U/l[c]	
Glutamate dehydrogenase (GLDH)	>30 U/l[b]	
Total bilirubin	<6.8 μmol/l[a]	Quantitative photometrically determination, reagent Roche, modular analyzer
Cholesterol	2.5–4.5 mmol/l[a]	Photometric determination, reagent Roche, modular analyzer
Urea	3.3–5.0 mmol/l[b]	Kinetic UV assay, reagent Roche, modular analyzer
β-Hydroxybutyrate	<1000 μmol/l[a]	Kinetic enzymatic method, reagent Ranbut, modular analyzer

[c]The threshold value of CK was chosen to exceed clearly the area of slightly elevated CK activities found in cows with dystocia (Sitzenstock, 2003).

Table 3. Frequency of a different diagnosis due to laboratory results of parturient cows of different age groups with the clinical diagnosis 'hypocalcaemia' in five practices (n = 770)

	A		B	C	D	E
Diagnosis after laboratory analysis	FV (n = 97)	HF (n = 50)	HF (n = 200)	HF (n = 102)	HF (n = 134)	HF (n = 187)
1st lactation	n = 12 (12.4%)	n = 3 (4.5%)	n = 1 (0.5%)	n = 2 (2.0%)	n = 5 (3.7%)	n = 21 (11.2%)
Hypocalcaemia[a]	1	–	–	1	2	12
Myopathy[b]	9	2	1	1	3	–
Ketosis/hepatosis[c]	–	–	–	–	–	1
No diagnosis[d]	2	1	–	–	–	8
2nd lactation	n = 6 (6.2%)	n = 3 (6.0%)	n = 4 (2.0%)	n = 11 (10.8%)	n = 6 (4.5%)	n = 21 (11.2%)
Hypocalcaemia	3	1	2	7	4	13
Myopathy	1	1	2	3	–	6
Ketosis/hepatosis	–	1	–	–	–	1
No diagnosis	2	–	–	1	2	1
3rd–5th lactation	n = 56 (57.7%)	n = 37 (70.1%)	n = 161 (80.5%)	n = 67 (65.7%)	n = 88 (65.7%)	n = 109 (58.3%)
Hypocalcaemia	43	34	130	58	73	85
Myopathy	7	1	10	3	11	8
Ketosis/hepatosis	–	1	7	1	3	2
No diagnosis	6	1	14	5	1	14
> 5th lactation	n = 23 (23.7%)	n = 7 (19.4%)	n = 34 (17.0%)	n = 22 (21.5%)	n = 35 (26.1%)	n = 36 (19.3%)
Hypocalcaemia	16	5	30	21	30	22
Myopathy	5	1	1	–	4	4
Ketosis/hepatosis	–		2	1	–	3
No diagnosis	2	1	1	–	1	7

[a]Clinical diagnosis hypocalcaemia confirmed by laboratory analysis: Ca < 2.00 mmol/l.
[b]Myopathy: ASAT acitivity or CK acitivity or both above the threshold values (tab. 1) and calcium concentrations in normal ranges.
[c]ß-Hydroxybutyrate or bilirubin concentrations or GLDH acitivity or any combination of the three parameters above the threshold values (tab. 1) and calcium concentrations in normal ranges.
[d]No diagnosis possible due to the laboratory results.

probable cause of the recumbency due to the clinical examination. The clinical examination did not suggest any other cause for the recumbency in the cows. The vast majority of the cows (78.0%) became recumbent in the first 24 h after calving, and the animals were treated the first time within 6 h after the cows' recumbency. Most of the cows (68.3%) were found lying in a sternal position and the vast majority (80.0%) had an undisturbed sensorium. In all regions, cows starting their third to fifth lactations were in the majority (57.7–80.5%). Except for practice E (11.2%) and the FV-cows in practice A (12.4%), the proportions of cows at their first calving were very small (Table 3). There was an age-dependent distribution of the diseases diagnosed, which was the same in all the regions (Table 3). When heifers became recumbent, they suffered more often from other diseases than hypocalcaemia except the heifers in practice E. In this practice, hypocalcaemia was the major cause of recumbency as it was found in all other lactation groups. Of all the parturient cows, 74.3% (65.0–85.3%) suffered from hypocalcaemia (Table 4). Moderate or severe muscle damage was diagnosed as the second most frequent (6.9–22.7%). Muscle damage also was the major concomitant disease (11.4–49.2%) in cows suffering from hypocalcaemia. Other than practices A–D, a higher incidence of ketosis was detectable in practice E. In this practice, ketosis was the most frequent concomitant disease (28.0%) in cows with hypocalcaemia.

The cure rate after the first treatment and the total cure rate were very high and showed similar values in all five practices (Table 5). The cure rate after the first treatment was at least 72.7%, and the total rate of cured animals was at least 89.4%. As the cows suffered from hypocalcaemia only, the total curing rate was at least 95% (Table 5). In comparing the laboratory results between the successfully and unsuccessfully treated cows, except for two cases, calcium and phosphorus concentrations did not differ significantly between the groups (Table 6). In practice A, FV-cows had higher phosphorus

Table 4. Frequency of concomitant diseases due to serum analysis of parturient cows with the clinical diagnosis 'hypocalcaemia' confirmed by serum analysis in five practices (n = 593)

	A		B	C	D	E
Diagnosis	FV	HF	HF	HF	HF	HF
Hypocalcaemia	n = 63 (65.0%)	n = 40 (80.0%)	n = 162 (81%)	n = 87 (85.3%)	n = 109 (81.3%)	n = 132 (70.6%)
No concomitant	22 (34.9%)	26 (65.0%)	57 (35.2%)	41 (47.1%)	36 (33.0%)	61 (46.2%)
Myopathy[a]	31 (49.2%)	12 (30.0%)	68 (42.0%)	26 (29.9%)	33 (30.3%)	15 (11.4%)
Ketosis[a]	6 (9.5%)	1 (2.5%)	24 (14.8%)	11 (12.7%)	18 (16.5%)	37 (28.0%)
Myopathy and ketosis	4 (6.4%)	1 (2.5)	13 (8.0%)	9 (10.3%)	22 (20.2%)	19 (14.4%)

[a]Definition see Table 2.

Table 5. Overall success rate and cure rate after the first treatment in parturient dairy cows with hypocalcaemia and different CK-activities ($n = 593$)

Practice	Number	Healing rate after first treatment (%)	Overall treatment rate (%)
Practice A FV	$n = 63$	$n = 38$ (94.8%)	$n = 59$ (93.7%)
No muscle damage	27	70.4%[a]	100%[a]
CK 300 < x < 1000 U/l	22	63.6%[a]	100%[a]
CK > 1000 U/l	14	35.7%[b]	71.4%[b]
		$P = 0.012$	$P = 0.002$
Practice A HF	$n = 40$	$n = 30$ (73.6%)	$n = 36$ (90.0%)
No muscle damage	24	87.5%[a]	95.8%[a]
CK 300 < x < 1000 U/l	12	58.3%[b]	91.7%[a]
CK > 1000 U/l	4	50.0%[b]	50.0%[b]
		$P = 0.027$	$P = 0.058$
Practice B	$n = 162$	$n = 134$ (82.7%)	$n = 156$ (92.3%)
No muscle damage	81	88.8%[a]	97.5%[a]
CK 300 < x < 1000 U/l	65	78.5%[a]	98.5%[a]
CK > 1000 U/l	16	68.8%[b]	81.3%[b]
		$P = 0.010$	$P = 0.030$
Practice C	$n = 87$	$n = 69$ (79.3%)	$n = 81$ (93.1%)
No muscle damage	56	85.7%[a]	96.4%[a]
CK 300 < x < 1000 U/l	24	70.8%[a]	95.8%[a]
CK > 1000 U/l	7	57.1%[a]	57.1%[b]
		$P = 0.083$	$P = 0.007$
Practice D	$n = 109$	$n = 91$ (83.5%)	$n = 104$ (95.4%)
No muscle damage	46	93.5%[a]	100%[a]
CK 300 < x < 1000 U/l	39	82.1%[a]	97.4%[a]
CK > 1000 U/l	24	66.7%[b]	83.3%[b]
		$P = 0.017$	$P = 0.004$
Practice E	$n = 132$	$n = 96$ (72.7%)	$n = 118$ (89.4%)
No muscle damage	98	79.6%[a]	95.9%[a]
CK 300 < x < 1000 U/l	23	56.5%[a]	73.9%[a]
CK > 1000 U/l	11	45.5%[b]	63.6%[b]
		$P = 0.002$	$P < 0.001$

[a,b]Cure rates with different superscripts in the same column differed significantly.

Table 6. Mean values and standard deviation of the mineral concentrations of successfully and unsuccessfully treated parturient dairy cows with hypocalcaemia prior to the first treatment

	n	Calcium (mmol/l)	Phosphorus (mmol/l)	Magnesium (mmol/l)
Practice A FV	63			
Cured	59	1.40 ± 0.3	0.65 ± 0.4	1.10 ± 0.3
		$P = 0.245$	$P = 0.021$	$P = 0.436$
Non-cured	4	1.61 ± 0.4	1.20 ± 1.0	0.99 ± 0.2
Practice A HF	40			
Cured	36	1.09 ± 0.3	0.52 ± 0.3	1.15 ± 0.2
		$P = 0.562$	$P = 0.644$	$P = 0.881$
Non-cured	4	1.19 ± 0.5	0.46 ± 0.3	1.13 ± 0.1
Practice B	162			
Cured	156	1.03 ± 0.3	0.93 ± 0.50	0.96 ± 0.22
		$P = 0.009$	$P = 0.456$	$P = 0.160$
Non-cured	5	1.40 ± 0.4	1.08 ± 0.6	0.82 ± 0.27
Practice C	87			
Cured	81	1.16 ± 0.34	0.74 ± 0.48	0.99 ± 0.19
		$P = 0.234$	$P = 0.968$	$P = 0.439$
Non-cured	6	0.99 ± 0.4	0.75 ± 0.3	1.05 ± 0.3
Practice D	109			
Cured	104	1.20 ± 0.3	0.59 ± 0.3	0.94 ± 0.2
		$P = 0.635$	$P = 0.544$	$P = 0.464$
Non-cured	5	1.12 ± 0.5	0.51 ± 0.2	1.01 ± 0.2
Practice E	132			
Cured	118	1.23 ± 0.4	0.80 ± 0.6	0.98 ± 0.3
		$P = 0.776$	$P = 0.149$	$P = 0.663$
Non-cured	14	1.20 ± 0.4	1.06 ± 0.8	1.0 ± 0.3

concentrations if they were not cured ($P = 0.021$). In practice B, non-cured cows had significantly higher calcium concentrations ($P = 0.009$). Magnesium concentrations did not differ between the cured and non-cured cows in any practice ($P > 0.05$). Glutamate-dehydrogenase activity and concentrations of β-hydroxybutyrate, bilirubin, urea and cholesterol showed significant differences ($P < 0.05$) in a few cases only (Table 7). Contrary to that, significant differences ($P < 0.004$) were detectable in the activities of ASAT and CK in all five practices (Table 7). Cows that could not be healed had higher activities of both enzymes at the time of first treatment. The increased activities of CK resulted in lower cure rates of the animals in all the practices (Table 5). The cure rate after the first treatment as well as the total cure rate differed significantly ($P < 0.03$) between the cows with very high CK-activities and those with normal or slightly elevated values. Based on these results, ROC-analyses were performed for these two parameters, which showed the same significant changes in all the practices. The threshold values obtained from the calculated ROC-curves (Table 8) varied between 65 U/l (practice A) and 144 U/l (practice B) for ASAT and for CK between 241 U/l (practice E) and 2128 U/l (practice A). No usable threshold values were obtained for the HF-cows of practice A. In using these threshold values, the positive predictive value (PPV) ranged between 0.147 and 0.323 for ASAT and between 0.080 and 0.400 for CK. The negative predictive values (NPV) were higher, ranging from 0.960 to 1.000 for ASAT and from 0.979 to 1.000 for CK (Table 8).

Discussion

Cows becoming recumbent around parturition need expeditious therapy, and the methods of treatment should lead to a good prognosis. In recumbent cows, the clinical symptoms are

Table 7. Mean values and standard deviation of the selected blood parameters of successfully and unsuccessfully treated parturient dairy cows with hypocalcaemia prior to the first treatment ($n = 593$)

	n	ASAT[a] (U/l)	CK[a] (U/l)	Bilirubin (μmol/l)	GLDH (U/l)	β-Hydroxybutyrate (μmol/l)	Urea (mmol/l)	cholesterol mmol/l
Practice A FV	63							
Cured	59	48 ± 2	343 ± 4	8.1 ± 5.5	7 ± 9	659 ± 288	4.96 ± 1.6	1.68 ± 0.4
		$P = 0.007$	$P = 0.002$	$P = 0.512$	$P = 0.984$	$P = 0.229$	$P = 0.049$	$P = 0.001$
Non-cured	4	124 ± 2	3483 ± 2	9.9 ± 5.8	7 ± 7	482 ± 132	6.68 ± 3.0	2.57 ± 0.4
Practice A HF	40							
Cured	36	36 ± 1	193 ± 3	9.8 ± 5.1	4 ± 2	580 ± 289	5.34 ± 1.4	1.51 ± 0.5
		$P = 0.029$	$P = 0.041$	$P = 0.182$	$P = 0.447$	$P = 0.467$	$P = 0.476$	$P = 0.396$
Non-cured	4	64 ± 3	721 ± 8	13.7 ± 8.9	5 ± 4	689 ± 177	4.81 ± 0.9	1.25 ± 0.2
Practice B	162							
Cured	156	106 ± 1	269 ± 2	5.27 ± 3.1	18 ± 13	730 ± 409	6.10 ± 1.8	2.0 ± 0.8
		$P < 0.001$	$P < 0.001$	$P = 0.335$	$P < 0.001$	$P = 0.255$	$P = 0.66$	$P = 0.006$
Non-cured	6	235 ± 2	1094 ± 6	6.71 ± 3.6	80 ± 68	939 ± 995	7.48 ± 2.3	2.87 ± 1.7
Practice C	87							
Cured	81	83 ± 1	227 ± 2	8.6 ± 4.7	13 ± 18	788 ± 390	4.72 ± 1.4	1.76 ± 0.5
		$P = 0.016$	$P = 0.001$	$P = 0.006$	$P = 0.071$	$P = 0.649$	$P = 0.057$	$P = 0.561$
Non-cured	6	123 ± 2	811 ± 5	14.3 ± 7.1	25 ± 37	864 ± 425	5.87 ± 1.7	1.78 ± 0.6
Practice D	109							
Cured	104	107 ± 1	352 ± 3	9.6 ± 7.6	9 ± 5	884 ± 440	5.93 ± 2.1	1.96 ± 0.7
		$P < 0.001$	$P < 0.001$	$P = 0.640$	$P < 0.001$	$P < 0.001$	$P = 0.530$	$P = 0.832$
Non-cured	5	263 ± 3	3241 ± 4	8.0 ± 3.2	27 ± 34	1926 ± 2094	5.34 ± 1.3	2.03 ± 0.7
Practice E	132							
Cured	118	48 ± 2	127 ± 3	8.4 ± 4.	15 ± 21	1004 ± 639	6.24 ± 3.9	–[b]
		$P < 0.001$	$P < 0.001$	$P = 0.145$	$P = 0.424$	$P = 0.777$	$P = 0.317$	
Non-cured	14	91 ± 2	613 ± 4	10.4 ± 7.7	19 ± 17	954 ± 517	7.31 ± 2.5	–

[a]The values for ASAT and CK are the inverted-log-values.
[b]Not tested.

Table 8. Threshold values of ASAT and CK for the prognosis of unsuccessful treatment in parturient dairy cows ($n = 593$)

	ASAT (U/l)					CK (U/l)				
	Threshold	SE	SP	PPV	NPV	Threshold	SE	SP	PPV	NPV
Practice A FV (63)	65 $P = 0.007$	1.000	0.831	0.286	1.000	2128 $P = 0.006$	1.000	0.898	0.400	1.000
Practice A HF (40)	38.5 $P = 0.279$	–	–	–	–	408.5 $P = 0.131$	–	–	–	–
Practice B HF(162)	144 $P = 0.001$	0.800	0.846	0.172	0.993	358 $P = 0.028$	0.800	0.705	0.080	0.982
Practice C HF(87)	104.5 $P = 0.027$	0.667	0.827	0.222	0.971	531.5 $P = 0.056$	–	–	–	–
Practice D HF (109)	118.5 $P = 0.007$	1.000	0.721	0.147	1.000	1075 $P = 0.001$	0.800	0.923	0.174	0.988
Practice E HF (132)	74.5 $P < 0.001$	0.714	0.822	0.323	0.960	241 $P < 0.001$	0.857	0.771	0.308	0.979

SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

sometimes insufficiently distinct to eliminate all but one possible diagnosis (Bostedt et al., 1979; Stolla et al., 2000). Therefore, the first treatment includes a degree of uncertainty of diagnosis (Martig, 2002). Recent studies revealed that hypocalcaemia is the major cause of recumbent paresis in dairy cows (Gelfert et al., 2005). But concomitant diseases, such as myopathy, ketosis, or neural lesions might complicate the healing process (Jönsson and Pherson, 1969; Waage, 1984). There are several studies in which the use of enzyme activities as a predictor of treatment outcome was investigated (Clark et al., 1987; Shpigel et al., 2003). The disadvantages of these studies were the high variability in the duration of recumbency at first sampling (Clark et al., 1987), the high variation in the causes of recumbency (Shpigel et al., 2003), and the high variability in the occurrence of recumbency during lactation in both studies. To eliminate this variability, the focus of the present study was placed on the peripartal period (1 day a.p.–2 days p.p.) and on hypocalcaemia, which is still the main cause of recumbency during those days (Gelfert et al., 2005). The main aspects of the present study were the possible shift in the causes of recumbency, which might be the reason for the recent reports (Abele and Wolf, 2000) of the increasing failure of the standard therapy (intravenous calcium infusion), and the search for other factors that influence the therapy outcome. The standardised procedure in the five practices guaranteed a low variability of the cows included in our study.

The results obtained in the five practices involved were very similar. Cows entering their first or second lactation seem to still have a very low risk of becoming recumbent around parturition, which confirms the findings of other studies

(Bostedt et al., 1979; Stolla et al., 2000; Metzner and Klee, 2005). In this age group, muscle damage was the main cause of recumbency, which was opposite to the other age groups (Table 3). Although the number of recumbent heifers was too small to generalize the findings, the results suggest that heifers have a minor risk of clinical hypocalcaemia. If this age group becomes recumbent, muscle damage or other health problems, such as neural lesions due to dystocia are likely to be the causes. There was one practice that showed a result that was opposite to the other four practices. This fact is an indicator that the reasons of recumbency may vary from region to region. Especially in recumbent heifers, a detailed clinical examination is indispensable, in which serum analysis should be carried out to confirm the clinical diagnosis.

Muscle damage was the main differential diagnosis in elder cows, which mainly suffered from hypocalcaemia in four of the five practices. Practice E again showed results against the current. The control of the feeding management revealed that the diet of the transition cows in this region had low energy content. Therefore, the cows had to mobilize their fat depots earlier and to a greater extent than normal, which is the reason for the high incidence of ketosis found in this region. However, muscle damage also occurs frequently in the cows of practice E as it was seen in the other four practices. It might be questionable as to whether the animals suffered from muscle damage. CK is a highly specific enzyme of the muscles but also occurs in other organs, such as the heart or uterus (Frahm et al., 1977; Sattler and Fürll, 2004). Studies of CK activities in non-recumbent cows, having calved normally, showed that CK activities increase in the first day postpartum (Bostedt, 1974; Sitzenstock, 2003). The values, however, were clearly below the threshold that we have chosen for our study. In the cows that needed obstetrics, the CK-activities were higher than in cows calved spontaneously but still below our threshold value (Sitzenstock, 2003). Cows suffering from heart diseases accompanied with elevated CK activities (Frahm et al., 1977) often showed typical clinical symptoms, such as cardia dysrhythmia, muffled heart sounds, peripheral oedema and jugular distension (Gründer, 2002; Reef and McGuirk, 2002). No indication of heart disease was shown from the clinical examination in any of the cows in the present study, in which we can conclude that the elevated CK activities are more likely the result of damage to the muscles.

The next problem to solve is whether the increased CK-activities found are only the result of the long lasting inability to rise. Cox et al. (1982) examined the development of CK-activity in the serum of experimental downer cows treated with halothane anaesthesia. They found that muscle damage occurs regularly in recumbent cows if they lay down for more than 12 h at a time. But up to 6 h of the CK-activities of these cows were still in physiological ranges and below the cut-off value (300 U/l) that we chose for the present study. The vast majority of the cows in our study were examined in-between 6 h after the onset of recumbency. Therefore, the elevated CK-activities found at the time of the first treatment can be taken as an indicator for autonomous muscle damage that was not the result of a long-running inability to rise.

The incidence of myopathy diagnosed by laboratory results varied between the races. In practice A, HF-cows with hypocalcaemia suffered less from myopathy than FV-cows at the same time. Additionally, the proportion of FV-cows suffering from myopathy alone was higher than that of HF-cows in all the practices. As the present study was performed under field conditions, no data were available regarding the body mass. We may assume, however, that the higher body mass of FV-cows in general might contribute to the higher risk of muscle damage, as was found to be the case in Cox et al. (1982) and Waage (1984).

The cure rates and success rates after the first treatment were sufficiently high in all five practices. After the first treatment, 72.7–94.8% of the cows were cured. The total rate of successful treatment was between 89.4% and 95.4%. Similar results were reported in previous studies (Breitenbuch von, 1985; Malz and Meyer, 1992; Shpigel et al., 2003; Siegwart and Niederer, 2005). In other studies, smaller curing rates after the first treatment were reported (Meschke, 1997; Roesch, 2000; Stolla et al., 2000). The authors assume that the low phosphorus levels may influence the healing process. Analysing the serum concentrations and enzyme activities of the cured and non-cured animals, the most surprising result was that the calcium and phosphorus concentrations at the time of the first therapy did not play a major role in the outcome of treatment. In the two cases that showed significant differences, the concentrations of calcium and phosphorus were higher in the non-cured cows. This result is in agreement with the results of Siegwart and Niederer (2005), but contrary to other studies (Bostedt, 1973; Malz and Meyer, 1992; Stolla et al., 2000). The most evident results were the significantly higher activities of ASAT and CK in the non-cured cows in all five practices. The cure rate was not significantly reduced unless the CK activity exceeded 1,000 U/l, which indicates that the muscle damage had to be sufficiently severe or had to last longer in order to influence the course of disease. CK is known to have a retarded increase in serum after damage to the muscle, and a small half-life. Therefore, the slightly elevated activities of CK in the serum samples obtained at the first clinical examination did not correlate with the extent of muscle damage. It also did not correlate to the size of functional deficiency. A rupture of the Mm adductors, M. gastrocnemius, or M. fibularis tertius more likely cause an inability to stand than damage to other muscles.

Because the activities of ASAT and CK were the only parameters that differed significantly in all the practices between cured and non-cured animals, ROC-curves were calculated to obtain cut-off-points of both enzymes so as to obtain help in deciding which cow to treat. In the cows, the calculated PPV of the failure of the treatment were very low for ASAT (0.147–0.323) and CK (0.080–0.400). On the other hand, the NPV of both enzymes was high in all the practices. A high NPV denotes that recumbent cows with activities below the threshold values had a high probability to be cured. Two facts are responsible for the results obtained. The first fact influencing the PPV is the high levels of ASAT and CK activities in the cured cows as was found in Clark et al. (1987). In some of the practices, the highest activity measured for the single enzyme occurred in the healed animals and not in the non-healed animals (Table 9). The result of this distribution is a moderate specificity of the single threshold value. The second and more important cause is the high curing rate, in turn resulting in a small number of non-cured cows in each practice. In the case of a low prevalence of any character, any test even with a high sensitivity and specificity would result in a high number of false positive cases (Table 10). This in turn results in a low PPV as seen in the present study. In all five practices,

Table 9. Maximum acitivities of ASAT and CK measured in recumbent cows prior to the first treatment and outcome of the therapy ($n = 593$)

	ASAT (U/l)		CK (U/l)	
Practice	Cured cows	Non-cured cows	Cured cows	Non-cured cows
A FV-cows	894	242	17080	10108
A HF-cows	110	347	3356	7962
B	391	346	4161	16473
C	440	246	5700	3840
D	355	1065	5775	33191
E	276	592	4091	7725

Table 10. Crosstabulation to calculate PPV and NPV in recumbent FV-cows in practice A

	Treatment outcome		
CK- threshold	Successfully	Unsuccessfully	
<2128	53	0	53
>2128	6	4	10
	59	4	63

PPV, $4/(6 + 4) = 0.400$; NPV, $53/(53 + 0) = 1.000$.

the threshold values calculated are useful to make a good prognosis for a successful treatment when the activities of both enzymes are below the threshold values. This confirms the results from Shpigel et al. (2003) who pointed out that the PPV and NPP change with the prevalence of the failure of recovery. In the case of a high success rate of therapy as found in the five practices, the NPV is very high and the PPV very low. Therefore, the serum analysis would not help to decide whether to treat an animal, because all the cows with low activities of the enzymes would be treated due to the high NPV and all the cows with high activities would be treated due to the low PPV.

The results of our study clearly show that hypocalcaemia is still the major cause of recumbency in the peripartal period in elder cows. The standard treatment of an infusion of organic calcium solutions is still recommended. In cows suffering from hypocalcaemia only, at least 95% regained the ability to stand. In the other cows, increased activities of CK and ASAT are an indication of the prevalence of muscle damage, but are not acting as an indicator for a prognosis of the recumbent cow. However, laboratory analyses are useful to confirm the clinical diagnosis in order to explain the failure of the first therapy and to improve the future treatment of the animal.

Conclusion

For the five regions chosen in the present study, it can be concluded that recumbent cows around parturition are suffering mostly from hypocalcaemia and often show other concomitant diseases, such as muscle damage and ketosis. Contrary to elder cows, muscle damage is the most frequent diagnosis in heifers. Severe muscle damage is the main reason for the failure of treatment. Serum analyses at the time of the first treatment do not provide additional information to improve the prognosis at that time if there is a high curing-rate. The serum samples can, however, provide hints about concomitant diseases and can improve subsequent treatments. Serum samples are useful in recumbent heifers, for in this age group hypocalcaemia is not the predominant cause of recumbency.

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References

Abele, U., and F. Wolf, 2000: Gebärparese - ein neues Therapiekonzept. Veterinärspiegel, **2000/1,** 197–201.

Baumgartner, W., 2005: Klinische Propädeutik der inneren Krankheiten und Hautkrankheiten der Haus- und Heimtiere. Parey, Stuttgart, Germany.

Bostedt, H., 1973: Blutserumuntersuchungen bei festliegenden Rindern in der frühpuerperalen Periode. 1. Mitteilung: untersuchungen über den Gehalt an Ca, anorganischen P und Mg im Blutserum festliegender Rinder. Berl. Münch. Tierärztl. Wochenschr. **86,** 344–349.

Bostedt, H, 1974: Enzymaktivitäten im Blutserum von Rindern in der Zeit um die Geburt. Berl. Münch. Tierärztl. Wochenschr. **87,** 365–371.

Bostedt, H., V. Wendt, and R. Prinzen, 1979: Zum Festliegen des Milchrindes im perpartalen Zeitraum - klinische und biochemische Aspekte. Prakt. Tierarzt **60,** 18–34.

Breitenbuch von, A., 1985: Zur prognostischen Bedeutung in der Praxis durchgeführter Erstuntersuchungen festliegender Rinder. Diss. vet. med., Tierärztliche Hochschule Hannover.

Cannon, R. M., and R. T. Roe, 1982: Livestock Disease Surveys: A Field Manual for Veterinarians. Austr. Bureau of Animal Health, Canberra, Australia, pp. 11–14.

Clark, R. G., H. V. Henderson, G. K. Hoggard, R. S. Ellison, and B. J. Young, 1987: Ability of biochemical and haematological tests to predict recovery in periparturient recumbent cows. N Z Vet. J. **35,** 126–133.

Cox, V. S., C. J. McGrath, and S. E. Jorgensen, 1982: The role of pressure damage in pathogenesis of the downer cow syndrome. Am. J. Vet. Res. **43,** 26–31.

Dirksen, G., 2002: Festliegen. In: Dirksen, G., H. D. Gründer, and M. Stöber (eds), Innere Medizin und Chirurgie des Rindes, pp. 863–871. Blackwell Verlag GmbH, Berlin, Wien.

Frahm, K., F. Graf, and H. Krausslich, 1977: Enzymaktivitäten in Rinderorganen. Zbl. Vet. Med. A, **24,** 81–87.

Gelfert, C. C., M. Dallmeyer, and R. Staufenbiel, 2004: Effekte einer zusätzlichen oralen Phosphorgabe auf den Behandlungserfolg festliegender Milchkühe. Prakt. Tierarzt **85,** 116–124.

Gelfert, C. C., S. Lesch, I. Alpers, M. Decker, A. Hüting, W. Baumgartner, and R. Staufenbiel, 2005: Untersuchungen zum Auftreten der Gebärparese in verschiedenen Regionen Deutschlands und zum Einsatz unterschiedlicher Therapien im Vergleich zur Kalziuminfusion. 1. Klinische Symptome und Verhalten der Mengenelemente. Tierärztl. Prax., **33**(G), 411–418.

Goff, J. P, 2004: Pathophysiology of calcium and phosphorus disorders. Vet. Clin. North Am. **16,** 319–337.

Goff, J. P., and R. L. Horst, 1997: Physiological changes at parturition and their relationship zu metabolic disorders. J. Dairy Sci. **80,** 1260–1268.

Greiner, M., D. Pfeiffer, and R. D. Smith, 2000: Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. **45,** 23–41.

Gründer, H. D., 2002: Unspezifisch bedingte Krankheiten des Herzens. In: Dirksen, G., H. D. Gründer, and M. Stöber (eds), Innere Medizin und Chirurgie des Rindes, pp. 163–173. Blackwell Verlag GmbH, Berlin, Wien.

Hunt, E., and J. T. Blackwelder, 2002: Bovine parturient paresis (milk fever, hypocalcaemia). In: Smith, B. P. (ed.), Large Animal Internal Medicine, pp. 1248–1254. Mosby Inc. St Louis, Baltimore, Philadelphia, Toronto.

Jönsson, G., and B. Pherson, 1969: Studies on the downer syndrome in dairy cows. Zbl. Vet. Med. A **16,** 757–784.

Kraft, W., and U. M. Dürr, 2005: Referenzbereiche. In: Kraft, W., and U. M. Dürr (eds), Klinische Labordiagnostik in der Tiermedizin, pp. 507–514. Schattauer Verlag, Stuttgart, Germany.

Malz, C., and C. Meyer, 1992: Neue Aspekte zur Pathogenese und Therapie der hypocalcämischen Gebärparese. Prakt. Tierarzt **73,** 507–515.

Martig, J., 2002: Hypokalzämische gebärlähmung. In: Dirksen, G., H. D. Gründer, and M. Stöber (eds), Innere Medizin und Chirurgie des Rindes, pp. 1245–1254. Blackwell Verlag GmbH, Berlin, Wien.

Meschke, A., 1997: Zur Gebärparese des Rindes: über die Wirksamkeit verschiedener Behandlungsmethoden und deren Einflüsse auf ausgewählte Blutparameter. Diss. vet. med., Justus-Liebig University Giessen, Giessen, Germany.

Metzner, M., and W. Klee, 2005: Klinische Befunde und Serumparameter bei festliegenden Kühen unter besonderer Berücksichtigung der Serumphosphorkonzentrationen. Tierärztl. Umsch. **60,** 13–22.

Reef, V. B., and S. M. McGuirk, 2002: Myocardial disease: myocarditis and cardiomyopathy. In: Smith, B.P (ed.), Large Animal Internal Medicine, pp. 460–463. Mosby Inc. St Louis, Baltimore, Philadelphia, Toronto.

Roesch, V., 2000: Untersuchungen zur Gebärparese bei Fleckviehkühen unter besonderer Berücksichtigung ausgewählter Parameter des Energiestoffwechsels und der Leberfunktion. Diss. vet. med., Justus-Liebig University Giessen, Giessen, Germany.

Rossow, N., U. Jacobi, M. Schäfer, R. Lippmann, and G. Furcht, 1987: Stoffwechselüberwachung bei Haustieren - Probleme, Hinweise und Referenzwerte. Institut für angewandte Tierhygiene Eberswalde.

Sattler, T., and M. Fürll, 2004: Creatine kinase and aspartate aminotransferase in cows as indicators for endometritis. J. Vet. Med. A **51,** 132–137.

Shpigel, N. Y., Y. Avidar, and E. Bogin, 2003: Value of measurements of the serum activities of creatine phosphokinase, aspartate aminotransferase and lactate dehydrogenase for predicting whether recumbent dairy cows will recover. Vet. Rec. **152,** 773–776.

Siegwart, N., and K. Niederer, 2005: Retrospektive Studie über den Einfluss des Kalzium- und Phoshporblutwertes auf den Therapieerfolg bei post partum festliegenden Kühen. Tierärztl. Umsch. **60,** 352–355.

Sitzenstock, A., 2003: Auswirkungen eines differenten Geburtsgeschehen bei Fleischrindern auf partalrelevante Parameter im peripheren Blut. Diss. vet. med., Justus-Liebig University Giessen, Giessen, Germany.

Staufenbiel, R., 1999: Hinweise. zur Therapie der Gebärparese der Milchkuh (Teil II). Veterinärspiegel, **1999/2,** 159–162.

Stolla, R., H. Schulz, and R. Martin, 2000: Veränderungen im Krankheitsbild des peripartalen Festliegens beim Rind. Tierärztl. Umsch. **55,** 295–299.

Waage, S., 1984: Milk fever in the cow – Course of disease in relation to the serum activity of aspartate aminotransferase, alanine aminotransferase, creatine kinase and gamma-glutamyltransferase. Nord. Vet. **36,** 282–295.