

Haematobiochemical profiles of cows shedding acid-fast bacilli vis-à-vis normal cows

R K BHARDWAJ¹, C S RANDHAWA², S S RANDHAWA³ and V K SINGLA⁴

Guru Angad Dev University of Veterinary and Animal Sciences, Ludhiana, Punjab 141004 India

Received: 23 March 2009; Accepted: 4 October 2010

ABSTRACT

The present study was undertaken to assess haematobiochemical alterations in 45 crossbred cows tested positive for JD in Johnin test and acid-fast bacilli in rectal pinch. The blood samples were collected from all cows under study to estimate haematobiochemical parameters. Hemoglobin and PCV was low in 53.3 and 64.4% of AFB shedding cows, respectively. Chronic diarrhoea was present in 42.2% of AFB shedding cows. The prevalence rate of anemia was high (64%) in diarrhoeic compared to non-diarrhoeic AFB shedding cows (36%). Anemia was of normocytic-normochromic type and of mild to moderate degree. Normal plasma iron and TIBC was seen in 48.8% of AFB shedding cows. Hypoproteinemia and hypoalbuminemia were observed in 15.5% of AFB shedding cows. Correlation coefficients between Hb-albumin and PCV-albumin were negative and nonsignificant. Low A: G ratio was observed in 15.5% and TPP: Fib. ratio was low in 40% of cows. Mean concentrations of plasma urea nitrogen and total plasma cholesterol was significantly low in cows shedding AFB. The study concluded that anemia was more prevalent in clinically JD cows. Anemia was of normocytic-normochromic type. Biochemical parameters, viz. plasma iron, TIBC, TPP, albumin, plasma urea nitrogen and total cholesterol were significantly low in JD cows.

Key words: Acid-fast shedder, Anemia, Diarrhoea, Johne's disease

Johne's disease (JD) or paratuberculosis, chronic progressive enteritis of ruminants, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is characterized by chronic continuous or intermittent diarrhoea, weight loss, emaciation and death. Though animals with clinical disease are often removed/culled from the herd, however it is subclinical that cause huge economic losses due to reduced productivity and lowered reproductive efficiency etc. Diagnosis of subclinical JD is difficult. Culture though most definitive, but is time consuming and labour intensive (Stabel 1998). Delayed type hypersensitivity (DTH) is detectable in early and subclinically infected carrier. As the disease progresses, DTH wanes and may be absent in clinical cases. Antibodies are detectable later than DTH and may also be present in carrier and animal recovered from infection. In the present paper haematobiochemical parameters of cows shedding acid-fast bacilli were studied.

Present address: ¹Assistant Professor, Division of Veterinary Clinical Medicine and Jurisprudence, Faculty of Veterinary and Animal Husbandry, R S pura, SKUAST Jammu.

^{2,3}Professor (³e mail: drswaran68@rediffmail.com), Department of Clinical Veterinary Medicine; ⁴Associate Professor, Department of Veterinary Gynecology and Obstetrics.

MATERIALS AND METHODS

Cows (45) positive for acid-fast bacilli in rectal pinch and scrapping smears and non acid-fast bacilli shedders positive in Johnin test were selected for this study. Fecal smears from cows were examined on 3 occasions for the presence of acid-fast bacilli by Ziehl's Neelson staining. Non-shedder cows for acid-fast bacilli were tested by Johnin test for the diagnosis of Johne's disease. History of the weight loss and consistency of feces was recorded. All animals were examined for general body condition, color of conjunctival mucous membrane.

Whole blood sample (2–3 ml) in dipotassium EDTA and (10–15 ml) in heparin were collected for hematology and biochemical parameters respectively. Hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC) were determined by standard methods (Jain 1986). Erythrocytic indices, viz. mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated. Peripheral blood smears were examined for red cell morphology, viz. size, staining characteristics and any red cell regeneration response of bone marrow. Rectal pinch and scrapings were taken from the cows for Ziehl's Neelson staining and demonstration of acid-fast bacilli as per Carter (1975). Total plasma Fe and total iron

binding capacity (TIBC) were determined as per Caraway (1962). Per cent transferrin saturation was calculated. Total plasma proteins and, albumin were estimated by Biuret method and BCG method, respectively. Globulins were determined by taking the difference in total plasma proteins and albumin and A/G ratio was derived. Plasma fibrinogen was estimated using heat total precipitation refractometer method as described by Jain (1986). Concentration of plasma total bilirubin (Malloy and Evelyn 1937), urea nitrogen (Ritcher and Laponite 1962) and total plasma cholesterol were estimated. Univariate analysis was done to compare for multiple comparison between groups to find out significant difference between them, using SPSS^{12.0} Software. Correlation coefficient was derived between albumin and Hb, albumin and PCV by using Hb and PCV as dependent and albumin as independent variable using Microsoft Excel computer program.

RESULTS AND DISCUSSION

Mean values of Hb and PCV of acid-fast bacilli (AFB) shedding cows are presented in Table 1. Hemoglobin and PCV was low in 53.3, 64.4% of AFB shedding cows, respectively. Anemia was of mild, moderate and severe in 33.3, 11.1 and 8.9% of AFB shedding cows, respectively (Table 1a). On account of PCV, anemia was mild and moderate in 42.2, 22.2% of AFB shedding cows, respectively (Table 1b). Clinical sign of chronic diarrhoea was noticed in 42.2% of AFB shedding cows. Anaemia was more common in diarrhoeic cows (63.0%) than non-diarrhoeic (36%) cows. Anaemia was mild, moderate and severe in 36.8, 15.7 and 10.5% of AFB shedding diarrhoeic cows, respectively. Whereas, anemia was mild, moderate and severe in 30.8, 7.7 and 3.8% of non-diarrhoeic AFB shedding cows, respectively. Anaemia was of mild, moderate and severe in 58.3, 25 and 16.7% in chronically diarrhoeic anemic cows, respectively. Whereas, it was mild, moderate and severe in 72.7, 18.1 and 9% of non- diarrhoeic anemic AFB shedding

Table 1 (a). Classification of anemia on basis of hemoglobin of AFB shedding cows (mean±SE)

Parameter	Mild (Hb=7–8) (n=15)	Moderate (Hb=6–7) (n=5)	Severe (Hb<6) (n=4)	Normal (Hb>8) (n=21)
Hb (gdl ⁻¹)	7.66±0.05 ^a	6.3±0.10 ^b	5.60±0.24 ^c	10.1±0.45 ^d
PCV (%)	23.0 0.41 ^a	21.4±0.23 ^b	20.6±0.33 ^b	27.3±0.96 ^c
TEC (10 ⁶ ml ⁻¹)	5.26±0.14 ^a	4.95±0.26 ^b	4.43±0.14 ^b	6.01±0.32 ^c
MCV (fl)	43.8±1.36 ^a	43.5±2.45 ^a	46.6±0.83 ^b	47.1±1.23 ^b
MCH (pg)	14.9±0.37 ^a	12.8±0.73 ^b	12.2±0.33 ^b	16.7±0.65 ^c
MCHC (%)	33.2±0.59 ^a	29.4±0.68 ^b	27.0±0.77 ^b	35.2 ± 0.51 ^c

Table 1 (b). Classification of anemia on basis of packed cell volume in AFB shedding cows (mean±SE)

Parameter	Mild (22–24%) (n=19)	Moderate (19–22%) (n=10)	Normal (>24%) (n=16)
Hb (gdl ⁻¹)	7.79±0.18 ^a	7.06±0.52 ^a	10.5±0.58 ^b
PCV (%)	23.1 0.21 ^a	20.6±0.16 ^b	29.4±0.82 ^c
TEC (10 ⁶ ml ⁻¹)	5.19±0.14 ^a	4.81±0.17 ^a	6.41±0.38 ^b
MCV (fl)	44.8±1.23 ^a	44.7±1.28 ^a	47.0±1.59 ^b
MCH (pg)	15.3 0.51 ^a	14.5±1.03 ^a	18.3±1.21 ^b
MCHC (%)	33.5 0.74 ^a	32.7±1.88 ^a	34.8±1.89 ^a

Means with different superscripts in row differ significantly ($P \leq 0.05$). Figures in parentheses show range. (n) refers to number of cows.

cows, respectively. Murlidharan *et al.* (2010) reported decreased values of haematological parameters in sheep affected concurrently with JD and haemonchosis. Total erythrocyte count was below $5 \times 10^6 \text{ ml}^{-1}$ in 31.1% of AFB shedding cows as compared to 52.6% of AFB shedding diarrhoeic cows. Mean values of MCV, MCH and MCHC were significantly low in AFB shedding animals compared to non-shedder cows, but were within normal physiological range as suggested by Radostits *et al.* (2000).

Senturk *et al.* (2009) also reported significantly low level of Hb, PCV and TEC in JD cows. Predominantly anemia was normocytic-normochromic in 60.9%, followed by normocytic-hypochromic in 26.1% and microcytic-normochromic in 13.0% of AFB shedding anemic cows. Anemia was normocytic-normochromic, normocytic-hypochromic and normocytic-hypochromic in 73.7, 21.0 and 5.3% of AFB shedding diarrhoeic cows, respectively. Similarly, anemia was normocytic-normochromic, microcytic-normochromic and normocytic- hypochromic in 65.4, 23.1 and 11.5% of AFB shedding non-diarrhoeic cows, respectively. Morphological classification based on direct microscopy of blood smear revealed normocytic-normochromic, microcytic-hypochromic and microcytic-hypochromic in 82.2, 8.9 and 8.9% of AFB shedding cows, respectively. Anemia was more likely to be present in

Table 1. Red cell parameters of non-anemic and AFB shedding cows (mean±SE)

Parameter	Non-anemic (n=35)	AFB shedder (n=45)
Hb (gdl ⁻¹)	10.6±0.21 ^a (9.10–14.6)	8.51±0.28 ^b (5.11–14.1)
PCV (%)	33.6±0.76 ^a (28.0–48.0)	24.8±0.61 ^b (20.0–36.0)
TEC (10 ⁶ ml ⁻¹)	6.63±0.19 ^a (5.00–9.84)	5.54±0.18 ^b (4.09–9.96)
MCV (fl)	50.9±0.92 ^a (38.3–60.6)	45.6±0.82 ^b (35.2–55.4)
MCH (pg)	16.2±0.34 ^a (12.4–20.1)	15.5±0.36 ^a (11.0–21.8)
MCHC (%)	32.3±0.72 ^a (24.8–41.9)	34.3±0.69 ^b (25.5–48.3)

clinically affected cows. Mean plasma Fe concentration of AFB shedding cows was 99.7 ± 5.24 mgdl⁻¹ and was significantly low compared to non-AFB shedder cows. Plasma Fe concentration was very low (< 50 mgdl⁻¹) and low (50 – 100 mgdl⁻¹) in 6.7 and 37.8% of AFB shedding cows, respectively. Low plasma Fe concentration (< 100 mgdl⁻¹) was observed in 63.1 and 30.8% AFB shedding diarrhoeic and non-diarrhoeic cows, respectively. Mean values of TIBC of AFB shedding cows were significantly higher as compared to non-AFB shedder cows (Table 2). Normal plasma Fe and normal TIBC concentration was detected in 48.8% of AFB shedding cows. Low-normal plasma Fe and normal TIBC, low plasma Fe and high TIBC, normal plasma Fe and high TIBC and low plasma Fe and normal TIBC were observed in 15.5, 13.3, 8.88 and 13.3% of AFB shedding cows, respectively. Percent transferrin saturation was significantly low in AFB shedding cows. Low plasma iron concentration was reported by Lepper *et al.* (1989) and Oikawa *et al.* (2000) in experimentally JD infected cows. Low TIBC and transferrin saturation with normal to decreased plasma Fe concentration occurred in anemia of chronic or non-infectious inflammatory diseases (Mc gillivray *et al.* 1985). Senturk *et al.* (2009) reported significantly low plasma iron and TIBC in JD cows. Mean concentration of TPP and albumin was significantly low in AFB shedding cows compared to non-AFB shedder cows (Table 2). Hypoproteinemia (TPP < 6.5 gd/l) and hypoalbuminemia (albumin < 2.4 gd/l) was recorded in 15.5% of AFB shedding cows. Hypoproteinemia and hypoalbuminemia was a feature in 36.8% AFB shedding diarrhoeic cows. Concurrent hypoproteinemia and hypoalbuminemia and anemia were observed in 4 AFB shedding anemic cows. Our results suggested that hypoproteinaemia and hypoalbuminemia was not a consistent feature in AFB shedding cows. In contrast Zinkl (2001) had suggested hypoalbuminemia as a characteristic clinico-pathological finding.

Szilagy *et al.* (1989) reported reduced concentration of TPP due to decreased digestion and absorption in experimentally induced JD. Furthermore the correlation coefficient derived between Hb-albumin (0.145) and PCV-albumin (0.138) was nonsignificant and negative. Hypoalbuminemia had either minor or no effect on erythropoiesis.

Concentration of globulins of AFB shedding cows did not differ significantly between non-AFB shedder cows and AFB shedder cows. However plasma globulins concentration was high in 14 of 45 AFB shedding cows and A : G ratio was low in 7 of 45 of AFB shedding cows. Increased globulins and low A : G ratio was more common in non-anemic AFB shedding cows compared to anemic AFB shedding cows. High globulins and low A : G ratio was probably not associated with anemia in AFB shedding cows.

Mean plasma concentration of fibrinogen was

Table 2. Comparative biochemical parameters of healthy and AFB shedding cows

Parameter	Non-AFB shedding cows (N=35)	AFB shedding cows (N=45)
Plasma iron (mgdl ⁻¹)	156.1 ± 7.08^a (92.5–267)	99.7 ± 5.24^b (45.6–161.5)
Total iron binding capacity (mgdl ⁻¹)	433.5 ± 18.4^a (240–680)	587.4 ± 35.7^b (594.0–961.1)
Per cent transferrin saturation (%)	39.4 ± 2.71^a (13.9–76.2)	21.9 ± 1.98^b (3.84–59.6)
Protein (gdl ⁻¹)	8.25 ± 0.09^a (7.36–9.16)	7.76 ± 0.18^b (4.42–10.2)
Albumin (gdl ⁻¹)	3.19 ± 0.03^a (2.80–3.72)	2.81 ± 0.06^b (2.10–4.00)
Globulin (gd/l)	5.02 ± 0.10^a (3.84–6.03)	4.95 ± 0.17^a (1.52–7.20)
A : G ratio	0.64 ± 0.17^a (0.48–0.91)	0.60 ± 0.03^a (0.39–1.90)
Fibrinogen (mgdl ⁻¹)	328.5 ± 18.6^a (200–600)	493.3 ± 23.9^b (200–800)
TPP: Fib. ratio	28.1 ± 1.71^a (13.3–45.0)	17.7 ± 1.19^b (8.25–44.6)
Total plasma bilirubin (mgdl ⁻¹)	0.41 ± 0.05^a (0.09–1.20)	0.29 ± 0.01^a (0.04–1.20)
Plasma urea nitrogen (mgdl ⁻¹)	17.8 ± 0.67^a (11.6–26.7)	10.9 ± 0.86^b (3.47–30.5)
Total plasma cholesterol (mgdl ⁻¹)	113.8 ± 6.66^a (59.7–210)	77.7 ± 5.31^b (26.6–171)

Means with different superscripts in row differ significantly ($P < 0.05$). Figures in parentheses show range. (n) refers to number of cows.

significantly high in AFB shedding cows compared to non-AFB shedder cows. Fibrinogen was high (< 600 mg/dl) in 38.6% of AFB shedding cows. Total plasma protein to fibrinogen (TPP: Fib. ratio) was significantly low in AFB shedding cows as compare to non-AFB shedder cows (Table 2). Low TPP: Fib. ratio was observed in 40% of cows shedding acid-fast bacilli. Low TPP: Fib. ratio ($< 15: 1$) was feature of chronic inflammatory disease or chronic infections in cattle (Morris and Johnston 2001). Mean total plasma bilirubin was comparable to non-AFB-shedder cows. Mean concentration of PUN was significantly low ($P < 0.05$) in AFB shedding cows compared to non-AFB shedder cows (Table 2). PUN was lesser than 7.5 mgdl⁻¹ in 14 of 45 AFB shedding cows. Low PUN was observed in 10 of 23 anemic AFB shedding cows and in 4 of 22 non-anemic AFB shedding cows. Low PUN concentration was more commonly observed in AFB shedding anemic diarrhoeic cows than anemic non-diarrhoeic AFB shedding cows. The AFB shedding cows with normal haemoglobin ($Hb > 8$ gdl⁻¹) had normal PUN level. Needless to say malabsorption was the cause of low PUN concentration. Similar findings were confirmed by Szilagyi *et al.* (1989) and Oikawa *et al.* (2000) in paratuberculosis affected cows. Mean value of total plasma cholesterol was

significantly lower in AFB shedder cows (Table 2). However, it was observed that 48.8% (22/45) of AFB shedder cows had low total plasma cholesterol. Low total plasma cholesterol was observed in 14 of 19 diarrhoeic cows. Low total plasma cholesterol was observed in 13 of 23 anemic AFB shedding cows and 9 of 22 non-anemic AFB shedding cows. Low total plasma cholesterol was attributed to decreased absorption. Szilagyi *et al.* (1989) also detected low total plasma triglycerides and cholesterol in paratuberculosis cows. The cause was attributed to intestinal malabsorption. Similarly low concentration of total cholesterol and phospholipids had been reported by Oikawa *et al.* (2000).

ACKNOWLEDGEMENT

The authors are highly thankful to the Dean, College of Veterinary Science for providing necessary facilities.

REFERENCES

- Caraway W T. 1962. Clinical diagnostic specificity of laboratory tests. *American Journal of Clinical Pathology* **37**: 445.
- Carte G R. 1975. *Diagnostic Procedures in Veterinary Microbiology*. 2nd edn, pp. 251–53. Charles C Thomas Publishers Springfield, Illinois, USA.
- Jain N C. 1986. *Schalm's Veterinary Haematology*. 6th edn, Pp. 31, 506, 610. Lea and Febiger, Philadelphia, USA.
- Lepper W D, Embury D H, Anderson D A and Lewis V M. 1989. Effect of cultured dietary iron intake in *Mycobacterium paratuberculosis* infected dairy cattle: sequential observations on growth, iron and copper metabolism and development of paratuberculosis. *Research in Veterinary Sciences* **46**: 289–96.
- Malloy and Evelyn. 1937. *Microanalysis in Medical Biochemistry*. 4th edn. J and A Churchill Ltd., 10W Gloucester Palace, London W I.
- Mc gillivray S R, Seary G P and Hirsch V M. 1985. Serum iron, total iron binding capacity, plasma copper and hemoglobin types in anemic and poikilocytic calves. *Canadian Journal of Companion Medicine* **49**: 286–90.
- Muralidharan J, Saravanan S, Ramesh V, Ramesh V and Kumar S. 2010. Incidence of Johne's disease and haemonchosis in a sheep. *Indian Journal of Field Veterinarians* **5**.
- Oikawa S, Katoh N, Watanabe A, Nishi H, Kurosawa T and Satoh H. 2000. Serum apolipoprotein AI evaluated as a possible marker in cows with Johne's disease. *Journal Rakuno-Gakuen University; Natural Sciences* **25** (1): 37–42 (Original not seen, Abstract in CAB abstracts).
- Radostits O M, Gay C C, Blood D C and Hinchcliff K W. 2000. *Veterinary Medicine: A Textbook of Diseases of Cattle, Sheep, Pig, Goat and Horses*. 9th edn, Pp. 414. W B Saunders, Harcourt Publishers Ltd., London.
- Ritcher H and Laponite Y. 1962. New reagent for use in determination of blood urea nitrogen with special reference to manual analysis. *Clinical Chemistry* **8**: 335.
- Senturk S, Metcitolu Z, Ulgen M, Borum, Temizel E and Kasap S. 2009. Evaluation of serum iron and iron binding capacity in cows with paratuberculosis. *Tierärztliche Praxis* **37**: 375–78.
- Stabel J R. 1998. Johne's disease: A hidden threat. *Journal of Dairy Science* **81**: 283–88.
- Szilagyi M, Kormendy B, Suri A, Tulboly S and Nagy G. 1989. Experimental Johne's disease studies on biochemical parameter in cattle. *Archives of Experimental Veterinary Medicine* **43**: 463–70 (Original not seen).