Research Square

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Frequency and diagnostic reliability of laboratory variables in cows with traumatic reticuloperitonitis and type 1, 2, 3, 4 and 5 abomasal ulcer

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Abstract

Background: A number of laboratory abnormalities occur in cows with traumatic reticuloperitonitis (TRP) as well as in those with abomasal ulcers (AU). This prompted us to compare the frequencies of laboratory abnormalities of healthy cows and cows with TRP and cows with abomasal ulcers and to calculate diagnostic sensitivities and specificities, predictive values and positive likelihood ratios for laboratory findings. The study included 182 healthy control cows, 503 cows with TRP, 94 cows with U1, 145 cows with U2, 60 cows with U3, 87 cows with U4 and 14 cows with U5. Haematocrit, total leukocyte count, concentrations of total protein, fibrinogen, urea, potassium and chloride, base excess and rumen chloride concentration were analysed.

Results: Values outside the reference interval occurred in 2 to 24% of control cows (rumen chloride 2%, urea 6%, serum chloride 11%, haematocrit 13%, base excess 18%, fibrinogen 20%, total protein 21%, total leukocyte count 22% and potassium 24%), which made differentiation of healthy and ill cows difficult. Therefore, the variables best suited for distinguishing healthy and ill cows were rumen chloride and blood urea concentration. This was also supported by an LR_+ of 14 to 27 for rumen chloride >30 mmol/l and 6 to 15 for blood urea >6.5 mmol/l in cows with ulcers. Urea also had a high diagnostic specificity and like rumen chloride was suited for differentiation of healthy and diseased cows. The urea concentration was >8.5 mmol/l in only 0.5% of controls, and the LR_+ for a urea concentration >8.5 mmol/l ranged from 11 in cows with TRP to 128 in cows with U2. Except for cows with TRP, azotaemia was significantly more frequent in ill cows than in controls. Cows with U2 (70%) had urea concentrations >8.5 significantly more often than cows of the other groups, which may have been prerenal azotaemia attributable to hypovolaemia. Even though the groups of ill cows differed significantly with respect to several variables, no variables were identified to reliably differentiate the various groups.

Conclusions: Isolated results are not suitable to distinguish among groups of ill cows and instead the history, the clinical findings and results of additional diagnostic techniques such as ultrasonography are required.

Background

Traumatic reticuloperitonitis (TRP) and abomasal ulcers are important intestinal disorders of cattle [1, 2]. Abomasal ulcers are classified as type 1 (U1), 2 (U2), 3 (U3), 4 (U4) or 5 (U5) [3–7]. Type 1 ulcer is non-perforating and accompanied by minimal haemorrhage, and is subdivided into subtypes 1a, 1b, 1c and 1d [8, 9]. Type 2 ulcer is characterised by the erosion of a large blood vessel causing intraluminal haemorrhage and anaemia. Type 3 ulcer is perforating and accompanied by localised peritonitis, U4 is perforating and accompanied by generalised peritonitis and U5 has perforated into the omental bursa. Laboratory findings in cows with TRP [10], U1 [11], U2 [12], U3 [13], U4 [14] and U5 [7] were recently described in detail. Laboratory findings reported in older studies of cows with TRP and abomasal ulcer have also been discussed [7, 10]. Of 94 cows with U1, the majority had hypokalaemia (68%), positive base excess (60%) and azotaemia (51%) [11], and of 145 cows with U2, the most frequent laboratory findings were azotaemia (89%), low haematocrit (82%), hypokalaemia (81%), hypoproteinaemia (74%) and metabolic acidosis (61%) [12]. The only laboratory abnormality that was commonly seen in 60 cows with U3 was hypokalaemia (75%) [13]. Haemoconcentration (69%) and azotaemia (56%) [14] were the main abnormalities in 87 cows with U4, and hypokalaemia (71%), haemoconcentration (57%) and metabolic acidosis (57%) were the most common abnormalities in 14 cows with U5 [7]. Hyperfibrinogenaemia (69%) and hyperproteinaemia (64%) were the major laboratory abnormalities in 503 cows with TRP [10]. The degree of increase in rumen chloride concentration also varied among the different diseases. The abovementioned studies showed that hypokalaemia and azotaemia are common to all groups, whereas haemoconcentration is typically seen in cows with U4 and U5, haemodilution in cows with U2, hyperproteinaemia in cows with TRP and hypoproteinaemia in cows with U2. Furthermore, the frequency with which the laboratory abnormalities occur varied greatly among the groups. The prognosis also differed significantly and was usually good in cows with TRP, often fair in cows with U2, but generally poor in cows with U4 and U5. Finally, TRP and abomasal ulcer require different therapeutic approaches. The diagnostic reliability of clinical signs in cows with TPR and abomasal ulcers was recently investigated [15]. From a diagnostic standpoint, it would be desirable to use also laboratory variables to aid in differentiation of cows with TRP and various types of abomasal ulcers. To investigate this, the frequencies of laboratory abnormalities recorded in cows with TRP and abomasal ulcer were compared and the parameters diagnostic sensitivity and specificity, predictive values and positive likelihood ratio (LR_+) calculated.

Methods

Animals

A total of 1,085 cows including 182 healthy controls, 503 cows with TRP, 94 cows with U1, 145 cows with U2, 60 cows with U3, 87 cows with U4 and 14 cows with U5 were used. All animals were privately owned and transported to the Veterinary Teaching Hospital of the University of Zurich for clinical examination. The sample size differed between groups and was dictated by the case load, which varied depending of the incidence of the disease in the population. The frequency and diagnostic reliability of the clinical findings of these cows were the subject of a recent publication [15].

Tentative and definitive diagnoses of TRP and type 1, 2, 3, 4 or 5 abomasal ulcer, treatment and response to treatment

The criteria for tentative and confirmed diagnoses of TRP and abomasal ulcers, inclusion and exclusion criteria, and treatment and response to treatment of the cows in this study were recently published [15].

Euthanasia

Euthanasia was done with pentobarbital (Esconarkon, Streuli Pharma), 80 mg/kg body weight intravenously.

Analysis of laboratory variables

The laboratory findings of the controls [16] and the cows with TRP [10], U1 [11], U2 [12], U3 [13], U4 [14] and U5 [7] have been described in detail. The variables haematocrit, total leukocyte count, concentrations of total protein, fibrinogen, urea, potassium and chloride, base excess and rumen chloride concentration were analysed. The reference intervals for total leukocyte count (5,000–10,000 cells/μl) and fibrinogen (4–7 g/l), urea (2.0-6.5 mmol/l) and potassium concentrations (4–5 mmol/l) have been published previously [10, 12–14, 17]. The reference interval for the haematocrit of 30–35% [10,12-14.17] was modified to 27–37% because otherwise 41% of the control cows would have had a value outside the reference interval. For similar reasons, the reference interval for total protein concentration was expanded from 60–80 g/l [10, 12–14, 17] to 60–85 g/l, serum chloride concentration from 96–105 mmol/l [13, 17] to 91–105 mmol/l, base excess from − 2 to + 2 [10, 12–14, 17] to -4 to + 4 mmol/l and rumen chloride concentration from 15–30 [18] to 6–30 mmol/l.

Statistical analysis

The program SPSS Version 25 was used for analysis. The Shapiro-Wilk test showed that only total protein and urea concentrations had normal distribution and therefor the medians and 25th and 75th percentiles were calculated. The medians underwent one-factor analysis of variance and pair-wise comparison using the Kruskal-Wallis test, and frequency distributions of all variables were calculated for the controls and six disease groups. Laboratory results were arbitrarily divided into appropriate ranges (see Table 2, example haematocrit; 27–37%, ≤ 20 and > 20%, < 27 and ≥ 27%, ≤ 37 and > 37%, ≤ 44 and > 44%), and the ranges compared among groups using the chi-square and the Bonferroni post-hoc tests. A P-value < 0.05 was considered significant. For each variable, the diagnostic sensitivity (a/[a + c]), diagnostic specificity (d/[b + d]), positive likelihood ratio (LR_+, sensitivity/[1-specificity]), positive predictive value (a/[a + b]) and negative predictive value (d/[c + d]) were calculated (a, true positive; b, false positive; c, false negative; d, true negative). A false positive result was an abnormal finding in a control cow and a false negative result was a normal finding in a cow with TRP or abomasal ulcer. The occurrence of a decreased (< 27%), normal (27–37%) and elevated (> 37%) haematocrit accompanied by decreased (< 60 g/l), normal (60–85 g/l) and elevated total protein concentration (> 85 g/l), and the occurrence of decreased, normal and elevated total protein concentration accompanied by decreased (< 4 g/l), normal (4–7 g/l) and elevated (> 7 g/l) fibrinogen concentration were investigated.

Table 2
Frequency distributions of the laboratory findings in healthy control cows and in cows with traumatic reticuloperitonitis (TRP), abomasal ulcer type 1 (U1), type 2 (U2), type 3 (U3), type 4 (U4) and type 5 (U5)

Variable	Classification	Controls	TRP	U1	U2	U3	U4	U5	Chi^2
Haematocrit (normal)	27–37%	159 (87%)[2,3,4,5,6,7]	378 (75%)[1,3,4,6.7]	54 (57%)[1,2,4,6]	21 (15%)[1,2,3,5]	41 (68%)[1,4,6]	26 (30%)[1,2,3,5]	5 (36%)[1,2]	274
	< 27 or > 37%	23 (13%)	123 (25%)	40 (43%)	124 (85%)	19 (32%)	61 (70%)	9 (64%)	
Haematocrit decreased	< 27%	7 (4%)[2,4]	86 (17%)[1,4]	7 (7%)[4]	115 (79%)[1,2,3,5,6,7]	3 (5%)[4]	6 (7%)[4]	2 (14%)[4]	366
	≥ 27%	175 (96%)	415 (83%)	87 (93%)	30 (21%)	57 (95%)	81 (93%)	12 (86%)	
	≤ 20%	0 (0%)[4,7]	7 (1%)[4]	0 (0%)[4]	102 (70%)[1,2,3,5,6,7]	1 (2%)[4]	4 (5%)[4]	1 (7%)[1,4]	
	> 20%	182 (100%)	494 (99%)	94 (100%)	43 (30%)	59 (98%)	83 (17%)	13 (93%)	631
Haematocrit increased	> 37%	16 (9%)[3,5,6,7]	37 (7%)[3,5,6,7]	33 (35%)[1,2,4,6]	9 (6%)[3,5,6,7]	16 (27%)[1,2,4,6]	55 (63%)[1,2,3,4,5]	7 (50%)[1,2,4,	232
	≤ 37%	166 (91%)	464 (93%)	61 (65%)	136 (94%)	44 (73%)	32 (37%)	7 (50%)	
	> 44%	0 (0%)[6.7]	7 (1%)[6]	3 (3%)[6]	1 (1%)[6]	3 (5%)[6]	29 (33%)[1,2,3,4,5]	1 (7%)[1]	213
	≤ 44%	182 (100%)	494 (99%)	91 (97%)	144 (99%)	57 (95%)	58 (67%)	13 (93%)	
Total leukocyte count (normal)	5000–10'000/µl	141 (78%)[2,4,6]	272 (54%)[1]	58 (62%)	78 (55%)[1]	38 (63%)	40 (47%)[1]	10 (71%)	39
	< 5000 or > 10'000/µl	41 (22%)	229 (46%)	36 (38%)	65 (45%)	22 (37%)	46 (53%)	4 (29%)	
Total leukocyte count	< 5000/µl	7 (4%)[6]	20 (4%)[3,4,6]	12 (13%)[2,6]	17 (12%)[2,6]	7 (12%)[6]	30 (35%)[1,2,3,4,5]	2 (14%)	
decreased	≥ 5000/µl	175 (96%)	481 (96%)	82 (87%)	126 (88%)	53 (88%)	56 (65%)	12 (86%)	98
	< 2500/µl	1 (1%)	1 (0.2%)[6]	2 (2%)	1 (1%)	0 (0%)	4 (5%)[2]	0 (0%)	
	≥ 2500/µl	181 (99%)	500 (99.8%)	92 (98%)	142 (99%)	60 (100%)	82 (95%)	14 (100%)	20

[1] Different from controls P < 0.01

[2] Different from TRP P < 0.01

[3] Different from U1 P < 0.01

[4] Different from U2 P < 0.01

[5] Different from U3 P < 0.01

[6] Different from U4 P < 0.01

[7] Different from U5 P < 0.01

Variable	Classification	Controls	TRP	U1	U2	U3	U4	U5	Chi^2
Total leukocyte count	> 10’000/µl	34 (19%)[2,4]	209 (42%)[1,6]	24 (26%)	48 (34%)[1]	15 (25%)	16 (19%)[2]	2 (14%)	
increased	≤ 10’000/µl	148 (81%)	292 (58%)	70 (74%)	95 (66%)	45 (75%)	70 (81%)	12 (86%)	49
	> 15’000/µl	3 (2%)[2]	46 (9%)[1]	5 (5%)	11 (8%)	5 (8%)	7 (8%)	1 (7%)	
	≤ 15’000/µl	179 (98%)	455 (91%)	89 (95%)	132 (92%)	55 (92%)	79 (92%)	13 (93%)	12
Total protein (normal)	60–85 g/l	144 (79%)[2,4]	272 (54%)[1,4,5]	66 (70%)[4]	29 (20%)[1,2,3,5,6,7]	48 (80%)[2,4]	58 (67%)[4]	11 (79%)[4]	145
	< 60 or > 85 g/l	38 (21%)	229 (46%)	28 (30%)	116 (80%)	12 (20%)	29 (33%)	3 (21%)	
Total protein	< 60 g/l	3 (2%)[4,6,7]	5 (1%)[4,6,7]	2 (2%)[4,6,7]	108 (75%)[1,2,3,5,6,7]	2 (3%)[4,6]	25 (29%)[1,2,3,4,5]	3 (21%)[1,2,3,4]	
decreased	≥ 60 g/l	179 (98%)	496 (99%)	92 (98%)	37 (25%)	58 (97%)	62 (71%)	11 (79%)	578
	< 50 g/l	0 (0%)[4,6]	2 (0.4%)[4,6]	1 (1%)[4]	77 (53%)[1,2,3,5,6,7]	0 (0%)[4]	9 (10%)[1,2,4]	0 (0%)[4]	
	≥ 50 g/l	182 (100%)	499 (99.6%)	93 (99%)	68 (47%)	60 (100%)	78 (90%)	14 (100%)	458
Total protein increased	> 85 g/l	35 (19%)[2,4,6]	224 (45%)[1,3,4,5,6,7]	26 (28%)[2,4,6]	8 (6%)[1,2,3]	10 (17%)[2]	4 (5%)[1,2,3]	0 (0%)[2]	144
	≤ 85 g/l	147 (81%)	277 (55%)	68 (72%)	137 (94%)	50 (83%)	83 (95%)	14 (100%)	
	> 100 g/l	2 (1%)	23 (5%)	3 (3%)	1 (1%)	2 (3%)	0 (0%)	0 (0%)	
	≤ 100 g/l	180 (99%)	478 (95%)	91 (97%)	144 (99%)	58 (97%)	87 (100%)	14 (100%)	13
Fibrinogen (normal)	4–7 g/l	145 (80%)[2,4,5,6]	131 (26%)[1,3,4,5,6,7]	59 (63%)[2]	82 (57%)[1,2]	31 (52%)[1,2]	39 (45%)[1,2]	9 (64%)[2]	182
	< 4 g or > 7 g/l	37 (20%)	368 (74%)	34 (37%)	61 (43%)	29 (48%)	47 (55%)	5 (36%)	
Fibrinogen	< 4 g/l	13 (7%)[4]	23 (5%)[4]	7 (8%)[4]	49 (34%)[1,2,3,5,6]	3 (5%)[4]	8 (9%)[4]	2 (14%)	

[1] Different from controls P < 0.01

[2] Different from TRP P < 0.01

[3] Different from U1 P < 0.01

[4] Different from U2 P < 0.01

[5] Different from U3 P < 0.01

[6] Different from U4 P < 0.01

[7] Different from U5 P < 0.01

Variable	Classification	Controls	TRP	U1	U2	U3	U4	U5	Chi^2
decreased	≥ 4 g/l	169 (93%)	476 (95%)	86 (92%)	94 (66%)	57 (95%)	78 (91%)	12 (86%)	116
	< 3 g/l	7 (4%)[4]	15 (3%)[4]	6 (7%)[4]	32 (22%)[1,2,3,6]	3 (5%)	3 (4%)[4]	2 (14%)[1,4]	
	≥ 3 g/l	175 (96%)	484 (97%)	87 (93%)	111 (78%)	57 (95%	83 (96%)	12 (86%)	76
Fibrinogen	> 7 g/l	24 (13%)[2,3,5,6]	345 (69%)[1,3,4,5,6,7]	27 (29%)[1,2,4]	12 (8%)[2,3,5,6]	26 (43%)[1,2,4]	39 (45%)[1,2,4]	3 (21%)[2]	
increased	≤ 7 g/l	158 (87%)	154 (31%)	66 (71%)	131 (92%)	34 (57%)	47 (55%)	11 (79%)	283
	> 9 g/l	3 (2%)[2,3,5,6]	224 (45%)[1,3,4,5,6,7]	10 (11%)[1,2]	7 (5%)[2,5]	14 (23%)[1,2,4]	14 (16%)[1,2]	0 (0%)[2]	
	≤ 9 g/l	179 (98%)	275 (55%)	83 (89%)	136 (95%)	46 (77%)	72 (84%)	14 (100%)	206
Urea (normal)	2–6.5 mmol/l	171 (94%)[3,4,5,6,7]	431 (86%)[3,4,5,6]	46 (49%)[1,2,4]	16 (11%)[1,2,3,5,6,7]	39 (65%)[1,2,4]	38 (44%)[1,2,4]	8 (57%)[1,4]	392
	> 6.5 mmol/l	11 (6%)	70 (14%)	48 (51%)	128 (89%)	21 (35%)	48 (56%)	6 (43%)	
Urea	> 6.5 mmol/l	11 (6%)[3,4,5,6,7]	70 (14%)[3,4,5,6]	48 (51%)[1,2,4]	128 (89%)[1,2,3,5,6,7]	21 (35%)[1,2,4]	48 (56%)[1,2,4]	6 (43%)[1,4]	
increased	≤ 6.5 mmol/l	171 (94%)	431 (86%)	46 (49%)	16 (11%)	39 (65%)	38 (44%)	8 (57%)	392
	> 8.5 mmol/l	1 (0.5%)[3,4,5,6,7]	30 (6%)[3,4,6,7]	26 (28%)[1,2,4]	101 (70%)[1,2,3,5,6,7]	8 (13%)[1,4,6]	35 (41%)[1,2,4,5]	4 (29%)[1,2,4]	
	≤ 8.5 mmol/l	182 (99.5%)	471 (94%)	68 (72%)	43 (30%)	52(87%)	51 (59%)	10 (71%)	374
Potassium (normal)	4–5 mmol/l	139 (76%)	132 (26%)[1]	27 (29%)[1]	25 (18%)[1]	15 (25%)[1]	17 (20%)[1]	3 (21%)[1]	190
	< 4 or > 5 mmol/l	43 (24%)	368 (74%)	67 (71%)	118 (83%)	45 (75%)	69 (80%)	11 (79%)	
Potassium	< 4 mmol/l	42 (23%)[2,3,4,5,6,7]	361 (72%)[1]	64 (68%)[1]	116 (81%)[1]	45 (75%)[1]	62 (72%)[1]	10 (71%)[1]	
decreased	≥ 4 mmol/l	140 (77%)	139 (28%)	30 (32%)	27 (19%)	15 (25%)	24 (28%)	4 (29%)	173
	< 3 mmol/l	1 (0.5%)[2,3,4,5,6,7]	63 (13%)[1]	20 (21%)[1]	29 (20%)[1]	15 (25%)[1]	17 (20%)[1]	2 (14%)[1]	
	≥ 3 mmol/l	181 (99.5%)	437 (87%)	74 (79%)	114 (80%)	45 (75%)	69 (80%)	12 (86%)	46
Chloride (normal)	91–105 mmol/l	161 (89%)[3,4,5]	405 (81%)[3,4,5]	49 (52%)[1,2,6]	91 (64%)[1,2]	34 (58%)[1,2]	64 (74%)[3]	10 (71%)	74
	≤ 90 or > 105 mmol/l	21 (11%)	94 (19%)	45 (48%)	51 (36%)	25 (42%)	22 (26%)	4 (29%)	
Chloride	≤ 90 mmol/l	4 (2%)[3,5,6]	36 (7%)[3,5,6]	17 (18%)[1,2]	11 (8%)	13 (22%)[1,2]	17 (20%)[1,2]	2 (14%)	
decreased	> 90 mmol/l	178 (98%)	463 (93%)	77 (82%)	131 (92%)	46 (78%)	69 (80%)	12 (86%)	45
	≤ 80 mmol/l	2 (1%)[3]	8 (2%)[3]	8 (9%)[1,2]	2 (1%)	2 (3%)	4 (5%)	1 (7%)	

[1] Different from controls P < 0.01

[2] Different from TRP P < 0.01

[3] Different from U1 P < 0.01

[4] Different from U2 P < 0.01

[5] Different from U3 P < 0.01

[6] Different from U4 P < 0.01

[7] Different from U5 P < 0.01

Variable	Classification	Controls	TRP	U1	U2	U3	U4	U5	Chi^2
	> 80 mmol/l	180 (99%)	491 (98%)	86 (91%)	140 (99%)	57 (97%)	82 (95%)	13 (93%)	21
Chloride increased	> 105 mmol/l	17 (9%)[3,4]	58(12%)[3,4]	28 (30%)[1,2,6]	40 (28%)[1,2,6]	12 (20%)	5 (6%)[3,4]	2 (14%)	51
	≤ 105 mmol/l	165 (91%)	440 (88%)	66 (70%)	102 (72%)	47 (80%)	81 (94%)	12 (86%)	
Base excess (normal)	-4 to + 4 mmol/l	145 (82%)[2,3,4,5,6]	254 (55%)[1]	39 (44%)[1]	61 (52%)[1]	32 (57%)[1]	34 (47%)[1]	9 (64%)	57
	< -4 or > +4 mmol/l	31 (18%)	210 (45%)	49 (56%)	56 (48%)	24 (43%)	38 (53%)	5 (36%)	
Base excess	< -4 mmol/l	10 (6%)[4,6]	26 (6%)[4,6]	11 (13%)	25 (21%)[1,2]	3 (5%)	13 (18%)[1,2]	2 (14%)	
decreased	≥ -4 mmol/l	166 (94%)	438 (94%)	77 (87%)	92 (79%)	53 (95%)	59 (82%)	12 (86%)	36
	< -8 mmol/l	1 (0.5%)[4]	2 (0.4%)[4,6,7]	3 (3%)	13 (11%)[1,2]	0 (0%)	4 (6%)[2]	1 (7%)[2]	
	≥ -8 mmol/l	175 (99.5%)	462 (99.6%)	85 (97%)	104 (89%)	56 (100%)	68 (94%)	13 (93%)	55
Base excess	> +4 mmol/l	21 (12%)[2,3,4,5,6]	181 (39%)[1]	38 (43%)[1]	31 (27%)[1]	21 (38%)[1]	25 (35%)[1]	3 (21%)	
increased	≤ +4 mmol/l	155 (88%)	283 (61%)	50 (57%)	86 (73%)	35 (62%)	47 (65%)	11 (79%)	53
	> +7 mmol/l	1 (0.5%)[2,3,4,5,6,7]	86 (19%)[1]	25 (28%)[1]	16 (14%)[1]	15 (27%)[1]	18 (25%)[1]	2 (14%)[1]	
	≤ +7 mmol/l	190 (99.5%)	378 (81%)	63 (72%)	101 (86%)	41 (73%)	54 (75%)	12 (86%)	55
Rumen chloride (normal)	6–30 mmol/l	178 (98%)[2,3,4,5,6,7]	375 (87%)[1,3,5]	51 (64%)[1,2]	80 (75%)[1]	30 (56%)[1,2]	52 (78%)[1]	6 (60%)[1]	94
	< 6 or > 30 mmol/l	3 (2%)	58 (13%)	29 (36%)	27 (25%)	24 (44%)	15 (22%)	4 (40%)	
Rumen chloride	> 30 mmol/l	3 (2%)[2,3,4,5,6,7]	58 (13%)[1,3,5]	29 (36%)[1,2]	27 (25%)[1]	24 (44%)[1,2]	15 (22%)[1]	4 (40%)[1]	
increased	≤ 30 mmol/l	178 (98%)	375 (87%)	51 (64%)	80 (75%)	30 (56%)	52 (78%)	6 (60%)	94
	> 40 mmol/l	1 (0.5%)[3,4,5,6]	26 (6%)[3,5]	15 (19%)[1,2]	11 (10%)[1]	14 (26%)[1,2]	6 (9%)[1]	1 (10%)	
	≤ 40 mmol/l	181 (99.5%)	407 (94%)	65 (81%)	96 (90%)	40 74%)	61 (91%)	9 (90%)	53

[1] Different from controls P < 0.01

[2] Different from TRP P < 0.01

[3] Different from U1 P < 0.01

[4] Different from U2 P < 0.01

[5] Different from U3 P < 0.01

[6] Different from U4 P < 0.01

[7] Different from U5 P < 0.01

Results

Haematocrit

The median haematocrit ranged from 14 to 40% among all groups (Table 1). Cows with TRP and U2 had a significantly lower haematocrit (TRP 30%, U2 14%) and cows with U4 a significantly higher haematocrit (40%) than controls (33%). The percentage of cows with a haematocrit in the reference interval was 87% in controls, and was significantly lower in all other groups (Table 2, Fig. 1). Anaemia with haematocrits < 27 and < 20% occurred in 4 and 0% of controls, respectively. Significantly more cows with TRP and U2 had a haematocrit < 27% (TRP 17%, U2 79%) than controls, and there were

significantly more cows with a haematocrit < 20% in the U2 group compared with all other groups (70% vs 0–7%). The LR_+ for a haematocrit < 27% and ≤ 20% in cows with U2 was 21 and 70, respectively (Table 3). Haemoconcentration with a haematocrit > 37% occurred in 9% of controls and significantly more often in cows with U1 (35%), U3 (27%), U4 (63%) and U5 (50%). A haematocrit > 44% was seen in 33% of cows with U4 (33%) but not in controls (Table 2). The LR_+ for a haematocrit > 44% in cows with U4 was 33 (Table 3).

Table 1
Laboratory variables in healthy control cows and cattle with traumatic reticuloperitonitis (TRP), abomasal ulcer type 1 (U1), type 2 (U2), type 3 (U3), type 4 (U4) and type 5 (U5) (medians, 25 and 75% percentiles in brackets)

Variable	Controls	TRP	U1	U2	U3	U4	U5
Haematocrit (%)	33[2,4,6] (30–35)	30[1,3,4,5,6] (27–33)	34[2,4,6] (30–39)	14[1,2,3,5,6,7] (11–23)	34[2,4,6] (31–38)	40[1,2,3,4,5] (33–48)	38[4,6] (28–41)
Leukocytes (/µl)	8000[2,6] (6500–9400)	9400[1,6] (7400–11850)	7800 (5900-10'200)	9000[6] (6200-11'600)	8150[6] (6375-10’375)	6250[1,2,4,5] (4475–9200)	6100 (5000–8150)
Total protein (g/l)	78[2,4,6,7] (72–84)	84[1,4,5,6,7] (78–90)	78[4,6,7] (72–87)	48[1,2,3,5,6,7] (41–60)	76[2,4,6,7] (70–82)	65[1,2,3,4,5] (54–74)	66[1,2,3,4,5] (60–71)
Fibrinogen (g/l)	5.0[2,3,4,5,6,] (4.0–6.0)	8.0[1,3,4,5,6,7] (6.0–10.0)	6.0[1,2,4] (4.0–8.0)	4.0[1,2,3,5,6,7] (3.0–6.0)	6.5[1,2,4] (6.0–9.0)	6.0[1,2,4] (4.8–8.0)	5.4[2,4] (4.8–6.5)
Urea (mmol/l)	4.3[3,4,5,6] (3.3–5.4)	4.0[3,4,5,6] (3.0–5.3)	6.6[1,2,4] (4.7–8.8)	10.1[1,2,3,5,6] (8.0–12.4)	5.7[1,2,4] (3.9–7.4)	7.3[1,2,4] (5.0–11.4)	6.1 (4.2–9.4)
Potassium (mmol/l)	4.2[2,3,4,5,6] (4.0–4.4)	3.6[1] (3.3–4.0)	3.5[1] (3.1–4.0)	3.4[1] (3.0–3.9)	3.4[1] (2.9–4.0)	3.6[1] (3.1–4.0)	3.6 (3.2–4.2)
Chloride (mmol/l)	100[4,6] (97–103)	100[6] (97–103)	100[6] (93–106)	103[1,5,6] (99–106)	98[4] (92–103)	96[1,2,3,4] (92–101)	101 (95–104)
Base excess (mmol/l)	1.7[2] (-0.4–3.3)	3.1[1,4] (-0.3–5.7)	3.6 (-1.0–7.9)	0.3[2] (-3.1–4.2)	2.4 (-1.2–8.2)	2.1 (-2.0–6.9)	1.1 (-1.2–3.9)
Rumen chloride (mmol/l)	13[2,3,4,5,6] (12–16)	20[1,5] (15–26)	25[1] (19–36)	22[1,5] (18–31)	29[1,2,4,6] (21–43)	22[1,5] (17–29)	28 (18–34)

[1] Difference to Controls P < 0.05, Kruskal Wallis test

[2] Difference to TRP P < 0.05, Kruskal Wallis test

[3] Difference to U1 P < 0.05, Kruskal Wallis test

[4] Difference to U2 P < 0.05, Kruskal Wallis test

[5] Difference to U3 P < 0.05, Kruskal Wallis test

[6] Difference to U4 P < 0.05, Kruskal Wallis test

[7] Difference to U5 P < 0.05, Kruskal Wallis test

Table 3.

Diagnostic sensitivity and specificity, positive and negative predictive values and positive likelihood ratio of laboratory variables in cows with traumatic reticuloperitonitis (TRP), abomasal ulcer type 1 (U1), type 2 (U2), type 3 (U3), type 4 (U4) and type 5 (U5) (in percent)

Variable	Reliability indices	TRP	U1	U2	U3	U4	U5
Haematocrit	Diagn. sensitivity	17	7	79	5	7	14
< 27 %	Diagn. specificity	96	96	96	96	96	96
	Pos. pred. value	92	50	94	30	46	22
	Neg. pred. value	30	67	85	75	68	94
	LR_+	4	2	21	1	2	4
Haematocrit	Diagn. sensitivity	1	0	70	2	5	7
≤ 20 %	Diagn. specificity	100	100	100	100	100	100
	Pos. pred. value	100	-	100	100	100	100
	Neg. pred. value	27	66	81	76	69	93
	LR_+	1[1]	0[1]	70[1]	2[1]	5[1]	7[1]
Haematocrit	Diagn. sensitivity	7	35	6	27	63	50
> 37 %	Diagn. specificity	91	91	91	91	91	91
	Pos. pred. value	70	67	36	50	77	30
	Neg. pred. value	26	73	55	79	84	96
	LR_+	1	4	1	3	7	6
Haematocrit	Diagn. sensitivity	1	3	1	5	33	7
> 44 %	Diagn. specificity	100	100	100	100	100	100
	Pos. pred. value	100	100	100	100	100	100
	Neg. pred. value	27	67	56	76	76	93
	LR_+	1[1]	3[1]	1[1]	5[1]	33[1]	7[1]
Total leukocyte count	Diagn. sensitivity	4	13	12	12	35	14
< 5’000/µl	Diagn. specificity	96	96	96	96	96	96
	Pos. pred. value	74	63	71	50	81	22
	Neg. pred. value	27	68	58	77	76	94
	LR_+	1	3	3	3	9	4
Total leukocyte count	Diagn. sensitivity	0	2	0	0	5	0
< 2’500/µl	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	50	67	50	0	80	0
	Neg. pred. value	27	66	56	75	69	93
	LR_+	0	4	1	0	8	0
Total leukocyte count	Diagn. sensitivity	42	26	34	25	19	14
> 10’000/µl	Diagn. specificity	82	82	82	82	82	82
	Pos. pred. value	86	42	59	31	33	6
	Neg. pred. value	34	68	61	77	68	93
	LR_+	2	1	2	1	1	1
Total leukocyte count	Diagn. sensitivity	9	5	8	8	8	7
> 15’000/µl	Diagn. specificity	98	98	98	98	98	98
	Pos. pred. value	94	63	79	63	70	25
	Neg. pred. value	28	67	58	76	69	93
	LR_+	6	3	5	5	5	4
Total protein	Diagn. sensitivity	1	2	74	3	29	21
< 60 g/l	Diagn. specificity	98	98	98	98	98	98
	Pos. pred. value	63	40	97	40	89	50
	Neg. pred. value	27	66	83	76	74	94
	LR_+	1	1	45	2	17	13
Total protein	Diagn. sensitivity	0	1	53	0	10	NA
< 50 g/l	Diagn. specificity	100	100	100	100	100	100
	Pos. pred. value	100	100	100	NA	100	NA
	Neg. pred. value	27	66	73	75	70	93
	LR_+	0[1]	1[1]	53[1]	0[1]	0[1]	NA
Total protein	Diagn. sensitivity	45	28	6	17	5	0
> 85 g/l	Diagn. specificity	81	81	81	81	81	81
	Pos. pred. value	86	43	19	22	10	0
	Neg. pred. value	35	68	52	75	64	91
	LR_+	2	1	0.3	1	1	1
Total protein	Diagn. sensitivity	5	3	1	3	0	0
> 100 g/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	92	60	33	50	0	0
	Neg. pred. value	27	66	56	76	67	93
	LR_+	4	3	1	3	0	0
Fibrinogen	Diagn. sensitivity	5	8	34	5	9	14
< 4 g/l	Diagn. specificity	93	93	93	93	93	93
	Pos. pred. value	64	35	79	19	38	13
	Neg. pred. value	26	66	64	75	68	93
	LR_+	1	1	5	1	1	2
Fibrinogen	Diagn. sensitivity	3	6	22	5	3	14
< 3 g/l	Diagn. specificity	96	96	96	96	96	96

	Pos. pred. value	68	46	82	30	30	22
	Neg. pred. value	27	67	61	75	68	94
	LR_+	1	2	6	1	1	4
Fibrinogen	Diagn. sensitivity	69	29	8	43	45	21
> 7 g/l	Diagn. specificity	87	87	87	87	87	87
	Pos. pred. value	93	53	33	52	62	11
	Neg. pred. value	51	71	55	82	77	93
	LR_+	5	2	1	3	3	2
Fibrinogen	Diagn. sensitivity	45	11	5	23	16	0
> 9 g/l	Diagn. specificity	98	98	98	98	98	98
	Pos. pred. value	99	77	70	82	82	0
	Neg. pred. value	39	68	57	80	71	93
	LR_+	27	7	3	14	10	0
Urea	Diagn. sensitivity	14	51	89	35	56	43
> 6.5 mmol/l	Diagn. specificity	94	94	94	94	94	94
	Pos. pred. value	86	81	92	66	81	35
	Neg. pred. value	28	79	91	81	82	96
	LR_+	2	8	15	6	9	7
Urea	Diagn. sensitivity	6	28	70	13	41	29
> 8.5 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	97	96	99	89	97	80
	Neg. pred. value	28	73	81	78	78	95
	LR_+	11	50	128	24	74	52
Potassium	Diagn. sensitivity	72	68	81	75	72	71
< 4 mmol/l	Diagn. specificity	77	77	77	77	77	77
	Pos. pred. value	90	60	73	52	60	19
	Neg. pred. value	50	82	84	90	85	97
	LR_+	3	3	4	3	3	3
Potassium	Diagn. sensitivity	13	21	20	25	20	14
< 3 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	98	95	97	94	94	67
	Neg. pred. value	29	71	61	80	72	94
	LR_+	23	39	37	46	36	26
Chloride	Diagn. sensitivity	7	18	8	22	20	14
≤ 90 mmol/l	Diagn. specificity	98	98	98	98	98	98
	Pos. pred. value	90	81	73	76	81	33
	Neg. pred. value	28	70	58	79	72	94
	LR_+	3	8	4	10	9	7
Chloride	Diagn. sensitivity	2	9	1	3	5	7
≤ 80 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	80	80	50	50	67	33
	Neg. pred. value	27	68	56	76	69	93
	LR_+	1	8	1	3	4	7
Base excess	Diagn. sensitivity	6	11	20	5	17	14
< -4 mmol/l	Diagn. specificity	95	95	95	95	95	95
	Pos. pred. value	74	53	72	25	57	18
	Neg. pred. value	29	70	66	77	75	94
	LR_+	1	2	4	1	4	3
Base excess	Diagn. sensitivity	0	3	11	0	6	7
< -8 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	67	75	93	0	80	50
	Neg. pred. value	29	69	65	77	74	94
	LR_+	1	7	21	0	11	14
Base excess	Diagn. sensitivity	39	42	26	36	35	21
> +4 mmol/l	Diagn. specificity	88	88	88	88	88	88
	Pos. pred. value	89	62	57	47	52	12
	Neg. pred. value	37	77	66	82	78	94
	LR_+	3	3	2	3	3	2
Base excess	Diagn. sensitivity	19	28	14	27	25	14
> +7 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	99	96	94	94	95	67
	Neg. pred. value	33	75	65	82	78	94
	LR_+	35	54	26	51	48	27
Rumen chloride	Diagn. sensitivity	13	36	25	44	22	40
> 30 mmol/l	Diagn. specificity	98	98	98	98	98	98
	Pos. pred. value	95	91	90	89	83	57
	Neg. pred. value	32	78	69	86	77	97
	LR_+	8	22	15	27	14	24
Rumen chloride	Diagn. sensitivity	6	19	10	26	9	10

> 40 mmol/l	Diagn. specificity	99	99	99	99	99	99
	Pos. pred. value	96	94	92	93	86	50
	Neg. pred. value	31	74	65	82	75	95
	LR_+	11	34	19	47	16	18

[1] Calculation of the LR_+ required the reduction of the diagnostic specificity from 100% to 99%

NA, not applicable

Values between 80 and 89% are in green

Values between 90 and 100% are in red

Values ≥10 are in blue

Total leukocyte count

The median total leukocyte count ranged from 6,100 to 9,400 cells/µl among all groups (Table 1). The total leukocyte count was significantly higher (9,400 cells/µl) in cows with TRP and significantly lower (6,250 cells/µl) in cows with U4 compared with controls (8,000/µl). The total leukocyte count was within in the reference interval in 78% (Table 2, Fig. 2), and leukopenia with < 5,000 and < 2,500 leukocytes/µl was seen in 4 and 1% of controls, respectively (Table 2). Leukopenia was significantly more common in cows with U4 (35%) than in controls (5%), cows with TRP (4%), U1 (13%), U2 (12%) and U3 (12%). The LR_+ for leukopenia was low (0–9; Table 3).

Total protein concentration

The median total protein concentration ranged from 48 to 84 g/l (Table 1) and was significantly higher in cows with TRP (84 g /l) and significantly lower in cows with U2 (48 g/l), U4 (65 g/l) and U5 (66 g/l) compared with controls (78 g/l). Seventy-nine percent of controls had a total protein concentration in the reference interval (Table 2, Fig. 3), 2% had hypoproteinaemia and 19% had hyperproteinaemia. Cows with U2 (75%), U4 (29%) and U5 (21%) had hypoproteinaemia with < 60 g/l significantly more often than controls (2%), and cows with U2 (53%) and U4 (10%) had total protein concentrations < 50 g/l significantly more often than controls (0%); concentrations < 50 g/l were also significantly more common in cows with U2 compared with all other groups. The LR_+ for hypoproteinaemia (< 60 g/l) was 45, 17 and 13 in cows with U2, U4 and U5, respectively (Table 3). Hyperproteinaemia > 85 g/l was significantly more common in cows with TRP (45%) and significantly less common in cows with U2 (6%) and U4 (5%) than in controls (19%). Total protein concentrations > 85 and > 100 g/l had positive predictive values of 86 and 92%, respectively, for TRP (Table 3)

Fibrinogen concentration

The median fibrinogen concentration ranged from 4 to 8 g/l (Table 1). The fibrinogen concentration was significantly higher in cows with TRP (8.0 g/l), U1 (6.0 g/l), U3 (6.5 g/l) and U4 (6.0 g/l) and significantly lower in cows with U2 (4.0 g/l) compared with controls (5.0 g/l). Eighty percent of control cows had a fibrinogen concentration in the reference interval (Table 2, Fig. 4), 7% had hypofibrinogenaemia and 13% had hyperfibrinogenaemia. Cows with U2 had fibrinogen concentrations < 4 (34%) and < 3 g/l (22%) significantly more often than controls. The LR_+ for these test results ranged from 1 to 6 (Table 3). Cows with TRP (69%), U1 (29%), U3 (43%) and U4 (45%) had fibrinogen concentrations > 7 g/l significantly more often than controls (13%). Cows with TRP also had fibrinogen concentrations > 7 and > 9 g/l significantly more often than all other groups. The LR_+ for a fibrinogen concentration > 9 g/l was 27 for TRP, 14 for U3 and 10 for U4 (Table 3).

Urea concentration

The median urea concentration ranged from 4.0 to 10.1 mmol/l (Table 1). Cows with U1 (6.6 mmol/l), U2 (10.1 mmol/l), U3 (5.7 mmol/l) and U4 (7.3 mmol/l) had significantly higher median urea concentrations than controls (4.3 mmol/l). Ninety-four percent of controls had a urea concentration in the reference interval (2.0–6.5 mmol/l), and 6% had azotaemia (Table 2, Fig. 5). Cows with U1 (51 and 28%), U2 (89 and 70%), U3 (35 and 13%), U4 (56 and 41%) and U5 (43 and 29%) had urea concentrations > 6.5 and > 8.5 mmol/l, respectively, significantly more often than controls (6 and 0.5%). Cows with U2 had azotaemia (89% >6.5 and 70% >8.5 mmol/l) significantly more often than cows of any other group. The LR_+ for a urea concentration > 8.5 mmol/l was > 10 in all groups and reached 128 and 74 for U2 and U4, respectively (Table 3).

Potassium concentration

The median potassium concentration ranged from 3.4 to 4.2 mmol/l (Table 1). Except for cows with U5, all cows from the other disease groups had significantly lower potassium concentrations than controls. Seventy-six percent of controls had a potassium concentration in the reference interval (Table 2, Fig. 6) and 23 and 0.5% had hypokalaemia with concentrations of < 4 and < 3 mmol/l, respectively. In cows of all disease groups, concentrations < 4 (68 to 81% of cows) and < 3 mmol/l (13 to 25% of cows) were significantly more common than in controls. The LR_+ for a potassium concentration < 3 mmol/l ranged from 23 to 46 in all disease groups (Table 3).

Chloride concentration

The median chloride concentration ranged from 96 to 103 mmol/l (Table 1). Eighty-nine percent of controls had normochloraemia (Table 2, Fig. 7) and 2 and 1% had hypochloraemia with concentrations ≤ 90 and ≤ 80 mmol/l, respectively. Cows with U1 (18%), U3 (22%) and U4 (20%) had hypochloraemia significantly more often than controls, and cows with U1 had concentrations ≤ 80 mmol/l significantly more often (9%) than controls. The LR_+ for a chloride concentration ≤ 90 mmol/l was 10 in cows with U3 (Table 3). Hyperchloraemia was significantly more common in cows with U1 (30%) and U2 (28%) than in controls.

Base excess

The median base excess ranged from 0.3 to 3.6 mmol/l (Table 1). Eighty-two percent of controls had normal values of -4 to + 4 mmol/l (Table 2, Fig. 8), 6% had values lower and 12% had values greater than the reference interval. A base excess smaller than − 4 and − 8 mmol/l was recorded in 6 and 0.5% of controls, respectively (Table 2); values lower than − 4 mmol/l were significantly more common in cows with U2 (21%) and U4 (18%), and values lower than − 8 mmol/l were significantly more frequent in cows with U2 (11%) than in controls. The LR_+ for a base excess lower than − 8 mmol/l was 21, 11 and 14 in cows with U2, U4 and U5, respectively (Table 3). A positive base excess greater than + 4 and + 7 mmol/l was significantly more frequent in cows with TRP, U1, U2, U3 and U4 (greater than + 4 mmol/l in 27 to 43%; greater than + 7 mmol/l in 14 to 28% including cows with U5) than in controls. The LR_+ for a base excess greater than + 7 mmol/l ranged from 26 to 54 for all groups (Table 3).

Rumen chloride

The median rumen chloride concentration ranged from 13 to 29 mmol/l (Table 1). Except for cows with U5, all disease groups had significantly higher rumen chloride concentrations than controls. Ninety-eight percent of controls had rumen chloride concentrations in the reference interval (Table 2, Fig. 9) and 2% had concentrations exceeding 30 mmol/l. The percentage of cows from all disease groups with concentrations > 30 mmol/l (13 to 44%) was significantly greater compared with controls. Except for TRP, the LR_+ for a rumen chloride concentration > 30 mmol/l was greater than 10 and ranged from 14 to 27 (Table 3).

Total protein concentration and haematocrit

The combined analysis of total protein concentration and haematocrit showed that 79% of cows with U2 had a decreased haematocrit, which was accompanied by hypoproteinaemia (30–59 g/l) in 73% and normoproteinaemia in 6% (Fig. 10A). In the other disease groups, a decreased haematocrit occurred in no more than 17% of the cows and was accompanied by normo-, hypo- or hyperproteinaemia. Most cows with a normal haematocrit had normoproteinaemia, some had hyperproteinaemia whereas hypoproteinaemia was rare (Fig. 10B). Most cows with increased haematocrit had normoproteinaemia and only rarely hypo- or hyperproteinaemia (Fig. 10C).

Total protein and fibrinogen concentrations

Seventy-five percent of cows with U2 had hypoproteinaemia, which was accompanied by normofibrinogenaemia in 42%, hypofibrinogenaemia in 32% and hyperfibrinogenaemia in 1% (Fig. 11A). Of the cows with normoproteinaemia (Fig. 11B), normofibrinogenaemia was most common in controls and cows with U1, U2, U3 and U5, and hyperfibrinogenaemia was most common in cows with TRP and U4. Most of the controls and cows with U1 with hyperproteinaemia had normofibrinogenaemia and most of the cows with TRP, U2, U3 and U4 with hyperproteinaemia had hyperfibrinogenaemia (Fig. 11C).

Discussion

Various studies have described the sensitivity, specificity and predictive value of total protein [19], total protein and fibrinogen [20] and fibrinogen, serum amyloid A and haptoglobin concentrations [21] for the diagnosis of cows with TRP; however, the results were compared with findings in ill cows and not healthy controls. Therefore, the calculated diagnostic parameters differ greatly from ours and a direct comparison is not possible. The laboratory variables used for comparison of healthy cows and cows with TRP, U1, U2, U3, U4 and U5 were selected from a comprehensive panel that included haematological, serum biochemical, blood gas and rumen fluid analyses and urinalysis. For a variable to be included in the study, it had to have been measured in the majority of cows and lie outside the reference interval in a large number of ill cows. Interestingly, numerous variables were outside the reference intervals in healthy cows including rumen chloride (2%), urea (6%), serum chloride (11%), haematocrit (13%), base excess (18%), fibrinogen (20%), total protein (21%), total leukocyte count (22%) and potassium (24%). This was despite the fact that the reference intervals of several variables had been modified and expanded. According to Constable and co-workers [22], healthy animals are assumed to have values within a certain range referred to as normal range or reference interval, and ill animals are assumed to have values outside this interval. A reference interval is established by using the results of a large number of healthy animals and usually includes 95% of the animals. Calculation of a reference interval is straightforward when the values have a normal distribution but requires transformation of non-normal data. In the present study, the variables had non-normal distributions with the exception of total protein and urea concentrations. Therefore, calculation of the reference interval assuming a normal distribution (mean ± 2 standard deviations) would result in an exaggerated interval that would include a considerable number of ill animals, erroneously considered healthy. When reference intervals published in the veterinary literature were used to assess healthy cows, the following values were outside the reference interval: haematocrit (41%), total protein (40%), serum chloride (26%), base excess (58%) and rumen chloride (64%). Therefore, the reference intervals of some variables were slightly expanded upward (total protein), downward (serum chloride and rumen chloride) or upward and downward (haematocrit, base excess). When healthy cows have values outside the reference intervals, they may be incorrectly diagnosed as ill (false positive). This problem can be mitigated by considering a combination of variables when making a diagnosis or to disregard certain values below the reference interval [22]. The extent of the deviation from the reference interval should also be considered because small deviations are less likely to reflect a disease than large ones. This can be quantified using the LR_+, which expresses the association between a

test result and the presence of a target disease [22]. The LR_+ expresses how much more likely an individual with the disease is to have a positive test result than if the individual is disease-free [23]. The LR_+ was calculated for all variables in the present study because it is an overall measure of the efficiency of a diagnostic test; it combines the diagnostic sensitivity and specificity and is not affected by the prevalence of the disease [22, 23]. The latter characteristic was of particular importance in the present study because the exact prevalence of TRP and abomasal ulcers is not known. Another drawback of using reference intervals is that not all diseased animals have values outside the interval. When a single variable with a value within the reference interval is used to make a diagnosis, a sick animal may be erroneously identified as not having the disease (false negative). This can be mitigated by shrinking the reference interval but in doing so the rate of false-positives increases. It would be better to consider groups of variables that all have diagnostic utility for the disorder in question. This was done in the present study by combining haematocrit and total protein, and total protein and fibrinogen concentrations.

Rumen chloride was the best variable for the differentiation of healthy and diseased cows because only 2% of controls had values outside the reference interval. This was also supported by an LR_+ of 14 to 27 in cows with ulcers. An LR_+ of 14 means that the likelihood of increased rumen chloride in an ill cow is 14 times more likely to occur than in a cow without an ulcer. The usefulness of rumen chloride for differentiating the different disease groups was limited. Only cows with TRP had rumen chloride concentrations < 40 mmol/l significantly less often (6%) than cows with U1 and U3. From a practical standpoint, this means that TRP is unlikely in a cow with a marked increase in rumen chloride concentration.

Although of slightly less importance than rumen chloride, urea had a high diagnostic specificity and was well suited for differentiation of healthy and diseased cows. The urea concentration was > 6.5 mmol/l in only 6% and > 8.5 mmol/l in only 0.5% of controls, and the LR_+ for a urea concentration > 8.5 mmol/l ranged from 11 in cows with TRP to 128 in cows with U2. Except for cows with TRP, azotaemia was significantly more frequent in ill cows than in controls. Cows with U2 (70%) had urea concentrations > 8.5 significantly more often than cows of the other groups, which may have been prerenal azotaemia attributable to hypovolaemia. Analogous to increased rumen chloride, an increase in urea concentration (above 8.5 mmol/l) is rare (6%) in cows with TRP because this is a localised disease with no direct systemic effects.

Even though 13% of controls had a haematocrit outside the expanded reference interval of 27 to 37%, none had a haematocrit > 44%. Likewise, no more than 5% of cows with TRP, U1, U2 and U3 had values > 44%, which was in contrast to cows with U4, in which 33% had a haematocrit > 44%. Thus, in cows with a high haematocrit, TRP or type 1, 2, 3 or 5 abomasal ulcers are unlikely. A haematocrit ≤ 20% occurred in 70% of cows with U2 and in only 0 to 7% of cows in the other groups and thus allowed differentiation of cows with U2.

Leukocytosis was a poor criterion for differentiating healthy and diseased cows and among cows of the different disease groups. The utility of leukopenia for this purpose was similar except that a total leukocyte count < 5000/µl was seen in 35% of cows with U4 but in only 4 to 14% of cows of the other groups. Persistent leukopenia is always a serious finding. Leukopenia in cows with U4 can be interpreted as a sequel to sepsis and leukocyte consumption [14]. Bone marrow production of leukocytes is insufficient in the face of severe inflammation because mobilisation of the marrow stem cell pool is slower in cattle than in other species [24].

A potassium concentration < 3 mmol/l is almost always a sign of disease because it occurred in only 0.5% of controls. However, hypokalaemia was non-specific and all disease groups were affected. The principal cause of hypokalaemia is decreased forage intake associated with illness [25].

A total protein concentration > 85 g/l was measured in 45% cows with TRP, which was significantly more frequent than in cows of the other groups. Similar observations were made for fibrinogen concentrations > 9 g/l. Diagnostic accuracy can be improved by considering multiple laboratory variables; 36% of cows with TRP had both hyperproteinaemia and fibrinogenaemia (Fig. 11C), which was in agreement with the results of previous reports [19–21]. Conversely, 75% of cows with U2 had hypoproteinaemia (< 60 g/l) and 32% had hypofibrinogenaemia (< 4 g/l, Fig. 11B). Cows with U4 also had hypoproteinaemia (< 60 g/l) significantly more frequently than cows of the other groups but only 4% had concurrent hypofibrinogenaemia (Fig. 11A). Interestingly, 79% of cows with U2 had anaemia accompanied by hypoproteinaemia in 73% (Fig. 10A), and 63% of cows with U4 had an increased haematocrit accompanied by normoproteinaemia in most cases (Fig. 10C).

Conclusions

Depending on the variable, 2 to 24% of healthy cows had values outside the reference intervals, which made the differentiation of healthy and diseased cows difficult based on laboratory variables alone. Rumen chloride and urea concentrations provided the best selectivity because they were outside the reference intervals in only 2 and 6% of healthy cows, respectively. Although there were significant differences between groups of cows with respect to several laboratory variables, there were no variables that reliably differentiated cows with different diseases. Instead of using a specific laboratory variable for diagnosis, a combination of multiple variables must be considered along with the history and clinical findings and often the results of additional techniques such as ultrasonography.

Abbreviations

TRP: Traumatic reticuloperitonitis; U1: Abomasal ulcer type-1; U2: Abomasal ulcer type-2; U3: Abomasal ulcer type-3; U4: Abomasal ulcer type-4; U5: Abomasal ulcer type-5; LR_+: Positive likelihood ratio; Fig: Figure.

Declarations

Ethics approval and consent to participate

The study was not submitted to our institutional ethics committee, because it was a retrospective study using medical records of cows that had been referred to our clinic for examination and treatment over a time period of 14 years (2001 – 2014). No experimental animals were used. Under these circumstances, ethical approval is not required in Switzerland. Written consent was obtained from all owners to participate.

Consent for publication

All owners signed a consent form allowing us to use the animals and all the associated medical data for scientific analysis and publication.

Availability of data and materials

The datasets used and analysed for this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Fundings

Not applicable since it was a retrospective analysis of medical records.

Author's contributions

UB initiated, planned and supervised the study and prepared the manuscript, SW and CR analysed the medical histories of the cows as part of her dissertation, KN and CG made contributions to acquisition and interpretation of data and were involved in drafting the manuscript. All authors read and approved the final manuscript.

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References

1. Dirksen G. Krankheiten von Haube und Pansen beim ruminanten Rind. In: Dirksen G, Gründer HD, Stöber M, editors. Innere Medizin und Chirurgie des Rindes. Berlin: Parey Buchverlag; 2002. p. 396–455.
2. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Diseases of the alimentary tract - ruminant. In: Constable PD, Hinchcliff KW, Done SH, Grünberg W, editors. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. St. Louis, Missouri: Elsevier; 2017. p. 436–621.
3. Whitlock RH. Bovine stomach diseases. In: Anderson NV, editor. Veterinary Gastroenterology. Philadelphia: Lea and Febiger; 1980. p. 425–8.
4. Smith DF, Munson L, Erb HN. Abomasal ulcer disease in adult dairy cattle. Cornell Vet. 1983;73:213–24.
5. Constable PD. Abomasal ulcers. In: Line S, editor. The Merck Veterinary Manual, 10th. edn., New York: Merck and Co., Inc.; 2010. p. 219–22.
6. Hund A, Wittek T. Abomasal and third compartment ulcers in ruminants and south american camelids. Vet Clin North Am Food Anim Pract. 2018;34:35–54.
7. Braun U, Reif C, Hilbe M, Gerspach C. Type-5 abomasal ulcer and omental bursitis in 14 cows. Acta Vet Scand. 2020;62:4.
8. Braun U, Eicher R, Ehrensperger F. Type 1 abomasal ulcers in dairy cattle. J Vet Med A. 1991;38:357–66.
9. Munch SL, Nielsen SS, Krogh MA, Capion N. Prevalence of abomasal lesions in Danish Holstein cows at the time of slaughter. J Dairy Sci. 2019;102:5403–9.
10. Braun U, Warislohner S, Torgerson P, Nuss K, Gerspach C. Clinical and laboratory findings in 503 cattle with traumatic reticuloperitonitis. BMC Vet Res. 2018;14:66.
11. Braun U, Gerspach C, Reif C, Hilbe M, Nuss K. Clinical, laboratory and ultrasonographic findings in 94 cows with type-1 abomasal ulcer. Schweiz Arch Tierheilk. 2020;162:235–44.

12. Braun U, Gerspach C, Nuss K, Hässig M, Hilbe M, Reif C. Clinical and laboratory findings, treatment and outcome in 145 cows with type-2 abomasal ulcer. Res Vet Sci. 2019;124:366–74.
13. Braun U, Gerspach C, Hilbe M, Devaux DJ, Reif C. Clinical and laboratory findings in 60 cows with type-3 abomasal ulcer. Schweiz Arch Tierheilk. 2019;161:523–31.
14. Braun U, Reif C, Nuss K, Hilbe M, Gerspach C. Clinical, laboratory and ultrasonographic findings in 87 cows with type-4 abomasal ulcer. BMC Vet Res. 2019;15:100.
15. Braun U, Nuss K, Warislohner S, Reif C, Oschlies C, Gerspach C. Frequency and diagnostic reliability of clinical signs in cows with traumatic reticuloperitonitis and abomasal ulcers. BMC Vet Res. 2020;16:359.
16. 16. Braun U, Amrein E, Estermann U, Egli J, Schweizer T, Lutz H, Ehrensperger F, Vandevelde M, Kihm Untersuchungen an 182 Nachkommen von an boviner spongiformer Enzephalopathie (BSE) erkrankten Kühen in der Schweiz. Teil 1: Klinische Befunde. Schweiz Arch Tierheilk. 1998;140:240–9.
17. Braun U, Beckmann C, Gerspach C, Hässig M, Muggli E, Knubben-Schweizer G, Nuss K. Clinical findings and treatment in cattle with caecal dilatation. BMC Vet Res. 2012;8:75.
18. Dirksen G. Pansensaftentnahme und -untersuchung. In: Dirksen G, Gründer HD, Stöber M, editors. Gustav Rosenberger. Die klinische Untersuchung des Rindes. Berlin, Hamburg: Paul Parey; 1990. p. 312–26.
19. Dubensky RA, White ME. The sensitivity, specificity and predictive value of total plasma protein in the diagnosis of traumatic reticuloperitonitis. Can J Comp Med. 1983; 47:241–4.
20. Jafarzadeh SR, Nowrouzian I, Khaki Z, Ghamsari SM, Adibhashemi F. The sensitivities and specificities of total plasma protein and plasma fibrinogen for the diagnosis of traumatic reticuloperitonitis in cattle. Prev Vet Med. 2004;65:1–7.
21. Nazifi S, Ansari-Lari M, Asadi-Fardqi J, Rezaei M. The use of receiver operating characteristic (ROC) analysis to assess the diagnostic value of serum amyloid A, haptoglobin and fibrinogen in traumatic reticuloperitonitis in cattle. Ve. J. 2009;182:315–9.
22. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Interpretation of laboratory data. In: Constable PD, Hinchcliff KW, Done SH, Grünberg W, editors. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. St. Louis, Missouri: Elsevier; 2017. p. 22–5.
23. Petrie A, Watson P. Additional techniques. In: Petrie A, Watson P, editors. Statistics for Veterinary and Animal Science. Chichester, West Sussex: Wiley-Blackwell; 2013. p. 200–29.
24. Tornquist SJ, Rigas S. Interpretation of ruminant leukocyte responses. In: Weiss DJ, Wardrop KJ, editors. Schalm's Veterinary Hematology. Ames-Iowa: Wiley-Blackwell; 2010. p. 307-13.
25. Sattler N, Fecteau G. Hypokalemia syndrome in cattle. Vet Clin North Am Food Anim Pract. 2014;30:351–7.

Figures

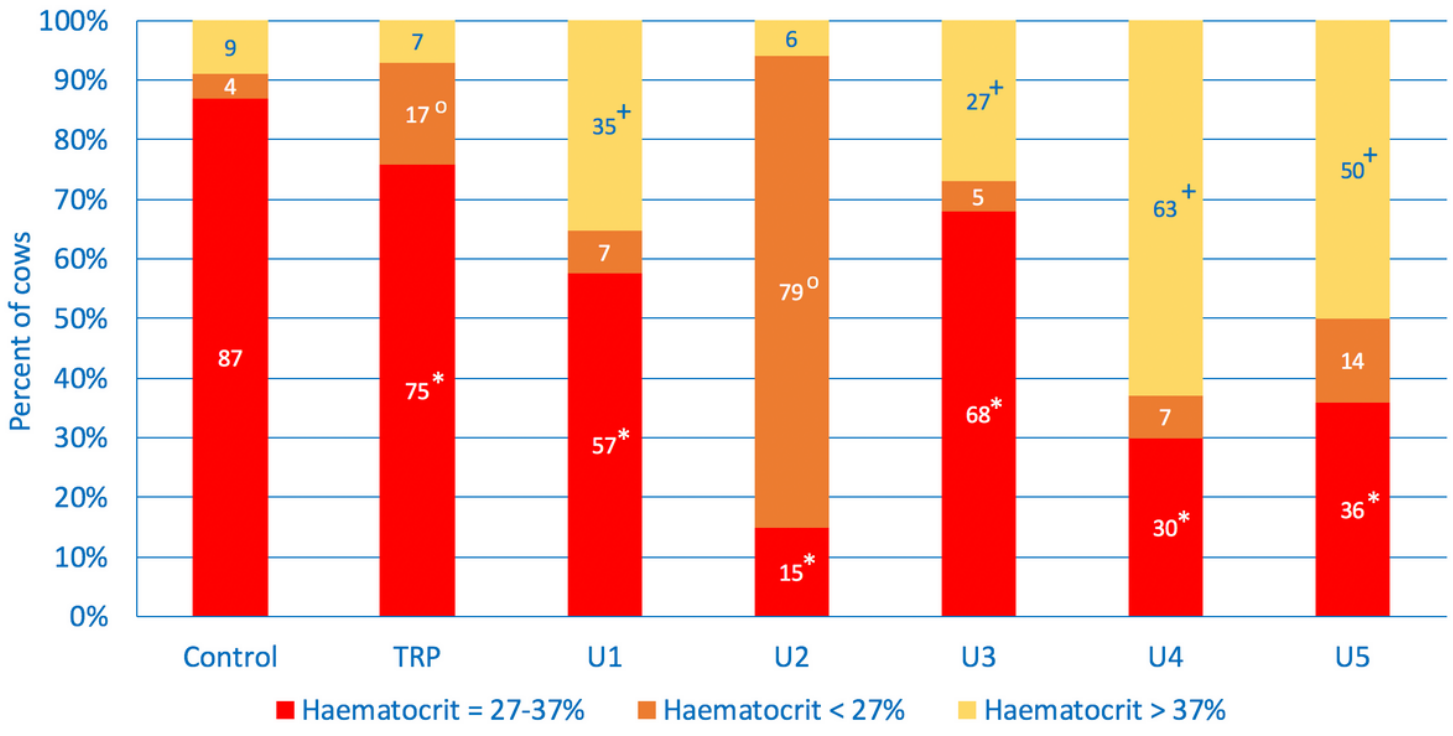

Figure 1

Frequency distribution of normal, decreased and increased haematocrit values in control cows and in cows with traumatic reticuloperitonitis (TRP) and type 1 (U1), type 2 (U2), type 3 (U3), type 4 (U4) and type 5 (U5) abomasal ulcer. *, o, + within classifications, percentages differ from controls ($P < 0.05$)

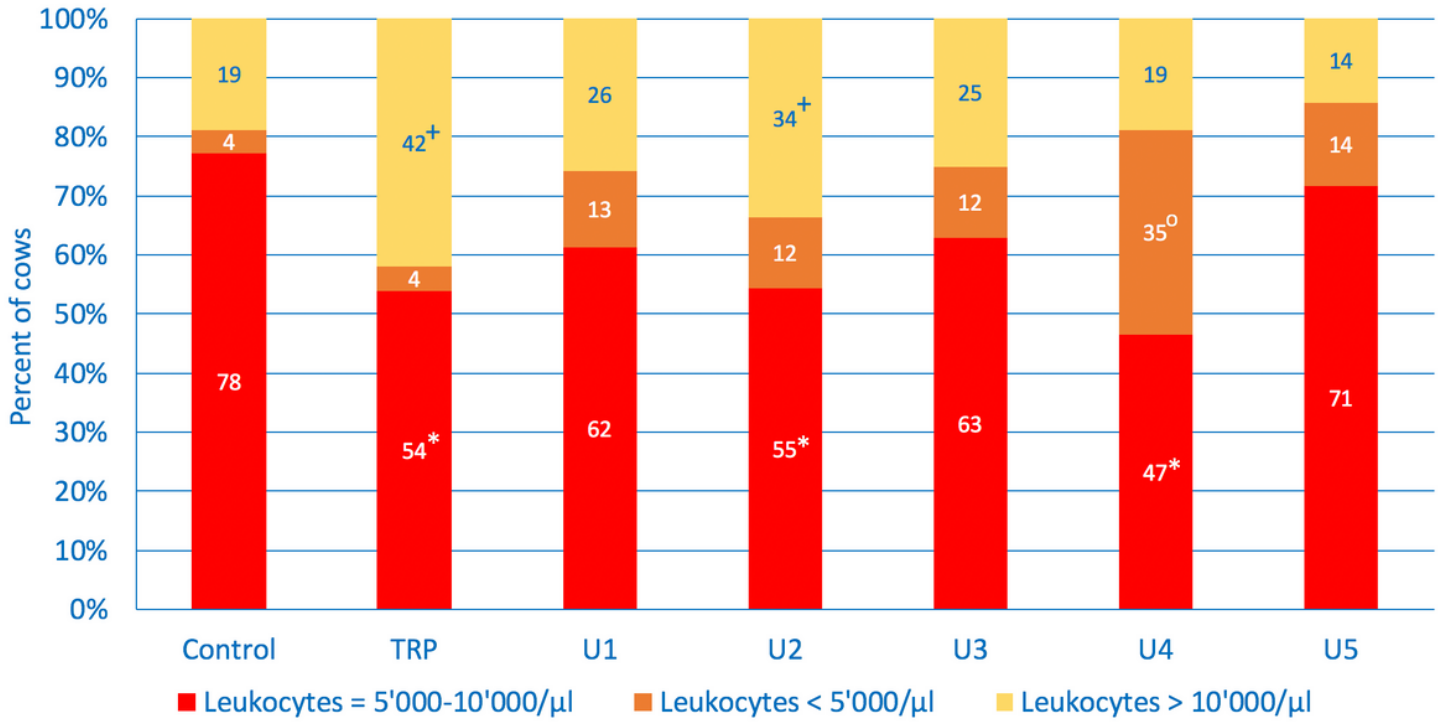

Figure 2

Frequency distribution of normal, decreased and increased total leukocyte counts in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

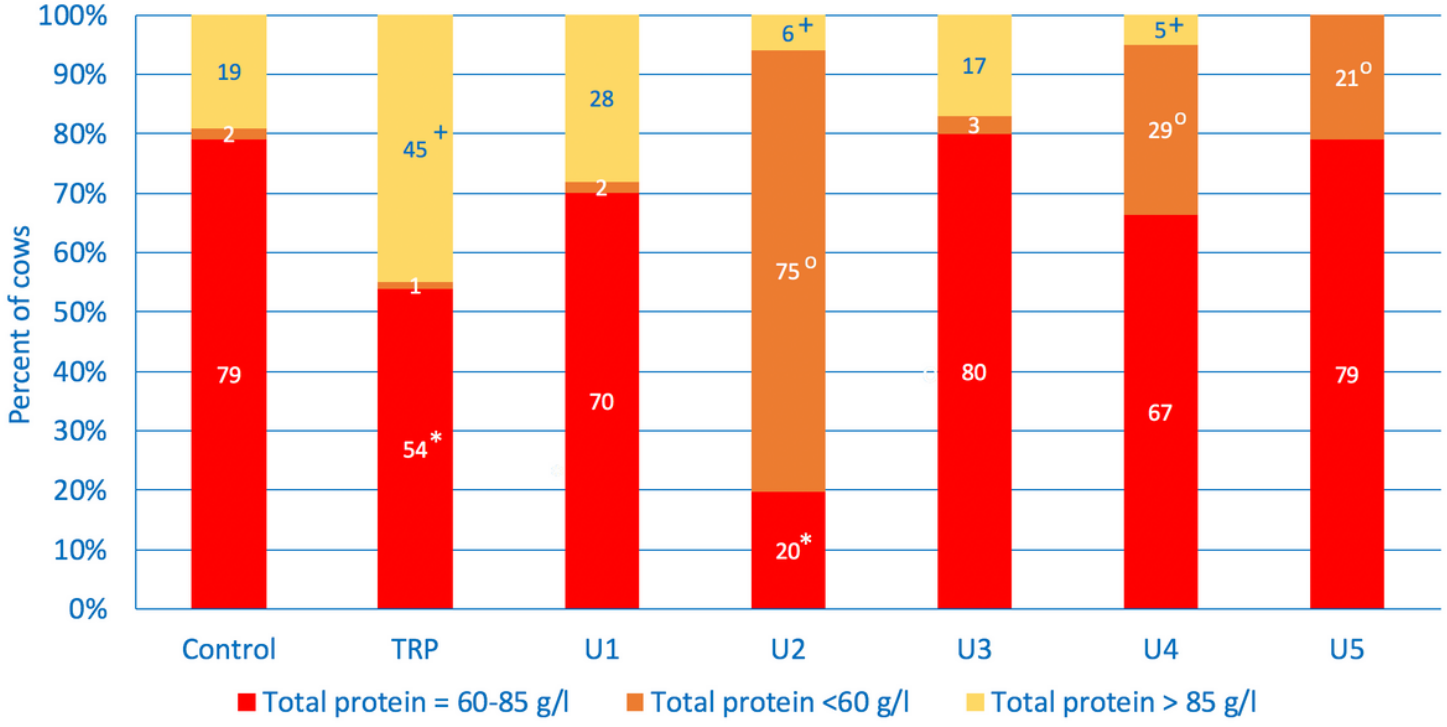

Figure 3

Frequency distribution of normal, decreased and increased total protein concentrations in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

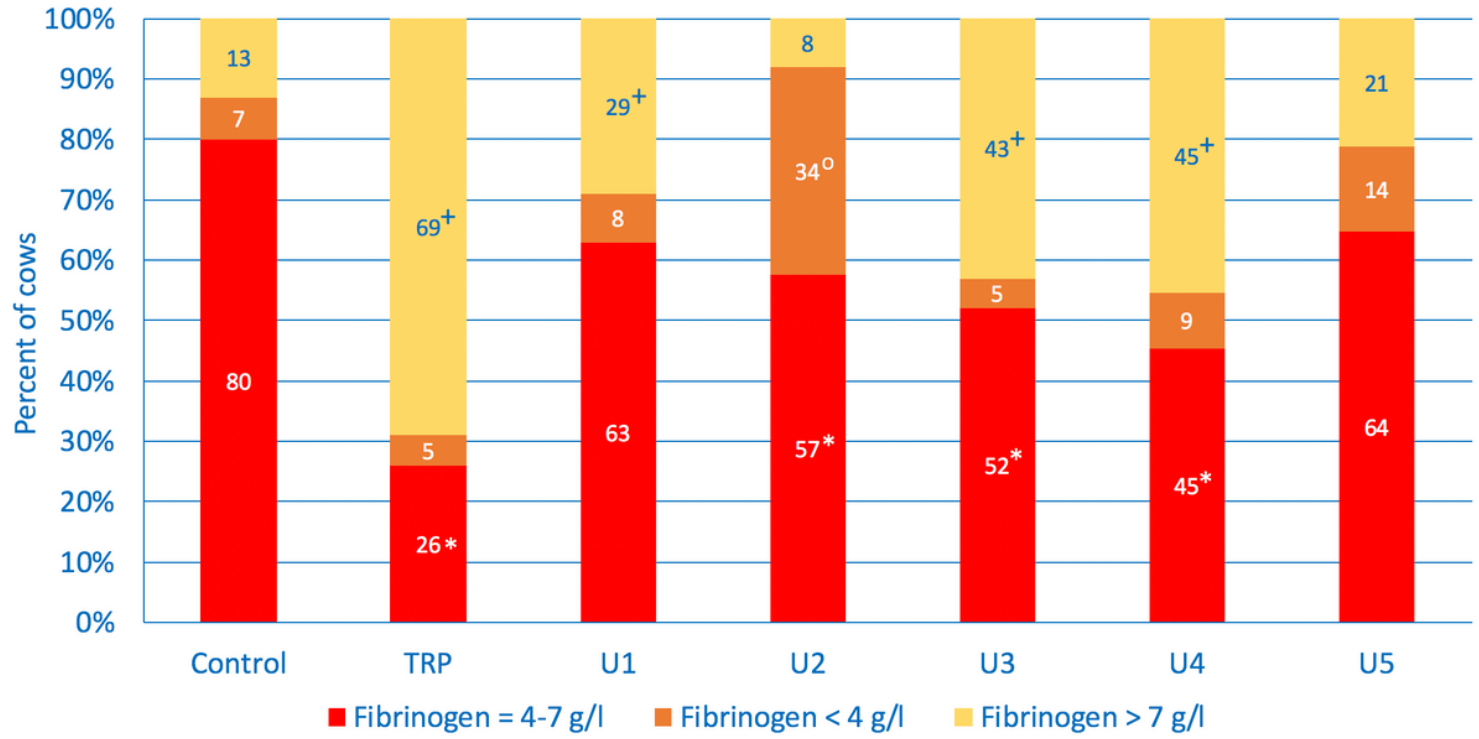

Figure 4

Frequency distribution of normal, decreased and increased fibrinogen concentrations in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

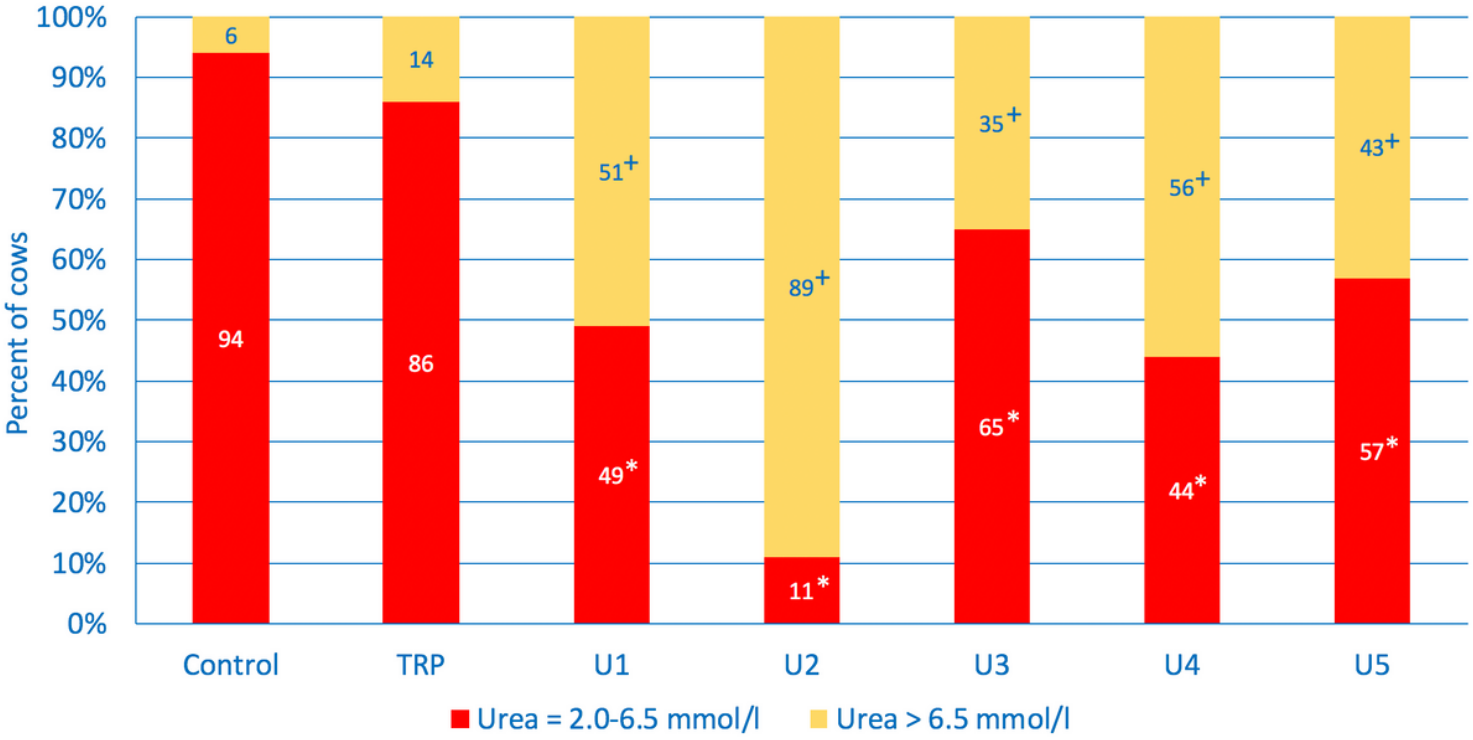

Figure 5

Frequency distribution of normal, decreased and increased urea concentration in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

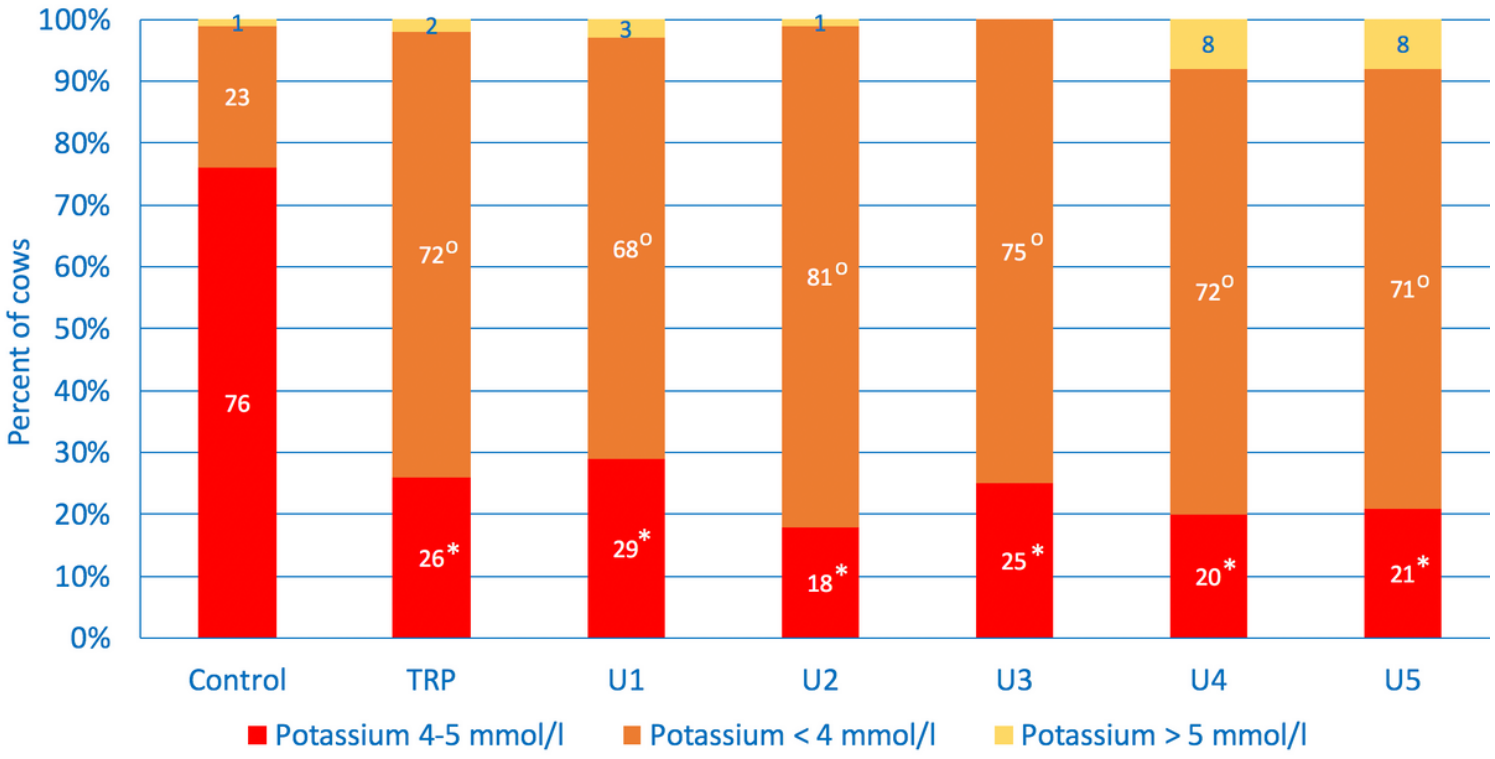

Figure 6

Frequency distribution of normal, decreased and increased potassium concentrations in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

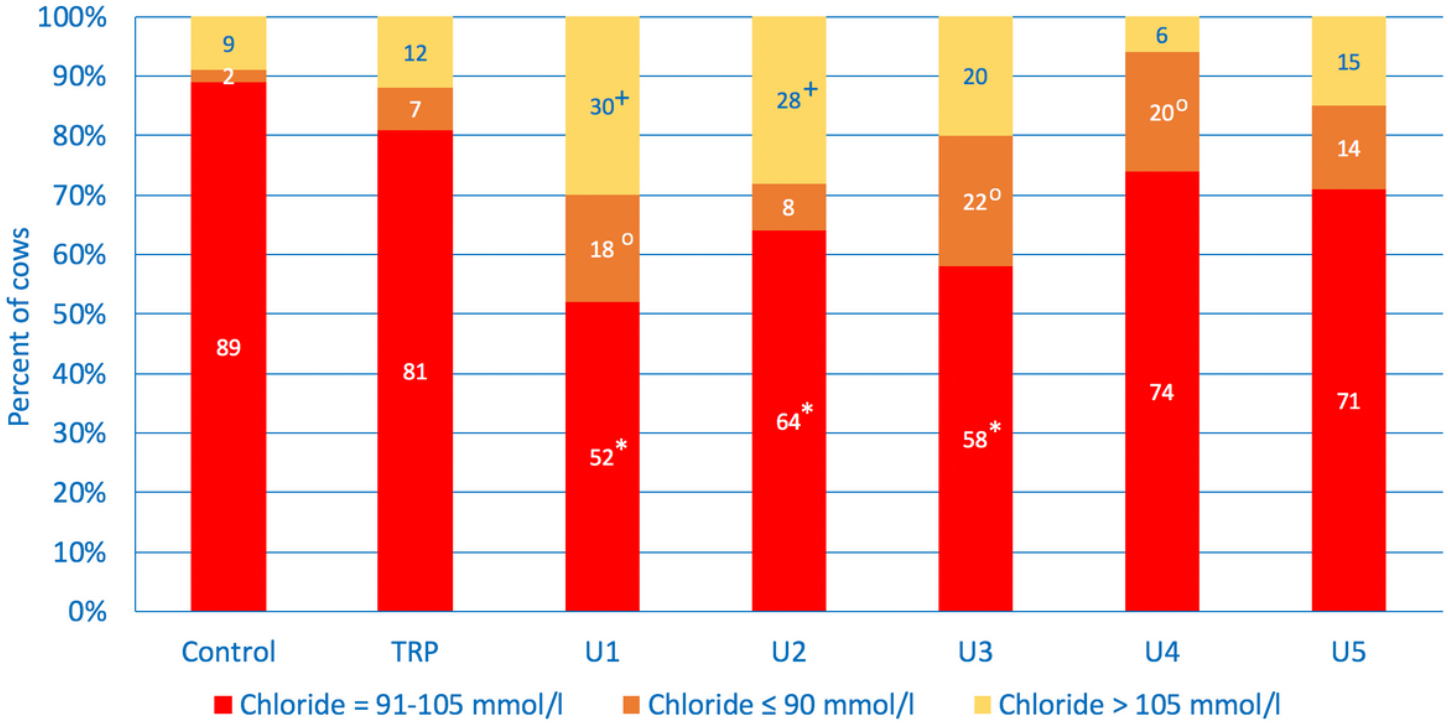

Figure 7

Frequency distribution of normal, decreased and increased chloride concentrations in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

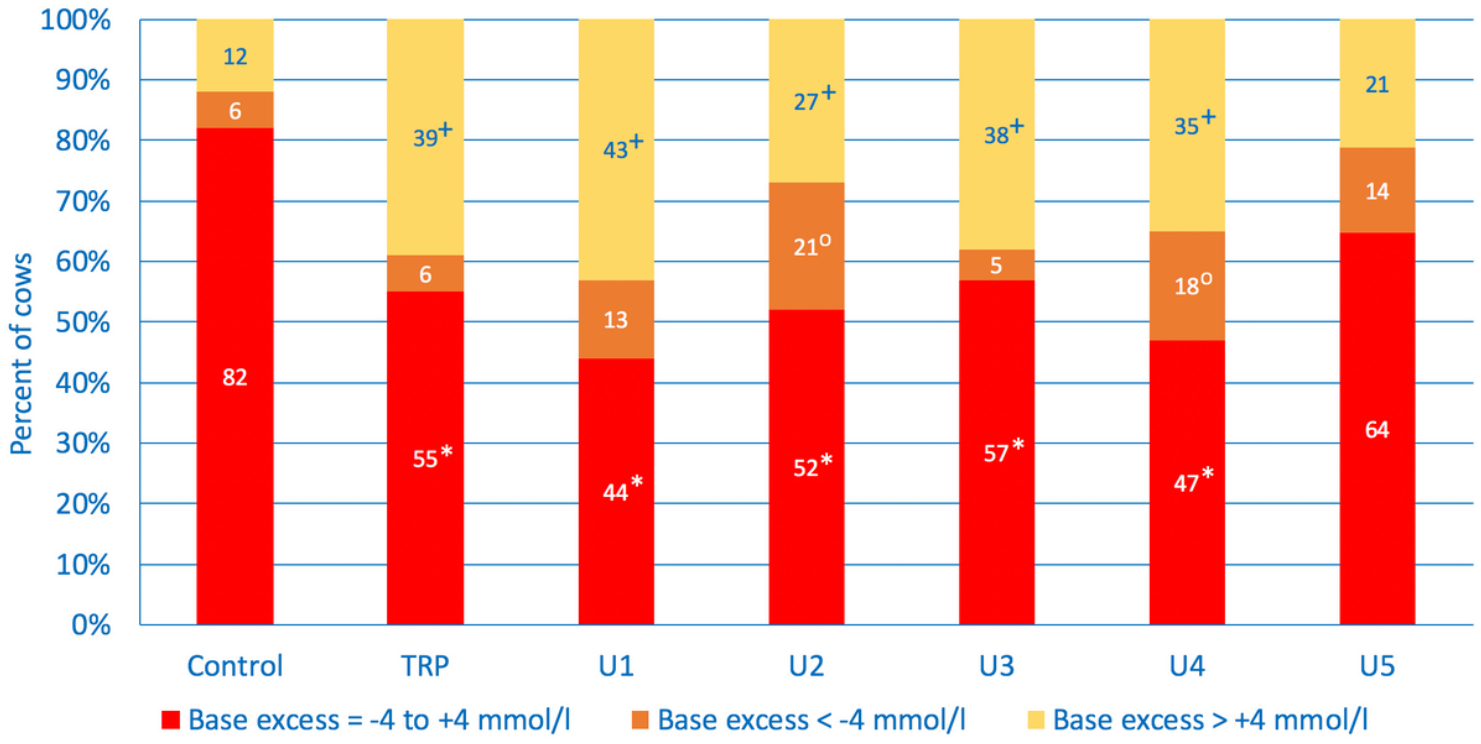

Figure 8

Frequency distribution of normal, decreased and increased base excess in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

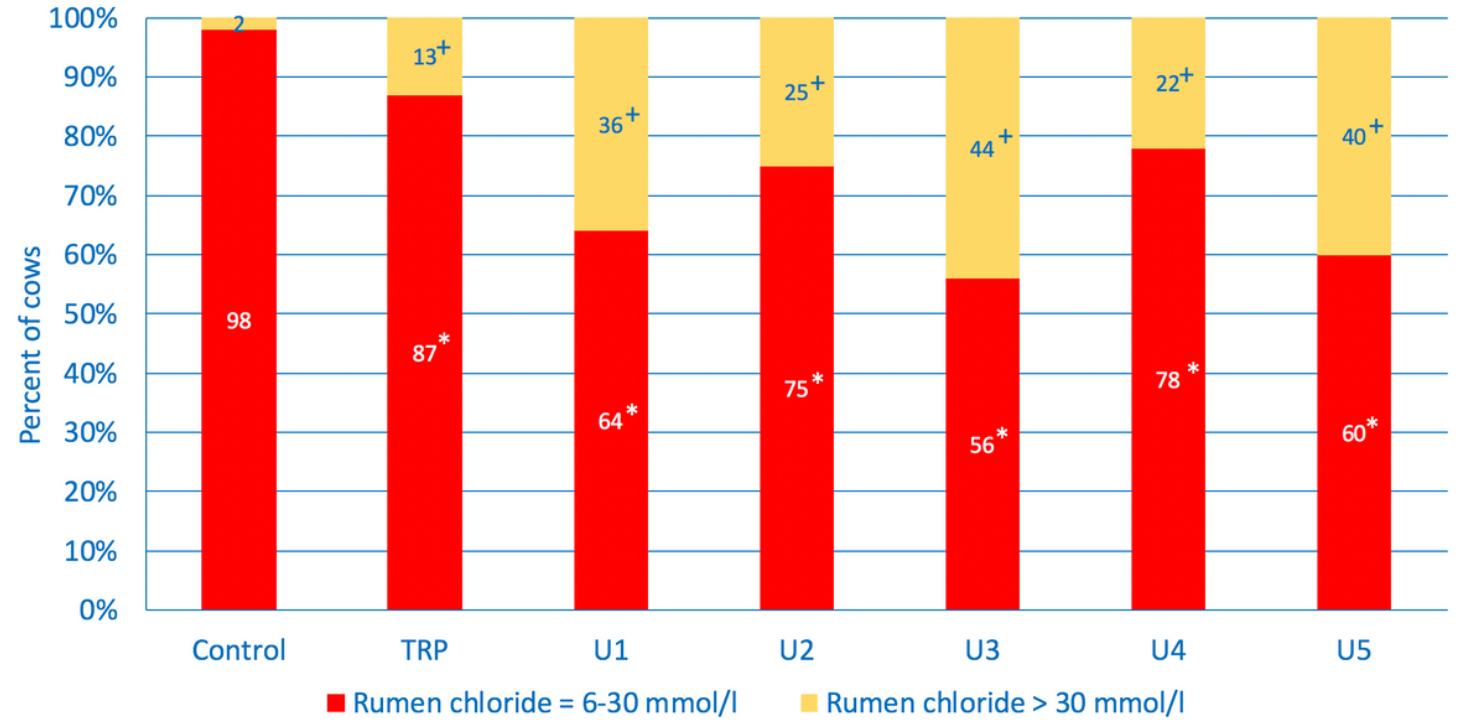

Figure 9

Frequency distribution of normal, decreased and increased rumen chloride concentrations in control cows and in cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

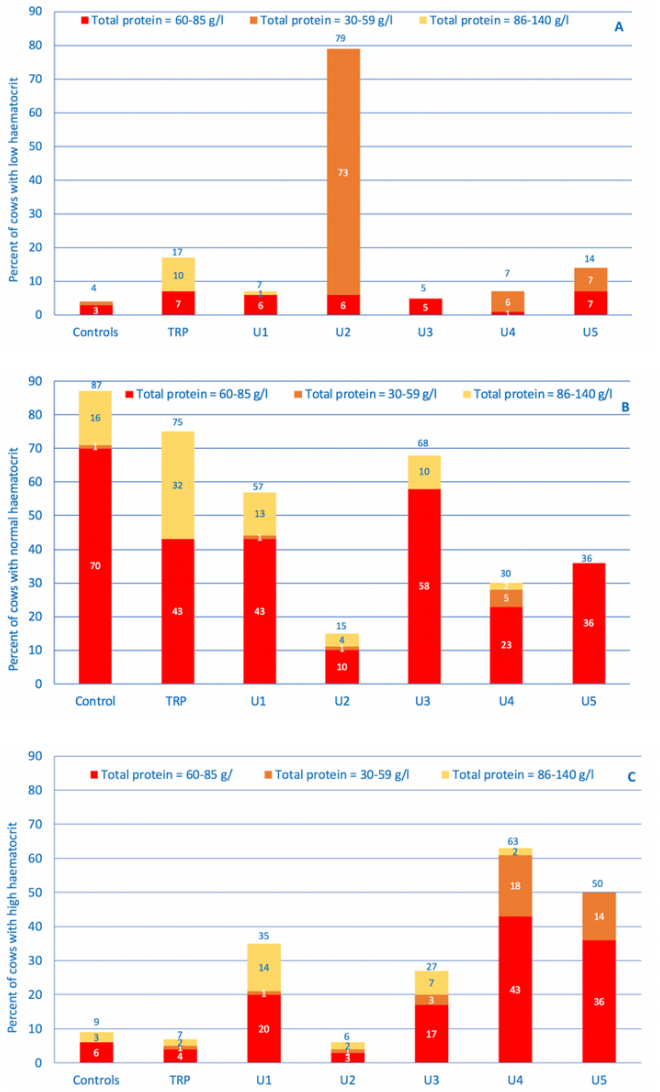

Figure 10

Frequency distribution of total protein concentrations in cows with decreased (< 27%, A), normal (27-37%, B) and increased haematocrit values (> 37%, C) in control cows and cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

Figure 11

Frequency distribution of fibrinogen concentrations in cows with decreased (< 60 g/l, A), normal (60-85 g/l, B) and increased total protein concentration (> 85 g/l, C) in control cows and cows with TRP, U1, U2, U3, U4 and U5. For key see Fig. 1

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