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Importance of Serum Protein Electrophoresis in Bovine Medicine (Easy Tool for Diagnosis)

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Abstract: This study aimed to search for a quick and inexpensive tool, especially available for the practitioner in bovine medicine, to detect inflammation by analysing the serum protein using electrophoresis (EPP). Sera of 200 animals underwent total bilirubin (Bil.T), gamma glutamyltranspeptidase (GGT) and alkaline phosphatase (PAL) for identifying liver function. Addionally, haptoglobin (Hp) and fibrinogen (Fb) were determined as the two main acute phase protein markers of inflammation:. The study of serum proteins by electrophoresis indicated that the majority of cattle (72.5%) had high albumin/globulin (A/G) ratio, but 38 animals (19%) had essential hypo-albuminemia. In addition, 97.5% of cattle had alpha1 globulins levels below (6gr/l). Alpha2 globulins were markedly high during bronchopneumonia. Beta-globulins increased in association to liver disease. Among the hyper-gamma globulin emitted, monoclonal peaks (8 animals) and 57 animals (28%) with poly clonal peaks were distinguished. The fibrinogen concentrations did not exceed the threshold of 5 gr/l. Haptoglobin concentration was greater than 0.41 gr/l for 10 (5%) of animals. The interpretation of the EPP associated with the assay of the other parameters, allowed the confirmation of clinically apparent cases aided to discover the subclinical liver Diseases animals from the clinical cases. In conclusion, electrophoresis of serum proteins can be considered as a practical tool for diagnosis in rural medicine.

Key words: Serum Protein Electrophoresis · Inflammation · Bovine Medicine

INTRODUCTION

The veterinary practitioner in cattle breeding research is in need for the diagnosis of diseases using practical tools, fast, inexpensive and above all available tools. For this reason, serum protein electrophoresis (EPP) was studied as a complementary support to clinical examinations, rather than other more bulky tools used in bovine medicine [1, 2]. The importance of this study lies in the reduction of various para-clinical examinations known in bovine medicine, often difficult to handle during a displacement in rural areas and which are also expensive.

MATERIALS AND METHODS

Animals: We studied 200 animals of cattle without distinction age, sex and breed. In the "Mitidja» region (north center of Algeria), distributed in 16 breeding sites, conducted above ground. Management is provided by family members with a hay diet, concentrates and market gardening derivatives. The ration often depends on the season and the practice is mixed (suckling and dairy farming).

Samples and Sampling: To arrive at the expected data,

Pre-analytical Step: on the condition of each animal was collected in an individual data sheet and included the clinically suspected pathology, CSC (body condition score) according to the method described by Edmondson *et al.* [3], age, sex and physiological stage (gestation, pre- or postpartum cows) and the conditions of production (fattening for bulls, drying up or lactation status for cows).

Analytical Step: Blood samples were collected from the jugular vein in citrate, heparin and blank tubes). After cooling according to Carlson's recommendations [4], the blood sample was centrifuged before hand at 3000 rpm for 10 minutes. The sera and plasma were harvested and stored at - 4°C. The biochemical analysis was launched on

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an automaton of biochemistry: Cobas 6000 (ROCHE), for the determination of enzymes of the liver function [5,6], Bilirubin total (Bil.T), alkaline Phosphatase (PAL), Gammaglutamyltranspeptidase (GGT), as well as two parameters for the indication of early inflammation [7,8,9]: Haptoglobin (Hb) and fibrinogen (Fb). Electrophoresis was performed on agarose gel (automated: SEBIA, Hydrasys gel).

Post-Analytic Step: For the validation of the results of veterinary clinical biology, the technical considerations of Radostits *et al*. was respected [5]. The values was compared with those reported by Kaneko [1], Smith [10] and Tourlomoussis *et al*. [11].

RESULTS AND DISCUSSION

Albumin and Globulin: Animals (55; 27.5%), had a low A/G (<0.7) compared to those previously stated [10, 12]. The higher gamma globulin (Figure 11) levels 26gr/l (M = 27.87 gr /l) observed during this study compared to other studies may refer to the difference in the breed and the gender of the animal [13, 14]. Moreover, the "sub-clinical" conditions and the chronic evolution had a peculiar effect on the bovine gamma globulin [15], 21 animals had a lack of albumin synthesis though the biochemical exploration of the liver did not reveal any hepatic insufficiency which may refer to parasitic spider infestation [6].

145 (72.5%) animals had A/G> 0.7 indicating high synthesis of albumin which is part of a hyperproteinemia, caused by rumen acidosis [10] resulting from a food imbalance (Figure 17).

Essential hypo-albuminemia (Figure 13): Nevertheless, the decrease of albumin below the threshold of 25gr/l [1,10] observed in 38 (19%) animals may refer to weight loss (SCC <3, outside lactation), a consequence of malnutrition (bad rationing). Moreover, the decrease of albumin is considered a negative marker of inflammation [16].

Alpha1 Globulin: The alpha 1 zone consists of the alpha 1 antitripsin, orosomucoid and alpha 1 anti chymotripsin. About 195 (97.5%) animal showed a low value of < 6gr/l [1, 10, 12, 14]. A congenital deficiency of α1 antitrypsin, major protein of the α1 zone may result in a normal decrease of its values. This anomaly did not lead to any specific clinical manifestation (Figures 8, 9). This is very well described in humans [17].

Alpha2 Globulin: Alpha zone 2 consists of haptoglobin, ceruloplasmin, Gc globulin, alpha 2 macroglobulin and alpha-lipoproteins. We noticed that its rate increases especially during bronchopneumonia where its concentration was higher than 9.5gr/l. Moreover, 28 (14%) of emaciated animals showed also high Alpha zone 2. The latter are suspected of developing chronic inflammatory conditions without clinical expression, which would prevent them from gaining weight [1, 10]. It was noted that this coincides with an increase in total proteinemia (Figures 10, 11). Moreover, on all the graphs we did not find any duplication of this zone, this confirms the absence of hemolysis of the collected samples [18, 19].

Beta-Globulins: They are composed by the fractions: Transférine, Heopexine, Betalipoprotein and Complement C3. This fraction showed a specific increase in hepatic diseases [10] because it is concomitant with an increase in enzymes specific for liver function (GGT, Bil.T, PAL) and total proteinemia (including the gamma globulin fraction, alpha2globulin). Only 3 (1.5%) animals on clinical examination revealed two cases of cows (6 and 8 years) postpartum, one lean (SCC less than 3) in negative energy balance and the other, with bronchopneumonia (Figures 18 and 11). In addition, the last was a case of heifer calves over 6 months old (Figure 12) that was fed a diet composed exclusively of concentrate. In this case we suspect a case of hepatic inflammation secondary to a state of rumen acidosis. Cases of hypo-beta globulin included 15 (7.5%) animal, occur simultaneously with cases of hypo albuminemia and this in the absence of increased liver function enzymes and without particular clinical signs (Figures 9, 13). Any lack of food intake is related to the physiological state and the real needs of these animals [16].

Gamma Globulins: They constitute the group of immunoglobulins (IgG, IgA, IgD, IgE and IgM): their increase is permanent during clinical affections such as lameness, bronchopneumonia and mastitis. They could not be revealed by the other biochemical parameters measured, regardless of the clinic; which makes them a good indicator of inflammation (Figures 19, 20). Their increase is not related to the clinical expression of pathology, but it sometimes coincides with that of haptoglobin, sometimes with fibrinogen. Hyper-gamma globulinemia with monoclonal peaks (narrow and homogeneous) was distinguished. This is the case of 6 months old. Except for a heifer, (Figures 1-7). The synthesis of antibodies is usually

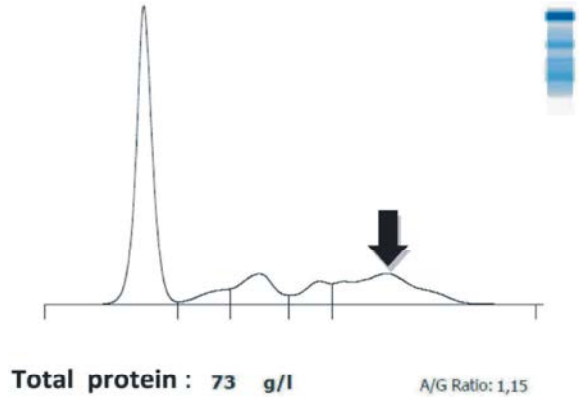

Fig. 1: Calf, under 2 months old, delay of growth, no exceptions at the level of the biochemical assays.

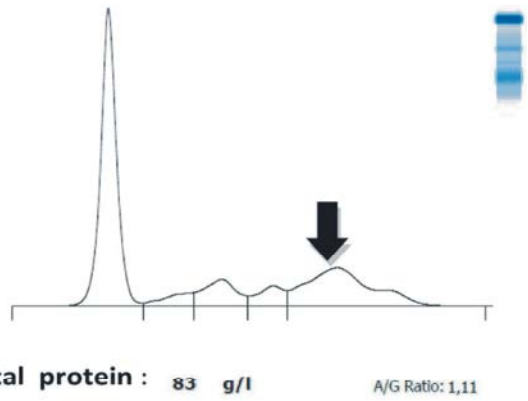

Fig. 2: Calf, less than 4 months old, delay of growth, no exceptions at the level of the biochemical assays.

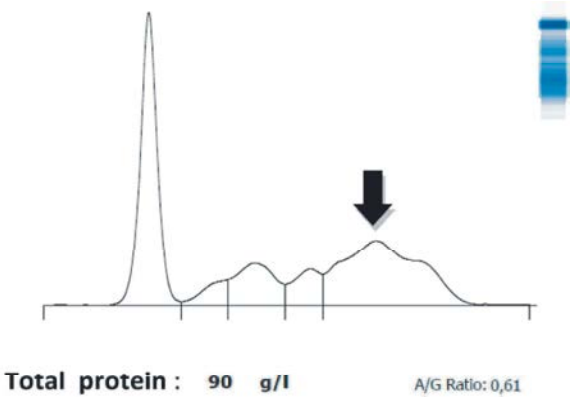

Fig. 3: Calf, aged under 3 months, delay growth, suffering from arthritis confirmed by Biochemistry: increase of proteinemia (90 gr/l), α2-globulin (10.8 gr/l) and γ globulins (34,56gr/l), the PAL (224 U/L).

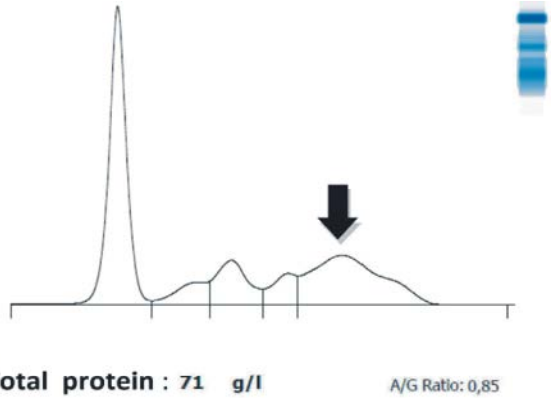

Fig. 4: Young bull, 6 months old, delayed growth, biochemistry reveals only an increase in PAL (146 U/l) that is witness to the bone growth, noting a peak in the globulins α2 without its value (8,31U/l).

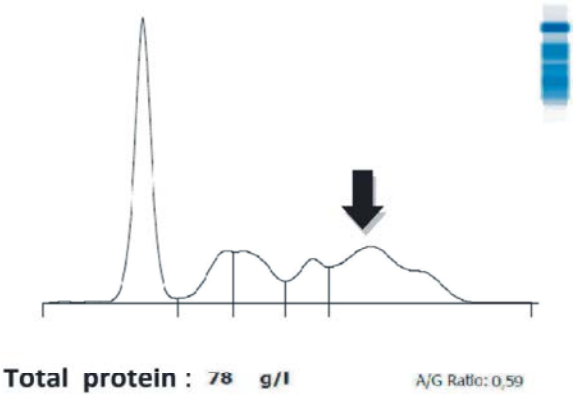

Fig. 5: Heifer, suffering from Broncho- pneumonia associated with liver disease revealed by an increase: alpha2-globulin (10, 3 gr/l), Bil.T (9 gr/l), GGT (42U/l).

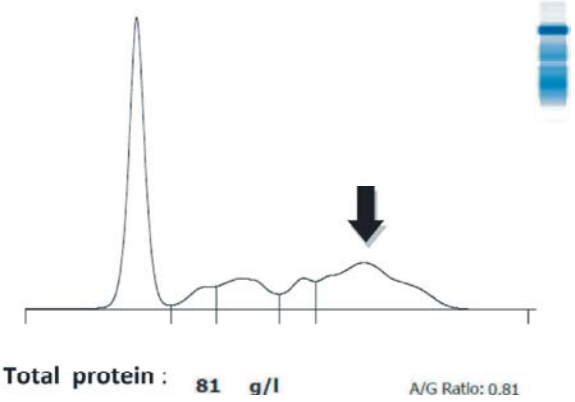

Fig. 6: Young heifer, aged 6 months, characterized by stunted growth. Biochemistry: increase of the α2 globulins (9,88 gr/l), of the PAL (281U/l) witness the bone growth.

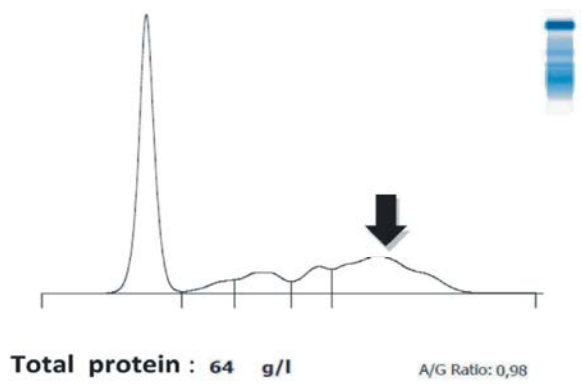

Fig. 7: Young Bull, aged less 6 months, characterized by stunted growth. Biochemistry: increase of the PAL (155 U/l) witness the bone growth.

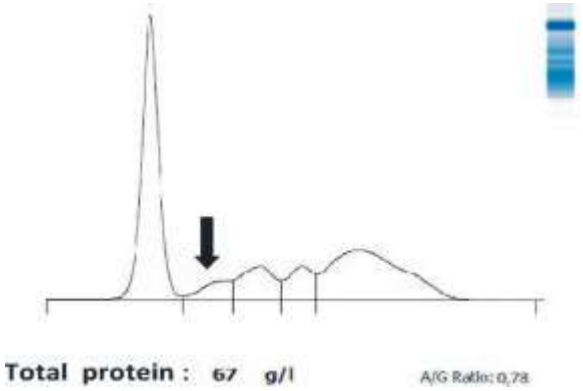

Fig. 8: Calf, aged 5 months, characterized by stunted growth. Biochemistry: lower α1-globulin (3.42 gr/l), increase the PAL (400 U l), witness to the bone fragility that is a consequence of food deficiency in minerals.

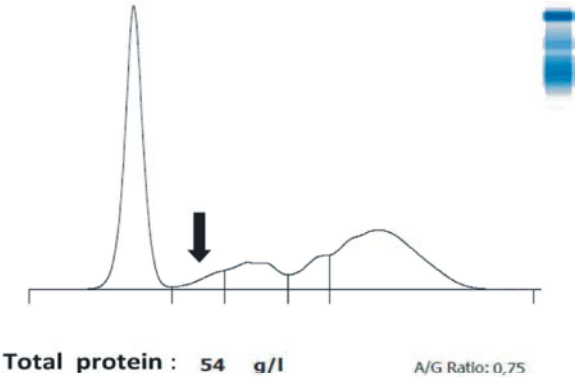

Fig. 9: Cow aged 3 years, 5 months pregnant, affect of mastitis. Biochemistry: decline of α1-globulin (1.94 gr/l) of albumin (23,22 gr/l), the β-globulin (3.83 g r/l).

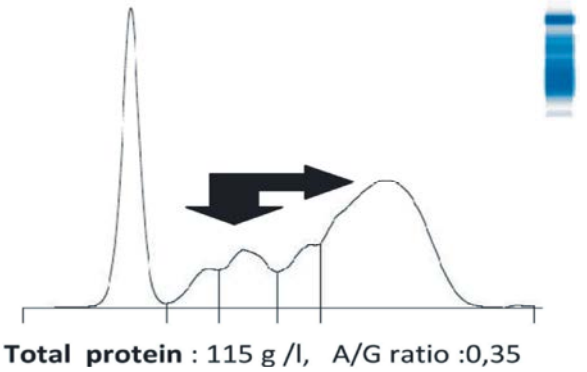

Fig. 10: Cow aged 3 years, suffering from lameness. Biochemistry: increase of the total proteins (115 gr/l), α2-globulin (12,31 gr/l) and γ globulins (57,04 gr/l). They are all witnesses of the severe inflammation.

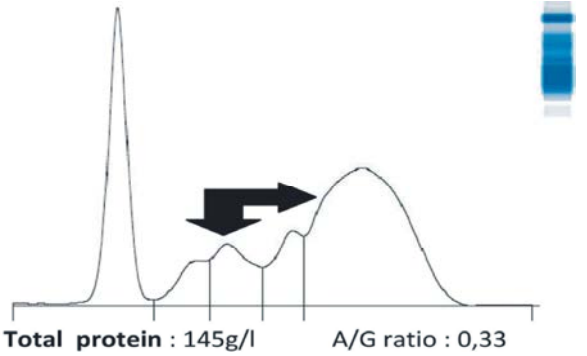

Fig. 11: Cow, 8 years old, suffering from rumen acidosis associated with liver failure revealed by Biochemistry: increase in the total proteins (145 gr/l), α2-globulins (13,78 gr/l), γ globulins (13,05 gr/l) and the β globulins (73,23 gr l).

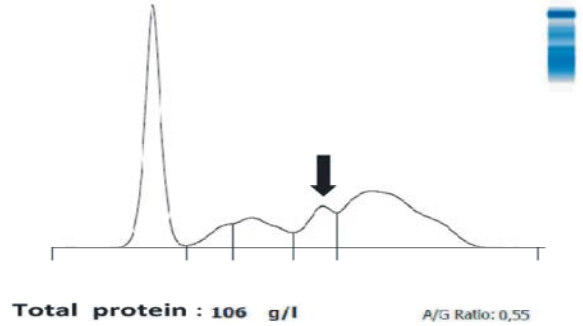

Fig. 12: Heifer aged 6 months, suffering from rumen acidosis associated with liver failure. Increased: of the total proteins (106 gr/l), the globulins α2 (11.55 gr/l), the γ globulins (11, 02 gr/l), the β-globulin (40,39 gr/l), the PAL (161U/l) and the GGT (274U/l).

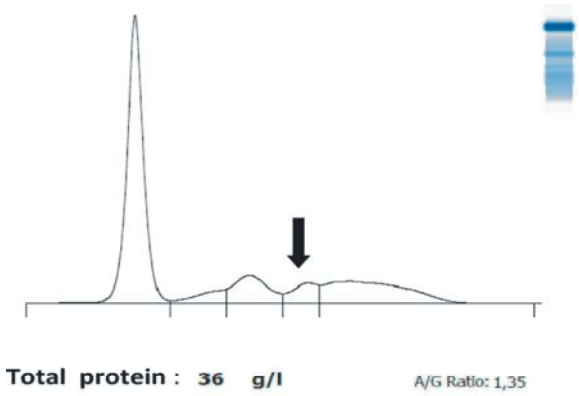

Fig. 13: Cow aged 6 years, suffering from a deficit in protein intake. Biochemistry: hypo albuminemia (20.66 gr/l).

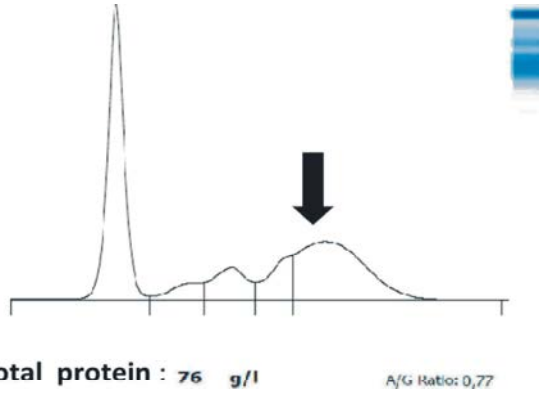

Fig. 14: Cow suffering from mastitis associated with bronchopneumonia, 8 years old. Biochemistry: increase GGT (40U/l), PAL (181 U/l), Hp (0.54 gr/l), γ globulins (25,38 gr/l).

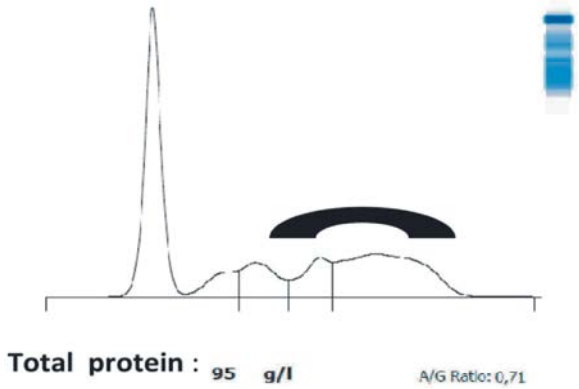

Fig. 15: Cow, 2 years old, with rumen acidosis associated with liver disease. Characterized by the formation of a block (β - γ). Revealed by an increase of the total proteins (95 g/l), the Bil.T (8 gr/l), the α2 globulins (9.75 gr/l) and γ globulins (30,02 gr/l).

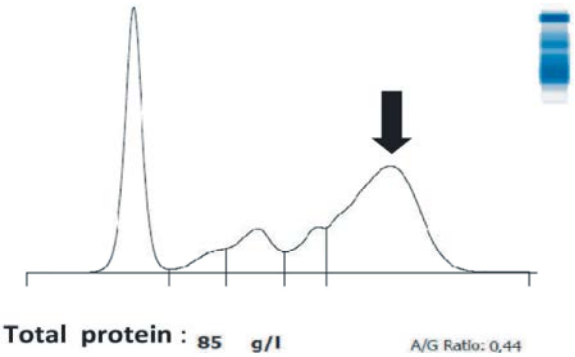

Fig. 16: Cow, 10 years old, suffering from chronic mastitis. Characterized by a peak of the γ globulins (40,04 gr/l).

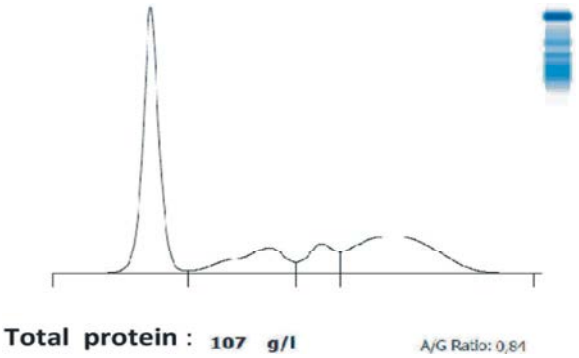

Fig. 17: Cow aged 6 years, CSC = 02, suffering from liver failure revealed by Biochemistry: increase in the total proteins (107 gr/l), α2 globulins (10, 7 gr/l), γ globulins (34.03 gr/l), the PAL (247 U/l), the GGT (51U (/ l).

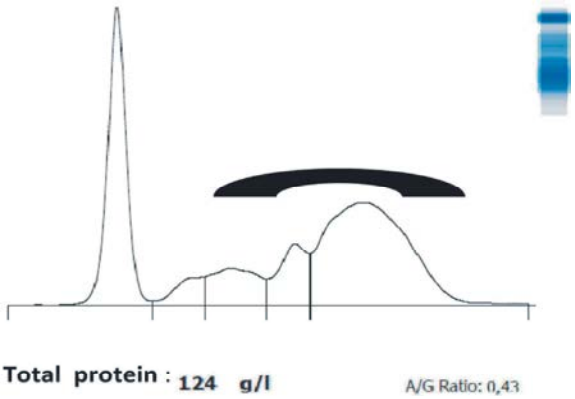

Fig. 18: Cow aged 6 years. She is suffering from bronchopneumonia associated with liver disease revealed by Biochemistry: increase of the α2 globulins (11.78 gr/l), the globulins (11.78 gr/l) β, the γ globulins (57,66 gr/l), the PAL (198 U/l), the GGT (106 U/l) and the total proteins (124 gr/l).

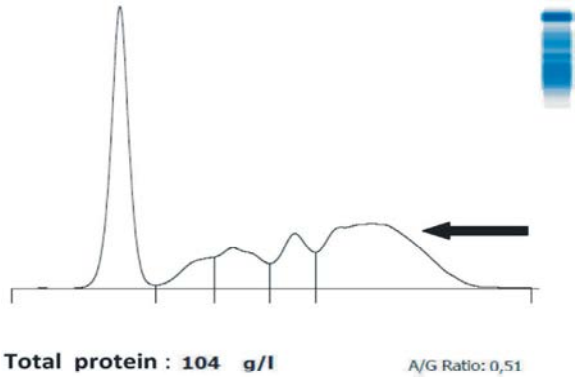

Fig. 19: Cow aged 5 years, pregnant for 5 months, affect of mastitis. Biochemistry: increase of the α2 globulins (11,13 gr/l), the γ globulins (40, 35 gr/l), the PAL (140U/l) witness of the fetal bone growth.

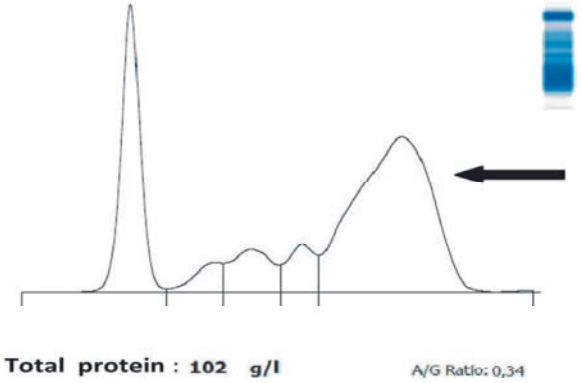

Fig. 20: Cow aged 8 years, affect of bronchopneumonia. Biochemistry: increase in the total proteins (102 gr/l), the γ globulins (57.3 gr/l) and the Hp (0.41 gr/l), witnesses of the strong inflammation.

directed against specific antigens caused by viral attacks, bacterial or parasitic. As a result, the poor overweight condition of these young animals is not explained. The monoclonal peaks in this species for older subjects >6 months was reported in animals having lymphocytic leukemia or reticulo-endothelial tumor system [1, 10].

The second group (57, 28%) showed polyclonal hyper-gamma globulinemia in cattle that develop general or special inflammatory diseases to an organ (Figures 10, 11, 16, 19, 20).

Interpretation of Results with Respect to Inflammation: The assay of fibrinogen revealed that its value did not exceed the threshold of 5 gr/l, even for animals expressing clinical pathological signs. This parameter was found to be less sensitive than the interpretation of the information provided by electrophoresis with respect to inflammation. Although its role is long recognized as a good indicator of inflammation [20], the non-existence of a significant increase in its value in the cases studied could be explained by the influence of the negative energy balance, which is the consequence of the non-application of the rationing techniques of the food necessary for the needs of the animals on the farms in question [8]. Haptoglobin concentration greater than 0.41g / l was observed in 10 cows (5%) having bronchopneumonia and belong to the same breeding. this feature has been revealed in other works [11, 21, 22]. Its value agrees with that found by those who place it between 0 and 60-70 mg / l [23 - 28]. For the rest of the cattle, the automaton could not fall below the threshold of 0.27gr/l. These unusable values are due to the degree of sensitivity of the analysis technique performed by the automaton. The latter is limited by a detection threshold And were low [19]. Through the interpretation of the EPP associated with the assay of other biochemical parameters, we have been able to confirm clinically apparent cases (Figures 14-16). Other asymptomatic cases were also observed (Figures 12, 17, 20). For the latter, it is about liver affections (an evolution without apparent clinical signs). The α2 fraction obtained by electrophoresis is still increasing during the inflammatory or infectious process, since the majority of APPs (proteins of the acute phase of inflammation) migrate to this zone [1, 10]. It would therefore be wise to consider this fact rather than having separate APPs that require more expensive techniques and to avoid the problem of detection sensitivity of the technique [20, 29-31].

CONCLUSION

Nevertheless, the simplicity of its method, the speed of its response (less than an hour), the quality of its information; Electrophoresis is a practical and economical alternative in the semiological exploration of ruminants. Subject to specific cases, it should be associated with other more specific biochemical assays (specific functional organ exploration) in order to prevent the practicing veterinarian from having to advocate uncertain treatments.

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