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An Investigation of the Ability of the Glutaraldehyde Test to Distinguish between Acute and Chronic Inflammatory Disease in Horses

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Brink P, Wright JC, Schumacher J: An investigation of the ability of the glutaraldehyde test to distinguish between acute and chronic inflammatory disease in horses. Acta vet. scand. 2005, 46, 69-78. – The glutaraldehyde test (GT), a rapid and inexpensive test, has been utilized empirically for many years in bovine practice for diagnosing inflammatory diseases. GT is used primarily to demonstrate increased serum concentrations of fibrinogen and globulin. Glutaraldehyde binds with free amino groups in fibrinogen and immunoglobulin to create a clot in a first degree chemical reaction. The clotting time of the GT estimates the content of proteins produced in response to inflammation. The applicability of GT for diagnosing inflammation in the horse has never been investigated. The objective of this study was to determine the ability of GT to distinguish between acute and chronic inflammatory disease in horses. Thirty-seven horses with suspected inflammatory diseases were evaluated using the GT, history, complete clinical examination and routine blood analysis. GT-times, laboratory results and clinical outcome were compared statistically. Horses that were determined to be acutely affected (based on history, clinical examination and routine blood analysis) tended to have a negative GT (75%). Results of the GT did not correlate with blood fibrinogen concentration. Positive GT also predicted a fatal outcome in 69% of the clinical cases. The results of this trial indicate that GT can be a useful screening test to distinguish between acute and chronic inflammatory disease in horses.

Glutaraldehyde test, inflammation, horse diseases, equine, diagnostic techniques, prognosis, immunoglobulin, globulin, blood clot, infectious diseases, hypergammaglobulinemia, serum biochemistries.

Introduction

The glutaraldehyde reagent in the glutaraldehyde test (GT) creates a clot with either fibrinogen or gammaglobulin in EDTA-stabilized blood by chemical reaction between the aldehyde groups in glutaraldehyde and free amino groups in fibrinogen and immunoglobulins (*Sandholm* 1974a, *Sandholm* 1974b, Martin et al. 1985). The process is believed to run as a first degree chemical reaction, where the reaction time is directly proportional to the concentration of fibrinogen and immunoglobulins (*Sandholm* 1974a, *Sandholm* 1974b, *Eriksen* 1984).

The rapid and inexpensive GT has been used with success empirically in Europe for many years for diagnosing inflammatory diseases in cattle (*Sandholm* 1974a, *Sandholm* 1974b, *Liberg et al.* 1975a, *Liberg et al.* 1975b, *Nielsen*

1975, *Martens* 1977, *Liberg* 1978, *Tennant et al.* 1979, *Liberg* 1981, *Liberg* 1982, *Eriksen* 1984, *Keulen et al.* 1984, *Doll et al.* 1985, *Larsson* 1985, *Mahlin et al.* 1985, *Chadli & Mahin* 1986, *Kovac* 1988, *Katholm & Jorgensen* 1992, *Kantor et al.* 1993, *Tyler et al.* 1996, *Sen et al.* 2000, *Ramprabhu et al.* 2002), pigs (*Liberg* 1979, *Hansen* 1985, *Kovac et al.* 1993), goats (*Satpathy et al.* 1996, *Vihan* 1989), mink (*Sandholm & Kangas* 1973), dogs (*Sandholm & Kivisto* 1975, *Wolff* 1986), and zoo animals (*O'-Rourke & Satterfield* 1981, *Carstairs-Grant et al.* 1988, *Juyal & Uppal* 1995). In these species, the test was used to indicate whether an inflammatory disease was acute or chronic (*Doll et al.* 1985, *Chadli & Mahin* 1986).

The GT, because of its simplicity, is very useful in bovine practice for rapidly diagnosing inflammation under circumstances where it is not practical or economically possible to have blood analyzed at a professional clinical laboratory (*Sandholm* 1974a, *Sandholm* 1974b, *Liberg et al.* 1975a, *Liberg et al.* 1975b, *Nielsen* 1975, *Martens* 1977, *Liberg* 1978, *Tennant et al.* 1979, *Liberg* 1981, *Liberg* 1982, *Eriksen* 1984, *Keulen et al.* 1984, *Doll et al.* 1985, *Larsson* 1985, *Mahlin et al.* 1985, *Chadli & Mahin* 1986, *Kovac* 1988, *Katholm & Jorgensen* 1992, *Kantor et al.* 1993, *Tyler et al.* 1996, *Sen et al.* 2000, *Ramprabhu et al.* 2002).

A negative GT can be used as a semiquantitative indicator of hypogammaglobulinemia caused by failure of passive transfer of colostrum in neonatal foals (*Beetson et al.* 1985, *Clabough et al.* 1989, *Saikku et al.* 1989, *Clabough et al.* 1991, *Kumaran & Bhuvanakumar* 1994, *Kalinbacak & Or* 1996, *Bruijn et al.* 2003), calves (*Tennant et al.* 1979, *Keulen et al.* 1984, *Larsson* 1985, *Kovac* 1988, *Tyler et al.* 1996, *Sen et al.* 2000), kids (*Vihan* 1989, *Satpathy et al.* 1996), and zoo ruminants (*O'-Rourke & Satterfield* 1981, *Carstairs-Grant et al.* 1988, *Juyal & Uppal* 1995). The GT also has been used to determine the content of IgG in mare colostrum (*Jones & Brook* 1995, *Ezhilan & Bhuvanakumar* 1998).

Clinical experience indicates that the GT may not be as reliable in horses as it is in cattle (*Nielsen* 1975). In horses, lack of reliability of the GT has been proposed to be caused by generally lower or delayed peaks of concentrations of fibrinogen and immunoglobulin or a different distribution of immunoglobulins (IgG, IgM, IgA) compared to cattle (*Bendixen* 1954, *Nansen & Nielsen* 1966, *Sandholm* 1974a, *Nielsen* 1975, *Aasted et al.* 1989).

The purpose of this clinical trial was to determine the ability of GT to distinguish between acute and chronic inflammatory disease in horses. During the trial we compared indicators of inflammation (the concentration of blood fibrinogen and serum globulin) to the GT.

Materials and methods

Thirty seven horses admitted for investigation of suspected inflammatory disease were evaluated using the GT (Glutarvac[a]), a complete clinical examination, CBC and routine serum biochemistries that included total protein, albumin, globulin and fibrinogen. Blood for the GT and laboratory analysis was collected at the same time either upon arrival at the hospital or the following day.

Horses having a history of clinical signs of inflammatory disease of total duration six days or less were arbitrarily classified as acutely inflamed. Horses with a history of clinical signs greater than six days were arbitrarily classified as chronically inflamed. The clinical examination leading to the diagnosis and etiology was also used to reinforce the distinction between acute and chronic disease (Table 1).

The GT was performed by adding equal amounts of fresh blood and glutaraldehyde in a test tube, mixing by slowly turning the test tube and visually observing and noting the time re-

Table 1: Diagnosis and outcome.

Horse #	Diagnosis	Duration	Outcome
1	Purulent, bilateral guttural pouch empyema	Chronic	Fatal (spontaneous)
2	Dorsal rectal abscess	Chronic	Discharged
3	Traumatic, infected joint capsular laceration	Acute	Discharged
4	Dorsal rectal abscesses	Chronic	Discharged
5	Purulent nephritis, lung abscesses, ulcerous dermatitis, myocarditis, fatty liver	Chronic	Fatal (euthanasia)
6	Severe, idiopathic, systemic infection	Acute	Fatal (spontaneous)
7	Purulent (jugular) thrombophlebitis (abscess)	Chronic	Discharged
8	Transportation syndrome, bronchitis/pleuritis, systemic infection	Acute	Discharged
9	Fibrinopurulent pleuropneumonia	Acute	Fatal (euthanasia)
10	Systemic, malign lymphoma, borrelia infection	Chronic	Fatal (euthanasia)
11	Infected tendovaginitis	Acute	Discharged
12	Intraabdominal abscess, squamous cell carcinoma (ventricle)	Chronic	Fatal (euthanasia)
13	Septic, purulent arthritis	Chronic	Discharged
14	Fibrinopurulent pleuropneumonia	Acute	Fatal (euthanasia)
15	Septicemia, pneumonia, peritonitis	Acute	Fatal (euthanasia)
16	Severe, purulent, traumatic muscle laceration	Chronic	Discharged
17	Severe, iatrogenic, muscle abscesses	Chronic	Discharged
18	Purulent osteomyelitis	Chronic	Fatal (euthanasia)
19	Severe subcutaneous infection/abscess, funiculitis	Chronic	Discharged
20	Humerus fracture, subcutaneous infection/abscess	Chronic	Discharged
21	Bacterial diarrhea	Acute	Fatal (euthanasia)
22	Abscess, inguinal region	Chronic	Discharged
23	Scrotal abscesses, postoperative castration	Chronic	Discharged
24	Necrotizing myositis, multiple subcutaneous abscesses	Chronic	Discharged
25	Fibrinopurulent septic bicipital bursitis, muscular septic cellulitis	Chronic	Fatal (euthanasia)
26	Pericarditis, mitral insufficiency, systemic infection	Chronic	Fatal (euthanasia)
27	Septic peritonitis	Chronic	Discharged
28	Septic meningitis	Acute	Discharged
29	Septicemia, premature foal	Acute	Discharged
30	M. Masseter, throat latch, parotid, jugular abscesses/fistulae	Chronic	Discharged
31	Systemic infection, septic myositis	Chronic	Fatal (euthanasia)
32	Systemic infection, possible abdominal/kidney abscess, emaciation	Chronic	Fatal (euthanasia)
33	Severe, multiple, purulent, septic arthritis	Chronic	Fatal (euthanasia)
34	Metritis, purulent peritonitis, abdominal abscesses, adherences	Chronic	Fatal (euthanasia)
35	Purulent, pharyngeal inflammation, choke	Acute	Discharged
36	Thrombosis pulmonary vessels, Cushing disease, laminitis	Chronic	Fatal (euthanasia)
37	Systemic intoxication, parasitic aneurysm, intestinal volvulus, paralysis	Acute	Fatal (euthanasia)

Table 2: Categorization of GT-time.

Group #	GT-times	Empiric categorization
1	0 < GT-time < 3 min.	High increase in concentration of fibrinogen and/or immunoglobulin
2	3 < GT-time < 6 min.	Moderate increase in concentration of fibrinogen and/or immunoglobulin
3	6 < GT-time < 15 min.	Low increase in concentration of fibrinogen and/or immunoglobulin
4	GT >15 min.	No increase in concentration of fibrinogen and/or immunoglobulin

Table 3: Blood values

Horse #	GT-time	Albumin (min)	Globulin (g/l)	Alb/Glob (g/l)	Fibrinogen (ratio)	Total prot. (g/l)	WBC (10.9/l) (g/l)	Differential cell count leukocytes						RBC (10.12/l)	Hemoglobin (g/l)	PCV (%)
								Bands (%)	Segm (%)	Eosin (%)	Mono (%)	Lymph (%)	Baso (%)			
1	2,0	30	58	0,5	7,1	88	9,4	1	63	0	4	32	0	8,8	113	31
2	NR	30	35	0,9	7,5	65	9,3	0	42	0	5	53	0	9,7	118	33
3	NR	38	30	1,3	3,6	68	9,4	0	67	1	1	31	0	8,5	123	34
4	NR	27	35	0,8	4,7	62	7,0	3	24	2	3	66	2	8,2	110	30
5	NR	41	33	1,2	2,3	74	16,7	0	85	0	5	9	1	11,6	178	48
6	3,5	31	40	0,8	8,3	71	10,2	2	79	0	4	15	0	7,3	117	33
7	NR	36	37	1,0	7,7	73	16,0	5	78	0	1	16	0	11,7	186	50
8	NR	34	42	0,8	4,4	76	9,3	2	68	1	3	25	1	7,3	125	35
9	NR	32	37	0,9	9,9	69	9,2	11	64	0	4	21	0	8,6	144	39
10	1,0	17	63	0,3	3,0	80	15,8	0	80	1	4	14	1	1,1	27	8
11	NR	31	48	0,6	8,2	79	6,2	0	61	1	4	34	0	5,9	102	28
12	6,0	32	60	0,5	5,2	92	7,0	0	74	1	5	20	0	7,0	130	34
13	NR	34	29	1,2	5,9	63	11,1	0	68	0	3	29	0	8,0	105	31
14	5,0	18	47	0,4	7,1	65	10,3	3	59	0	8	30	0	9,2	153	45
15	NR	23	27	0,9	6,4	50	2,8	7	11	0	5	77	0	12,8	176	50
16	NR	33	25	1,3	2,9	58	7,4	1	68	6	1	23	1	7,8	136	38
17	NR	31	29	1,1	5,4	60	7,3	0	56	2	6	36	0	10,1	168	48
18	NR	27	25	1,1	4,9	52	35,4	0	94	0	3	3	0	6,5	137	39
19	3,0	29	58	0,5	6,8	87	19,2	1	72	2	1	24	0	5,8	85	26
20	NR	33	20	1,7	12,2	53	15,0	0	75	0	6	19	0	9,1	121	35
21	NR	20	18	1,1	7,0	38	36,6	0	90	0	1	9	0	10,3	136	37
22	3,0	21	69	0,3	5,0	90	30,7	0	85	1	1	13	0	6,3	92	24
23	NR	21	36	0,6	6,0	57	7,5	0	54	2	2	41	1	6,3	107	28
24	NR	21	19	1,1	5,0	40	13,7	1	87	0	2	10	0	5,5	72	19
25	NR	36	31	1,2	6,6	67	8,6	0	77	0	8	14	0	5,6	101	27
26	NR	35	22	1,6	6,0	57	10,8	0	86	2	2	10	0	8,3	141	41
27	5,0	31	46	0,7	9,0	77	13,5	0	72	0	3	25	0	7,4	119	32
28	NR	25	41	0,6	10,0	66	33,4	0	93	0	6	1	0	10,5	126	32
29	NR	32	16	2,0	5,0	48	0,8	0	16	0	0	84	0	7,7	117	31
30	NR	28	21	1,3	14,7	49	26,4	0	72	0	9	18	1	8,1	98	25
31	15,0	40	27	1,5	7,3	67	11,5	1	78	1	2	17	1	6,8	118	31
32	NR	36	19	1,9	4,6	55	15,0	2	42	2	7	47	0	9,3	116	32
33	15,0	29	44	0,7	4,8	73	8,0	1	45	0	9	45	0	8,0	128	35
34	14,0	21	23	0,9	5,8	44	9,4	0	90	2	2	6	0	6,2	114	31
35	2,0	31	42	0,7	11,0	73	12,0	0	75	1	2	21	1	5,1	83	20
36	3,0	32	41	0,8	5,0	73	16,7	0	93	0	2	5	0	4,3	80	20
37	NR	33	39	0,8	6,3	72	11,9	0	76	0	7	17	0	11,7	185	53

* NR = no reaction

Table 4: GT result versus mean blood values (+/- standard deviation).

	Albumin (g/l)	Globulin (g/l)	Alb/Glo (ratio)	Fibrinogen (g/l)
GT-positive	27,9 (+/- 6,6)	47,5 (+/- 13,7)	0,7 (+/- 0,3)	6,6 (+/- 2,1)
GT-negative	30,7 (+/- 5,7)	29,3 (+/- 8,7)	1,1 (+/- 0,4)	6,6 (+/- 2,9)
All horses	29,7 (+/- 6,1)	36,0 (+/- 13,6)	1,0 (+/- 0,4)	6,6 (+/- 2,6)

Table 5: Clinical parameters versus mean blood values.

		GT-positive (%)	Albumin (g/l)	Globulin (g/l)	Alb/Glo (ratio)	Fibrinogen (g/l)
Duration	Acute	23,1	26,7	43,0	0,6	8,8
	Chronic	76,9	28,2	48,9	0,7	5,9
Outcome	Fatal	69,2	27,8	44,8	0,7	6,0
	Discharged	30,8	28,0	53,8	0,6	8,0

Table 6: GT-time groups versus mean blood values within groups (+/- standard deviation).

Group #	GT-positive (No)	Mean Globulin (g/l)	Mean Alb/Glo (ratio)	Mean Fibrinogen (g/l)
1: (0<GT-time<3 min.)	6	55,2 (+/- 11,3)	0,5 (+/- 0,2)	6,3 (+/- 2,7)
2: (3<GT-time<6 min.)	4	48,3 (+/- 8,4)	0,6 (+/- 0,2)	7,4 (+/- 1,7)
3: (6<GT-time<15 min.)	3	31,3 (+/- 11,2)	1,0 (+/- 0,4)	6,0 (+/- 2,0)
4: (GT-time>15 min.)	24	29,3 (+/- 8,7)	1,1 (+/- 0,4)	6,6 (+/- 2,9)

quired for full clot formation. The test result was categorized respectively as high, moderate, low or no increase in concentration of fibrinogen and/or immunoglobulin based on GT-time (Table 2).

The results of the GT and fibrinogen, globulin and albumin/globulin ratio were compared using regression and correlation. The association of the GT results with fatality was analyzed using chi-square. All data from the blood analysis were also tested for correlation with GT using principal component analysis.

Results

In Table 1, diagnoses, estimated duration of the diseases and outcome of the clinical cases are summarized.

In Table 3, the GT-times and results of blood analysis of the horses are summarized.

Table 4 shows the mean concentration of selected blood values for horses whose blood had positive reaction to the GT, compared to horses whose blood had a negative reaction to the GT.

Table 5 shows the comparison of selected clinical parameters and mean blood values of horses with positive GT.

The GT-times were divided into groups as listed in Table 6. Table 7 shows the correlation of GT-time and Group number versus globulin concentration and albumin/globulin ratio, respectively, by linear regression. The regression equations are also shown in Graphs 1-4. Group number did not correlate with the mean fibrinogen concentration within groups.

Among the hospitalized horses, there was a higher fatality rate in the GT positive horses (69% = 9/13) when compared to the GT negative horses (38% = 9/24); however, this finding was not statistically significant (p=0.06, Chi square test).

Table 7: GT-time and Group# correlation with globulin concentration and albumin/globulin. Linear regression and regression coefficient.

Dependent variable	Independent variable (equation)	r
GT-time	- 0,22 [globulin] + 16,33	0,61
GT-time	10,84 [albumin/globulin] - 1,21	0,67
Group #	- 0,10 [mean globulin within groups] + 6,50	0,96
Group #	4,06 [mean albumin/globulin within groups] - 0,83	0,96

Among the 37 horses, the proportion of test negatives of horses that were acutely inflamed was 75% (9/12). The proportion of acutely inflamed test negatives was significantly greater than the proportion of chronically inflamed test positives (p=0.04, Chi square test). The proportion of test positives of horses that were chronically inflamed was 40% (10/25).

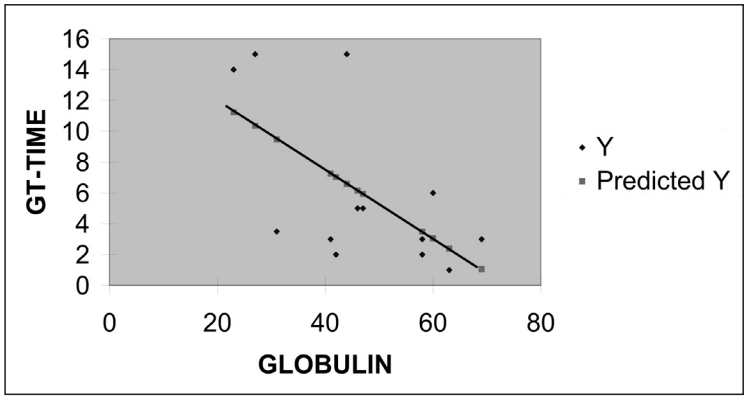
The GT did not show statistically significant correlation with the concentration of blood fibrinogen in acute or chronic diseases.

All results from the blood analyses (Table 3) were also compared to the GT using principal component analysis without finding any statistically significant correlation.

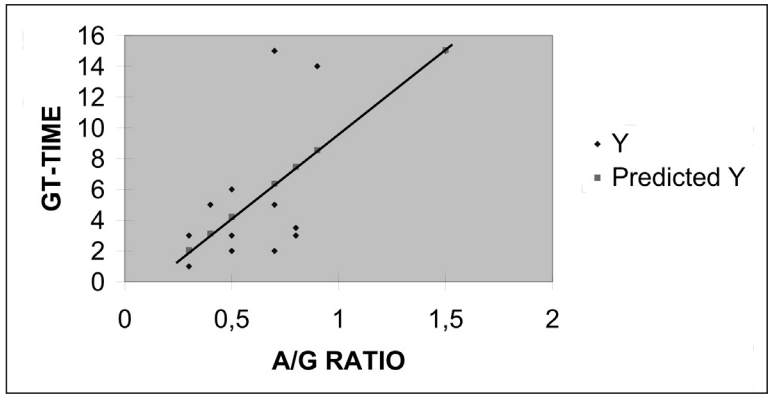
Discussion

The results of this study indicate that the GT can be used to quickly differentiate chronic from acute inflammatory disease in horses. The high proportion of test negatives of horses having acute inflammation indicates that horses with inflammatory disease and negative GT are likely to be acutely, rather than chronically, inflamed. Among GT positive horses, 77% were chronically inflamed as shown in Table 5. The GT was not reliable in predicting the blood concentration of fibrinogen in acute or chronic inflammatory diseases.

Useful clinical information could be obtained by dividing GT-times into categories (groups) as listed in Table 2 (*Liberg et al.* 1975a, *Liberg et al.* 1975b). Comparison of category and respectively globulin concentration and albumin/globulin ratio within a category seemed to correlate, although this tendency was not statistically significant. This could be due to the small number of data points. A larger number of horses included in a future study like ours would probably eliminate this statistical uncer-



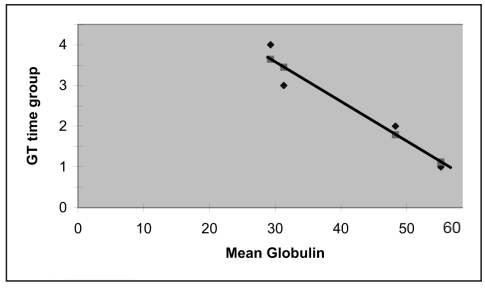
Graph 1. GT-time/globulin regression.



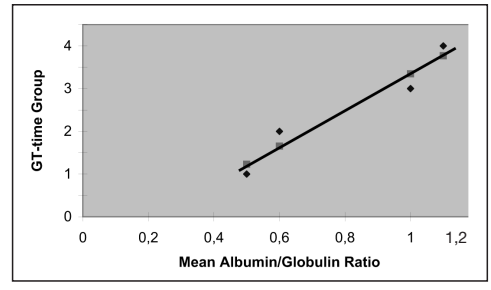
Graph 2. GT-time/A/G ratio regression.

tainty. The correlation above has been observed in cattle (*Sandholm* 1974a, *Liberg et al.* 1975a, *Liberg et al.* 1975b, *Nielsen* 1975, *Eriksen* 1984). The difference between other studies of other species and this study was that horses in Group 1 had only moderately increased globulin concentration and moderately decreased albumin/globulin ratio, Group 2 horses had a mildly increased globulin concentration and mildly decreased albumin/globulin ratio, and horses in Group 3 had a globulin concentration and albumin/globulin ratio within normal range.

If the clinical examination indicates systemic infection (eg. increased rectal temperature) and the GT is positive, the probability is high (77% likelihood) for chronic inflammatory disease. A positive GT acts then as an indicator for further laboratory analysis of blood to determine chronicity and etiology of the disease. If the test is negative, the disease is most likely acute or the systemic inflammatory response is either insignificant or absent. The GT can also be used as an additional diagnostic test to indicate prognosis because a positive test predicted fatal outcome in 69% of the clinical cases we studied. The test performance regarding the predictability of a fatal outcome might increase if only severe inflammatory diseases are included as compared to a study also including mild cases (selection bias). Also, the lack of controls will add bias to the percentages and will eliminate false positives. Because the study did not include a group of controls and a group of



Graph 3. Regression of GT time Group vs Mean Globulin.



Graph 4. Regression of GT-time Group vs Mean Albumin/Globulin Ratio.

horses suffering from non-inflammatory diseases, the data presented can only be considered valid for horses with inflammatory disease. For this reason, the conclusions are not valid for the entire population of horses. The selection of horses among patients submitted to a large referral hospital also might introduce spectrum bias as the hospitalized horses are more likely to be severely affected than horses treated in practice.

A positive GT in horses indicated the probability of increased serum concentration of globulin and a decreased albumin/globulin ratio, but the GT was not correlated with the blood concentration of fibrinogen.

Taking into consideration the low cost and rapid application of the GT and correlation of a positive test with increased concentration of globulin, the GT is a useful screening test for horses suspected to suffer from inflammatory disease.

a) Glutarvac Test tube; Jorgen Kruuse A/S, Marslev, Denmark.

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Sammendrag

Glutaraldehydprøvens evne til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest.

Glutaraldehydprøven (GP), en hurtig og billig test, har været anvendt empirisk gennem mange år i kvægpraksis for diagnosticering af inflammatoriske sygdomme. GP bliver primært brugt til at påvise øget serum koncentration af fibrinogen og globulin. Glutaraldehyd bindes til frie amino-grupper i fibrinogen og globulin, som derpå danner et blodkoagel ved en 1. grads kemisk reaktion. Koaguleringstiden af GP estimerer indholdet af de proteiner, som produceres i et inflammatorisk respons. Anvendeligheden af GP til diagnosticering af inflammatoriske tilstande i hestepraksis har aldrig været undersøgt før. Formålet med dette studie er at bestemme GPs evne til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest. 37 heste, mistænkt for inflammatorisk sygdom, blev evalueret på basis af GP, anamnese, fuldstændig klinisk undersøgelse samt rutinemæssig blodprøver. GP-tid, blodprøvesvar og klinisk udfald blev sammenlignet statistisk. De heste, som var bestemt til at være akut afficeret på basis af anamnese, klinisk undersøgelse og rutinemæssig blodprøve, tenderede mod at have negativ GP (75%). Der kunne ikke påvises sammenhæng mellem GP og fibrinogen koncentration i blodet. Positiv GP forudsagde også et fatalt udfald i 69% af de kliniske tilfælde. Resultaterne af dette studie indikerer, at GP kan være en brugbar praktisk test til at skelne mellem akut og kronisk inflammatorisk sygdom hos hest.

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