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Research Article

A Discriminant Analysis of Blood Parameters in Bovine Laminitis

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ABSTRACT

Although laminitis is seen in bovine species, due to its numerous etiological factors it is generally undiagnosed or wrongly diagnosed to be some other condition. At the same time, no confirmation could be given by laboratory method other than histamine analysis. Histamine is the confirmatory blood cytokine for laminitis, as the disease is due to production of higher level of blood histamine. But histamine analysis is expensive and not easily available; therefore, the study was conducted to find out a diagnostic index for confirmatory and low cost diagnosis of bovine laminitis. Private dairy farms in and around Khanapara, Guwahati of Assam was considered for the study. Blood samples for haematological and biochemical studies were collected from 12 clinically healthy and 12 acute laminitis animals; animals were selected after preliminary survey. Parameters studied were Haemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC), serum glucose, serum glucose, Calcium and Phosphorus (Ca and P). Serum Calcium & ESR values were found to increase significantly along with Eosinophilia and Neutrophilia in animals suffering from laminitis. On the other hand there was significant decrease in serum glucose in the diseased animals compared to control group. The results were statistically analyzed using Discriminant Analysis method. It provides a model base decision support in identifying an animal as normal or laminitis on the basis of blood parameters through the Discriminant function. Presence or absence of laminitis can be ascertained by putting values of different blood parameters in the Discriminant function as aid to posterior classification of normal or laminitis animal on the basis of blood sample in future. It could be concluded that the findings of the present investigation provides a diagnostic index or an easier way for ascertaining presence or absence of Laminitis by using average value of blood parameters.

Key words: Bovine laminitis, Haematological and biochemical parameter, Discriminent analysis, Diagnostic index

INTRODUCTION

Inflammation of the laminae is called Laminitis which results in impaired circulation of blood to the tissue of foot that produces horn. It is a disease usually seen in horses but reported in cattle as well (Sharma *et al.*, 2012; Sastry, 2004). Bovine laminitis was also reported in the area of investigation (4.289% out of total animal surveyed) with highest incidence of chronic Laminitis (2.761% out of total animal surveyed). Anamnesis also revealed that, out of different etiological factors highest incidence (73.556%, out of total animal affected) was reported for reproductive tract disorders (Sharma *et al.*, 2012). There is a noticeable economic loss in Laminitis as it is a major cause of discomfort due to severe pain and lameness in cattle (Mgasa, 1987). Many times Laminitis leads to invalidation of animal due to frequent undiagnosis or wrong diagnosis. The disease occurs due to increased production of histamines, an inflammatory cytokines, which may be due to multiplicity of the etiological factors (Garner *et al.*, 2003; Messick, 2007). Analysis of histamine from blood is costly; therefore, to give a confirmatory diagnosis is hard. For this reason clinical signs and symptoms along with laboratory analysis of blood are the only tool to make a basis of diagnosis. Several cases of this disease among the cows reared in confinement in and around Guwahati city of Assam have been detected but a thorough study, record and a diagnostic method based on haematological and/or biochemical findings of the disease is still lacking. Perhaps, due to this reason the disease is very often remain undiagnosed or wrongly diagnosed which leads the disease to chronic form. So, keeping the above mentioned fact in view, the present study has been undertaken to compare of haematological and biochemical parameters of healthy and acute

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laminitis animal in an attempt to establish a diagnostic index for the disease.

MATERIALS AND METHODS

Blood from twelve apparently healthy (control) and twelve acute laminitis animal were collected for biochemical and haematological analysis. Whole blood (2 ml) in EDTA (@ 1 mg/ml) for haematological and serum for (1.5ml) biochemical analysis were collected. Biochemical parameters estimated were Serum glucose {estimated by GOD/POD method (Trinder, 1969) and expressed in mg/dl}, Serum calcium {estimated by using Calcium Arsenazo III-Colorimetric Kit. Presentation: 2×125ml Ref.: 30160; 4×250ml Ref.: 30161} and Serum phosphorus {estimated by using Phosphorus Phospho-molybdate. U.V. Kit. Presentation: 2×125ml Ref.: 30330}. Both these kits were procured from Chemelex, S.A. Pol. Ind. Can Castells. C/ Industria 113, Nau J, 08420 Canovelles- BARCELONA. Haematological parameters viz. Hb (Haemoglobin), TLC (Total Leukocytic Count), DLC (Differential Leukocytic Count), ESR (Erithrocyte Sedimentation Rate) and (PCV) Packed Cell Volume were estimated by method stated by Jain (1986).

The data obtained were compared grossly and analyzed statistically (Snedecor and Cochram, 1967) and also by using Discriminant Analysis method (Johnson and Wichern, 2002).

The discriminant analysis is a multivariate statistical technique, which facilitates model base decision support particularly useful in classifying and separation of objects on the basis of individual characterization. It involves deriving a linear combination of predictor variables that maximizes the ratio of between groups to within group variability and subsequently discriminate the two non overlapping contrasting groups. Let X_1 and X_2 be matrices of deviation from average blood parameters *viz*. Glucose, Calcium, Phosphorus, Hb, ESR, PCV, TLC, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil in 24 animals respectively in 12 each from normal and laminitis group. If the Maximum Likelihood Estimate (MLE) of mean vector of blood parameters in normal and laminitis respectively are m_1 and m_2 in the same order then MLE for the 12 × 12 pooled variance covariance matrix,

$$S= (X_1^{/}X_1 + X_2^{/}X_2)/ (n_1+n_2-2).$$

Where, $X^{/}$ = (Glucose, Calcium, Phosphorus, Hb, ESR, PCV, TLC, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil) is a 1 × 12 vector of blood parameters.

The linear function of the form

$Z = X^{/}S^{-1}(m_1- m_2)$

is called a discriminant function (Rao, 1965). The classification rule states that if

$Z - (m_1+m_2)\ S^{-1}(m_1-m_2)/2 > 0$; an animal is assigned as laminitis animal

$Z - (m_1+m_2)\ S^{-1}(m_1-m_2)/2 < 0$ normal otherwise

Where m_1 and m_2 are mean vector of X.

The performance of discriminant function was examined by Wilk's Lamda = {|E|/|T|}; Where E is residual matrix and T is total variability matrix.

Wilks Lamda is also expressed as:

Wilk's Lamda= $i\prod/i=1\ 1/1+\lambda_i$, {i=min (p, k-1)} p is number of variable and k is number of observation per variable.

λ_i's are eigenvalues of $E^{-1}H$, where H is the hypothesized effect.

The lambda was then subjected to chi-square and F transformation for statistical test of significance for homogeneity of within-covariance matrices. Larger the between group dispersion, the smaller the value of Wilk's Lambda and greater the implied significance. The canonical correlation was computed to measures the relation between discriminant score and groups. The eigen value, percentage of variance and canonical correlation were also calculated. The reliability of the estimated discriminant function was examined by percentage of correct classification in the cross validated data in the confusion matrix. Data were analyszed by SPSS 10.0 package and interptretation of results were made by method describe above (Johnson and Wichern, 2002; Rao, 1965; Sharma and Das, 2003).

RESULTS

Clinical analysis

Compared to the control in affected animals, mean Hb values decrease insignificantly. ESR values increased significantly in the acute laminitis animals compared to the control group. In the present investigation there is significant decrease of PCV in acute laminitis group compared to the normal group. Neutrophil and Monocyte in DLC shows non-significant difference, yet significant difference was seen between Lymphocyte of normal and affected animals. Compared to the control group, in laminitis group there was significant increase of Eosinophil count.

The serum glucose value in diseased group decreased significantly to that of the control group. Though significantly increased Calcium value was found in laminitis animal compared to normal animals but no hypercalcaemia was noticed; the mean values of serum phosphorus in the of laminitis and normal animals did not differ statistically. Table no.1represents the blood profiles of normal and laminitis animals.

Statistical analysis

Blood analysis shows (table no.1) that the mean and standard error of glucose, PCV, TLC and lymphocyte were comparatively low in laminitis animals to that of the control group, on the other hand the value of Ca, ESR, neutrophil, monocyte and eosinophil were higher in laminitis animals than that of the normal animals. The differences in group means investigated by Wilks lamda as ratio of within group sums of squares to total sums of squares revealed the value of Wilk's lambda and F transformation for 1, 22 d.f. (digrees of freedom) in all individual characters as showed in the table no.1.

The test of functions Wilks Lamda and F ratio tested the hypothesis that the means of the Normal and Laminitis groups listed in the table were equal. Wilks' Lamda is the proportion of the total variance not explained by among the groups. Range of Wilks' Lamda varies from 0 and 1. Values close to 0 indicated that the group means in case of

Table 1: Showing blood profile of normal and laminitis animal

Parameters		Normal		Laminitis		Wilks'	F	P
		Mean	SE	Mean	SE	lambda	(1,22)	Value
Glucose		46.25	±0.83	35.08	±2.74	0.591	15.20**	0.00
Calcium		10.36	±0.35	11.49	±0.39	0.824	4.69*	0.04
Phosphorus		5.42	±0.17	5.26	±0.22	0.984	0.35	0.56
Hb		8.61	±0.23	8.23	±0.23	0.939	1.43	0.25
ESR		0.00	±0.00	0.67	±0.09	0.304	50.29**	0.00
PCV		28.17	±0.81	25.00	±1.22	0.826	4.63*	0.04
TLC		9.56	±0.94	7.38	±0.72	0.866	3.41	0.08
	Neutrophil	31.58	±0.84	33.83	±1.26	0.909	2.21	0.15
D	Lymphocyte	58.08	±0.87	49.92	±1.49	0.496	22.35**	0.00
L	Monocyte	1.67	±0.31	2.58	±0.66	0.932	1.59	0.22
C	Eosinophil	8.67	±0.40	13.67	±1.45	0.666	11.02**	0.00
	Basophil	0.00	±0.00	0.00	±0.00			

*indecates P(<0.05): significant ** indecates P(<0.01): highly significant

Table 2: Classification function coefficients

Blood parameters	Unstandardized coefficients	Standardized coefficients
Glucose (Gl)	-0.18	-1.23
Calcium (Ca)	0.32	0.41
Phosphorus (P)	0.13	0.09
Hb	0.08	0.07
ESR	6.14	1.41
PCV	-0.04	-0.15
TLC	0.01	0.02
Neutrophil (N)	0.16	0.59
Lymphocyte (Ly)	-0.07	-0.28
Monocyte (M)	0.50	0.90
Constant	-1.37	-
Eigenvalue	16.71	
Canonical Correlation	0.97	
Wilks' Lambda	0.056	
Chi square for 10 d.f.	48.861**	

Table 3: Functions at group centroids

Category	Function/Score
Normal	-3.91
Laminitis	+3.91

Table 4: Structure matrix showing pooled within-groups correlations between discriminating variables (blood parameters) and standardized canonical discriminant functions

Blood Parameter	Within-groups correlation
ESR	0.37
DLC Ly	-0.25
Glucose	-0.20
DLC E (a)	0.17
Calcium	0.11
PCV	-0.11
TLC	-0.10
DLC N	0.08
DLC M	0.07
Hb	-0.06
Phosphorus	-0.03

(a) This variable was discarded due to 0 tolerance.

Glucose, Calcium, ESR, PCV, Lymphocyte and Eosinophil were different. The other values close to 1 showed that group means in case of Phosphorus, Hb, TLC, Neutrophil and Monocyte were statistically at par.

Empirical discriminant function:

The standardized and unstandardized discriminant function derived to separate a normal animal and laminitis animal is displayed in the table no.2.

The unstandardized discriminant function may be written as

$Z_{\text{Unstandardized}}$ = - 1.37-0.18Gl + 0.32Ca + 0.13P + 0.08Hb + 6.14ESR - 0.04PCV + 0.01TLC + 0.16N - 0.07 Ly + 0.50 M

While the standardized discriminant function is

$Z_{\text{Standardized}}$ = -1.23Gl + 0.41Ca + 0.09P + 0.07Hb + 1.41ESR -0.15PCV + 0.02TLC + 0.59N - 0.28 Ly + 0.90 M

Eosinophil was not used in the analysis because of the fact that all variables passing the tolerance criteria were entered simultaneously while Eosinophil having Within-Groups Variance 13.61 had 0.000 tolerances in SPSS package which set minimal tolerance at 0.001. Further Basophil was also been discarded on the basis of 0.000 within-groups variance and similar minimal tolerance.

The classificatory function had eigen value 16.71 and canonical correlation coefficients computed by square root of between group sums of square to total sums of square at 0.97. The canonical correlation measured the association between the discriminant scores and the groups. Values close to 1 indicated a strong correlation between the discriminant scores and the groups. The highly significant canonical correlation indicated 97.00% of total variability was explained by differences between groups. The Wilks' Lambda of the derived discriminant function computed from the two blood parameter group (normal and laminitis) was found to be 0.056. The Chi-square transformation of the same found to be 48.86 for 10 d.f. was highly significant. The contribution of the blood parameters in separation of the animals was examined by the standardized canonical discriminant function. It revealed highest contribution from ESR **(**1.41**)** followed by Monocyte **(**0.90**)** and Neutrophil (0.59**)** and Ca **(**0.41**)** with lowest contribution of Glucose **(**-1.23**)** in derived classificatory function. The structure matrix of within-group correlations of each predictor variable with the canonical function, which provided another way to study the usefulness of each blood parameters in the derived function, also confirmed the same. The discriminant scores of the derived discriminant function in normal and laminitis animal were displayed in the table showing tests of equality of blood parameters in normal and laminitis animals**.** Now on the basis of such scores the animal separation decision rule was a positive score in laminitis animal and negative score in normal animal.

Table 5: Confusion matrix showing classification results

Category			Predicted Group Membership		Total
			Normal	Laminitis	
Original	Normal	Count	12	0	12
		Percentage	100.00	0.00	100.00
	Laminitis	Count	0	12	12
		Percentage	0.00	100.00	100.00
Cross-validated (a)	Normal	Count	12	0	12
		Percentage	100.00	0.00	100.00
	Laminitis	Count	1	11	12
		Percentage	8.30	91.70	100.00

[a]Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case; [b]100.0% of original grouped cases correctly classified; [c]95.8% of cross-validated grouped cases correctly classified.

Unstandardized canonical discriminant functions evaluated at group means found for the present investigation were mentioned on the table no.3.

The structure matrix (table no.4) contains within-group correlations of each blood parameters with the standardized canonical function. This matrix provided another way to study the usefulness of each variable in the discriminant function. It reveal that correlations with glucose, phosphorus, Hb, PCV, TLC and lymphocyte are positive while with calcium, ESR, neutrophil, monocyte and eosinophil are found to be negative. Highest positive correlation was recorded in case of ESR.

The decision rule in terms of derived function showed that linear discriminant function excellently predicted normal or laminitis group membership (Table no.4).

Further analysis by posterior probabilities of group association, squared Mahalanobis distance to centroid, and cross-validation of each animal classified by the derived function from all animals found that 100.0% of original grouped cases were correctly classified and 95.8% were cross-validated. The summery of the results is depicted in the table no.5 below.

The table no.5 measures the degree of reliability of the classification for the animals. The number and percentage of cases correctly classified and misclassified are displayed in the table. In this experiment, 24 or 100.0% of the total original animals were classified correctly. None was classified incorrectly. However, the original results provided over optimistic estimates. The cross-validation attempted to improve this problem. With cross-validation, each animal in the analysis was classified by the functions derived from all animal other than that particular animal. In this example, one laminitis animal was wrongly predicted as normal animal resulting in 95.8% of the cross-validated cases were classified correctly. It was seen that difference in the percentage of cross-validated cases with the original cases was significantly lower than 5% which advocated the use of discriminant function as aid to posterior classification of normal or laminitis animal on the basis of blood sample in future.

DISCUSSION

ESR values in the laminitis animals increases significantly compared to the normal animals, similar observation was also recorded in cattle by Nilsson, (1963). Acute inflammatory state of the laminae could be the cause of significant rise of ESR values (Sastry, 1983). In the present investigation statistically insignificant decrease Hb value was observed in affected animal, contrary to that Nilsson (1963) found statistically insignificant increase value. Significant decrease PCV value was seen in the laminitis animals compared to the normal; corroborative observation was reported in horse (Moore *et al.*, 1981). Contrary to this observation, Nilsson (1963) in cattle and Hussain *et al.*, (2004) in mules reported no statistical differences in the PCV of the laminitis and normal animals. Neutrophil and Monocyte count showed no significant difference, however mean values of Lymphocyte differ significantly between normal and laminitis animals. Conversely, there was high significant increase of Eosinophil count in the laminitis animals compared to the normal animals. Histamine, released in higher amounts in laminitis, act as chemotactic agent for eosinophil which could be the probable cause of higher eosinophil value (Messick, 2007; Kok Hin, 1972). Significant decrease in the number of Neutrophils and increased number of lymphocytes was observed in Andalusian horses affected with laminitis by Riber *et al.,* (1995).

Significantly decrease serum glucose value was found in laminitis animals compared to that of the normal animals. Increased blood glucose in acute laminitis cattle and in Andalusian horses was reported by Nilsson (1963) and Riber *et al.* (1995) respectively. Severe pain results in loss of appetite and starvation which perhaps consequently cause hypoglycemia. Calcium value of laminitis animals' increased significantly compared to that of the normal animals but there was no hypercalcaemia; at the same time there was no statistical difference between mean values of serum phosphorus in normal animals and laminitis animals. However, hypercalcaemia and hypophosphataemia was reported in cows suffering from lameness (Singh *et al.,* 1998). Blood profiles of normal and laminitis animals were presented in the table no. 1.

These above findings were not sufficient enough for confirmatory diagnosis of laminitis. Histamine analysis for confirmatory diagnosis is expensive, time consuming as well as not easily assessable in the area of the present investigation. Hence, there is a prime need of a diagnostic tool for Laminitis in fovour of the field veterinarians. Therefore, confirmation of laminitis is aimed by Discriminant Analysis. Discriminant and classification multivariate techniques was adopted because it considers all variables simultaneously resulting in increase in power

and confidence in inference with better representation of the system or event. Since all parameters in diseased and control are continuous, there were no qualitative variables, while their outcome is binary (disease or control), linear discriminant analysis was preferred over logistic regression.

Conclusion

It can be concluded from the present investigation that various haematological and biochemical changes occur in respect of laminitis in cattle. Since histamine analysis is expensive for conformation, the presence or absence of laminitis can be ascertained by putting values of different blood parameters in the discriminant function as aid to posterior classification of normal or laminitis animal on the basis of blood sample.

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