

# Development of a Radioimmunoassay for Bovine Pepsinogen A

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## Keywords

Cattle – Pepsinogen A –  
Radioimmunoassay – Blood plasma.

## Summary

Pepsinogen A is the most abundant zymogen found in blood, and its enzymatic measurement is used for the diagnosis of gastric lesions. The present study was conducted to develop a radioimmunoassay (RIA) specific to pepsinogen A in bovine plasma. The authors purified large amounts of three non-denatured isoforms of bovine pepsinogen A with high proteolytic activity. These homogeneous preparations were used to produce specific antisera in New Zealand White rabbits, and three antisera with high titers were obtained. In the present assay the antiserum #822 was used at a final dilution of 1/250,000. The detection limit of the assay was 20 ng/ml and the recovery ranged from 85.5 to 103.3%. The repeatability (intra-assay coefficient of variation) was lower than 6.6%, whereas the reproducibility (inter-assay coefficient of variation) was lower than 13.4%. The capacity of the RIA to detect pepsinogen A in blood was tested by measuring the concentrations in plasma of newborn calves (n = 6) serially sampled from birth to four months of age. The mean pepsinogen A value (mean  $\pm$  standard deviation) in the plasma of calves was 2071  $\pm$  752 ng/ml one day after birth. The concentration decreased progressively and was about 1196  $\pm$  307 ng/ml at day 21, and 677  $\pm$  109 ng/ml at day 120. The present study is the first report on pepsinogen A concentrations in bovine measured by RIA. Further investigations using the RIA should be performed in order to confirm these values and determine pepsinogen levels in older cattle in physiological and pathological conditions such as gastrointestinal helminthosis.

## INTRODUCTION

Gastric aspartic proteases (AP) are among proteolytic enzymes that are widely distributed in vertebrates. They are responsible for the digestion of dietary proteins and are synthesized as inactive precursors (prochymosin, pepsinogen and progastricsin), commonly known as zymogens. One part of their secretion by mucous neck and chief cells in the gastric mucosa (2, 22, 23) reaches the blood stream allowing their measurement in the peripheral circulation. The determination of plasmatic concentrations of gastric aspartic proteases is conventionally performed by proteolytic assays, the first report having been described by Anson and Mirsky (4). The widest accepted measurement techniques are based on the estimation of the

peptides released during incubation of the sample (at acidic pH) with a protein substrate (albumin or hemoglobin) added in high amounts (much higher than the endogenous content). The results are obtained by reading the optical density after addition of Folin-Ciocalteu's color reagent. For routine diagnosis purposes, the proteolytic method was recently simplified by the use of multiwell plates and multireaders for the determination of the optical density (11, 21).

Before the discovery of the multiplicity of the gastric AP family (15, 28), there was a general agreement that the measured protease activity in blood was exclusively due to pepsinogen. However, it is well known today that enzymatic assays are not specific to pepsinogen in that they detect other enzymes, which may be present in the sample and activated at acidic pH. These include at least two types of pepsinogens (A and C or progastricsin), two types of cathepsins (D and E) and different forms of prochymosin (28).

The diversity of human gastric enzymes was pointed out by Samloff (28), who suggested the necessity to develop specific immunoassays for the existing gastric proteases. In recent years, considerable progress has been made in identifying and characterizing these proteases. Different forms of pepsinogens and prochymosins

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were discovered and assumption was set out about distinct secretion sites of these zymogens in stomach mucosa. In humans, pepsinogen A was found to be secreted by fundic mucosa, while pepsinogen C was secreted by fundic, pyloric and proximal mucosae (14). In the same way, the hypothesis of a differential release of zymogens under physiological and pathological conditions stimulated the interest on their purification and on the development of specific assays. For example, by analyzing the ratio between pepsinogen A (PgA) and pepsinogen C (PgC) concentrations, several studies allowed a more precise diagnosis of gastric diseases such as duodenal ulcers with *Helicobacter pylori* infection, and gastric cancer (7, 9, 26, 37).

In ruminants, information concerning different forms of pepsinogen and chymosin progressed according to their social usefulness in cheese manufacturing, specially for chymosin. Some reports are available on biochemical isolation and characterization of zymogens in terms of amino acid sequences and activation processes (5, 10, 12, 16, 24). However, attempts to produce and characterize a specific antiserum for the development of specific immunoassays were limited to the studies of Turner and Shanks (35) in sheep, and Gomes et al. (16) in cattle.

In cattle, the measurement of blood pepsinogen by proteolytic assay has been used since 1960 in parasitological investigations concerning the severity of ostertagiosis infections (1, 13), or hemochosis in tropical areas. Later, this assay was also recommended to confirm clinical diagnosis of abomasal displacement (39) and gastric ulcers (25).

We recently described a simple procedure to prepare large amounts (> 10 mg) of bovine pepsinogen A, which are used for the production of antisera and in the development of a specific immunoassay. Three isoforms (bPgA-1, bPgA-2 and bPgA-3) with the same N-terminal sequence and differing in their phosphate content were isolated (31). A high proteolytic activity towards bovine serum albumin (5.15, 7.45 and 6.81 units of tyrosine per milligram of protein, bPgA-1, bPgA-2 and bPgA-3, respectively) indicated that the three zymogens were not denatured. The degree of homogeneity of these fractions was confirmed by electrophoresis and mass spectrometry. The aim of the present study was thus to develop a specific radioimmunoassay (RIA) for pepsinogen A determination in bovine peripheral blood. Pepsinogen has been closely related to gastric damage due to parasites. In tropical areas, *Haemonchus* is the most important gastric nematode that causes huge problems in cattle husbandry. The RIA will be helpful to manage these parasites. Thus, different antisera were raised against bovine pepsinogen A (bPgA) in New Zealand White rabbits. A specific radioimmunoassay (bPgA-RIA) was developed and validated in terms of sensitivity, specificity, accuracy and reproducibility. Finally, the RIA was used to determine plasma concentrations of pepsinogen A in calves serially sampled from birth to four months of age.

## ■ MATERIALS AND METHODS

### Reagents and equipment

The following reagents and equipments were purchased from the indicated sources: potassium monophosphate, methanol, and microcrystalline cellulose (Merck, Darmstadt, Germany); bovine serum albumin (BSA, ICN Biomedicals, Eschwege, Germany); Tween 20 (Fluka and Riedel-de Haën, Schweiz, Switzerland); polyethyleneglycol 6000, and sodium azide (PEG 6000, Vel, Leuven, Belgium); Freund's incomplete adjuvant (Difco Laboratories, Detroit, IL, USA); Freund's complete adjuvant, bovine chymosin B, human pepsinogen A, porcine pepsinogen A, pepstatin A, chloramine T, and sodium metabisulfite (Sigma-Aldrich, St Louis, MO,

USA); second antibody (Physiology of Animal Reproduction, University of Liege, Belgium); Na-<sup>125</sup>I, and Sephadex G-75 (Amersham Biosciences, Uppsala, Sweden); and automatic gamma counter 1261 Multigamma (Wallac, Turku, Finland).

### Bovine pepsinogen A (bPgA)

Pure preparations of bovine pepsinogen A (bPgA) were made available from previous studies (31) and used as standard, tracer and immunogen for antisera preparation.

### Antisera production

Antisera against bPgA were raised in New Zealand White rabbits according to the method of Vaitukaitis et al. (36). During two months, three rabbits aged between 3 and 5 months received intradermal injections (15 days' intervals) of 500 µg of bPgA dissolved in 500 µl of 0.05 M phosphate buffer (pH 7.5), and emulsified in an equal volume of Freund's complete adjuvant. Afterwards, rabbits received monthly booster doses of bPgA (500 µg) emulsified in Freund's incomplete adjuvant over a period of six months. The animals were bled two months after the first injection and then monthly. Blood was collected from the marginal vein of the ear and allowed to clot overnight at room temperature. The next day, the serum was transferred into clean tubes, centrifuged at 2500 g for 20 min, aliquoted into small fractions and stored at -20°C till tested.

### Radiolabeled protein (tracer)

The purified bPgA was labeled according to the chloramine T method (17). Briefly, a stock solution of bPgA dissolved in 0.05 M phosphate buffer, pH 7.5 (1.0 mg bPgA/ml), was prepared, aliquoted in small volumes (10 µl) and stored at -20°C till used for labeling. Chloramine T hydrate (5.0 mg/ml) and sodium metabisulfite (30.0 mg/ml) were dissolved in the same buffer just before use. The radio-iodination mixture was composed of 10 µg bPgA (10 µl), 10 µl 0.5 M phosphate buffer (pH 7.5), 10 µl <sup>125</sup>I-Na (1 mCi, approximately 3.7 x 10<sup>7</sup> disintegrations/s) and 10 µl of chloramine T solution. After 1 min of gentle stirring, 10 µl of metabisulfite solution was added. Unreacted iodine was separated from <sup>125</sup>I-bPgA by gel filtration on a Sephadex G-75 column (1 x 30 cm) equilibrated and eluted with 0.05 M phosphate buffer (pH 7.5). Eluted aliquots of 1.0 ml were collected, submitted onto a test with the antiserum, and selected according to the non-specific binding value (NSB) and binding/total count (B/T) ratio. The fractions exhibiting the highest performances in terms of specific binding were diluted, aliquoted (1.0 ml) and stored at -20°C until used in the RIA procedure.

### RIA buffer

Phosphate buffer (0.05 M, pH 7.5) containing 1.0 g/l BSA, 5.0 ml/l Tween 20, and 5.0 mg/l pepstatin A was used throughout the procedure.

### Second antibody precipitant system

The immunoprecipitation is based on the formation of a molecular network by the binding between the Ag and the Ac. The addition of a certain proportion of normal rabbit serum makes it possible to widen this network and obtain a visible precipitate. So, the second antibody was prepared by mixing sheep anti-rabbit IgG serum with normal rabbit serum (5:1; v:v). This solution was incubated for 16 h at 4°C before use at 1% in the precipitation system, which constituents were 0.05 M phosphate buffer (pH 7.5), 0.4% BSA, 4% PEG 6000, and 0.05% microcrystalline cellulose.

## Antisera binding test

The obtained three antisera were serially diluted in the RIA phosphate buffer (1/1000, 1/10,000, 1/20,000, 1/40,000, 1/80,000, 1/160,000, 1/320,000, 1/640,000 and 1/1,280,000) in order to obtain a tracer-binding ratio in the zero standard ( $B_0/T$ ) of approximately 30-50% and an NSB below 2%. For the binding test, 100  $\mu$ l of the diluted antiserum, 100  $\mu$ l  $^{125}$ I-bPgA and 300  $\mu$ l of the RIA buffer were incubated overnight before addition of the second antibody precipitation system, washing, centrifugation and count of the pellet radioactivity.

## Tracer stability

To test  $^{125}$ I-bPgA stability, the tracer was used for two weeks at short time intervals and the regression curve of B/T ratio was determined.

## Bovine PgA radioimmunoassay

As the concentrations of pepsinogen A in bovine blood are relatively high, the PgA-RIA system was developed without a preincubation step. The RIA was performed at 4°C in polystyrene assay tubes (75 x 12 mm). Pure stock bovine pepsinogen A (lyophilized powder) was diluted with assay buffer to give standard curves ranging from 19.5 to 10,000 ng/ml. Each tube contained 100  $\mu$ l of standard dilution or 100  $\mu$ l of unknown sample, 100  $\mu$ l of antiserum #822 diluted at 1/50,000 (1/250,000 final dilution), and 100  $\mu$ l of labeled pepsinogen (20,000 cpm). The incubation volume was made up to 500  $\mu$ l by addition of 200  $\mu$ l of assay buffer. In the zero standard tubes ( $B_0$ ), the standard dilution was replaced by assay buffer. NSB tubes contained 400  $\mu$ l buffer and 100  $\mu$ l tracer. All standard and unknown tubes were set up in duplicate. The mixture was incubated at 4°C from 18 to 24 h. After addition of 1.0 ml of the second antibody precipitation system, the mixture was allowed to react at 4°C for 1 h. Free and bound pepsinogen A were then separated by centrifugation at 3000 g for 20 min. The supernatant was discarded and the radioactivity of the pellet was determined in an automatic gamma counter with a counting efficiency of 75%.

## RIA characteristics

### Specificity and serial dilutions of a blood sample

Commercially available members of the aspartic protease family such as porcine pepsinogen A, chymosin B from calf stomach, and human pepsinogen A were tested in the wide range of 1.0 to 100  $\mu$ g/ml. When a cross-reaction was observed, the percentage in the RIA was estimated. In addition, different dilutions of a plasma sample containing detectable concentrations of pepsinogen were set up in buffer (1/1, 1/2, 1/4, 1/8; 1/16, 1/32 and 1/64).

### Accuracy

The accuracy test was carried out by adding defined concentrations of bovine pepsinogen A (78, 156 and 312 ng) to bovine plasma containing a known concentration. The percentage of recovery was calculated as follows: [observed value (ng/ml)/expected value (ng/ml)] x 100.

### Reproducibility

To test the reproducibility of the RIA, samples with low and higher concentrations of pepsinogen were tested. RIA precision was determined by estimating the intra- and inter-assay coefficients of variation (CV). To determine the intra-assay CV, a serum was assayed 10 times within the same assay. The inter-assay reproducibility was assessed by analyzing different sera in five consecutive assays.

### Sensitivity

The sensitivity was determined by measuring the least detectable dose of pepsinogen A in the developed RIA (32). The mean and

standard deviation of precipitate counts of 20 binding values at zero pepsinogen concentration were calculated. The pepsinogen value that corresponded to the mean count minus two standard deviations (SD) transposed onto the standard curve was defined as the sensitivity of the RIA.

## Animal and plasma samples

A total of six newborn calves were housed in the experimental farm of the Faculty of Veterinary Medicine (University of Liege, Belgium) from day 1 after birth to 120 days of age. Blood samples were serially taken from the jugular vein when the calves were 1, 7, 14, 21, 28, 60, 90 and 120 days old. Plasma was separated by centrifugation at 2000 g for 15 min and stored at -20°C until used.

## Statistical analysis

The data obtained were analyzed with Microsoft Excel. Inter- and intra-CVs were calculated as the SD divided by the mean value. Mean recoveries at each concentration were calculated as a percentage of the expected value.

## RESULTS

### Antisera binding ratios

The dilution curves of the three raised antisera (#821, 822, and 823) are presented in Figure 1. All the rabbits exhibited high antibody titers. For antiserum #822, 78% of labeled bPgA was bound and NSB was below 2% in the presence of excess antibody. A similarly high (over 70%) binding level (B/T) at 1/1000 was found in all antisera. These high binding levels point out the high degree of purity of the bPgA used as antigens. For the antiserum #822, a tracer-binding ratio in the zero standard of 40% (NSB below 2%) was obtained at a final dilution of 1:250,000.

### Tracer stability

The binding of the radiolabeled bPgA (B/T) declined progressively from 40 to 15% during the two weeks of running the RIA (Figure 2). During this period, there was no significant inter-assay variation of pepsinogen A concentrations as measured in the tested samples. However, a great variability was observed after these two weeks and the pepsinogen concentrations determined in these conditions were no more reliable.

### Standard curve and bPgA-RIA validation

Displacement curve of bPgA-RIA is presented in Figure 3. The standard inhibition curves ranged from 95 to 5% binding when serial dilutions of bPgA (ranging from 19.5 ng/ml to 10,000 ng/ml) were assayed.

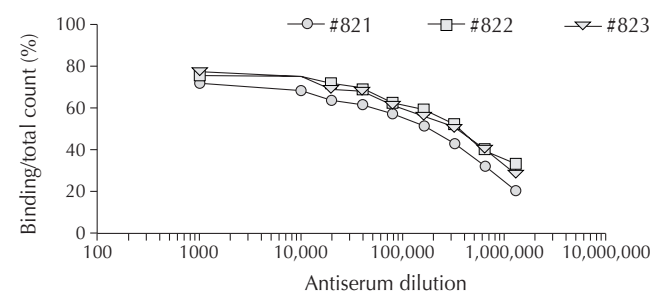


Figure 1: Binding ratio after serial dilution of three antisera raised against bovine pepsinogen A preparations.

A slight cross-reaction of bPgA-RIA was observed with calf chymosin B, porcine pepsinogen A and human pepsinogen A at concentrations ranging from 1.0 to 100 µg/ml (Figure 4). The cross-reactivity percentages at 100 µg/ml concentration were 0.50% for chymosin B, 0.16% for porcine pepsinogen A and 0.22% for human pepsinogen A. The serial dilution of the bovine plasma showed a dose-response curve parallel to the standard of the purified bPgA (Figure 3).

The recovery of the pepsinogen assay ranged from 85.5 to 103.3% (Table I). Concerning the reproducibility, mean bPgA concentrations and CVs are presented in Table II. The intra-assay coefficient of variation varied from 4.63 to 6.58% while the inter-assay ranged from 5.98 to 13.39%. The calculated least detectable dose was 2 ng/tube or 20 ng/ml of sample.

**Pepsinogen concentration in newborn calves**

Bovine pepsinogen A plasma concentrations gradually decreased with increasing calf age. The bPgA levels decreased from 2071 ± 752 ng/ml one day after birth to 677 ± 109 ng/ml at 120 days of age (Table III).

**Table I**

Recovery of purified bovine pepsinogen A (bPgA) added to four bovine samples containing different concentrations of bPgA

Initial serum sample bPgA concentration	Amount of bPgA (ng) added	Expected bPgA concentration (ng/ml)	Observed bPgA concentration (ng/ml)	Recovery* (%)
26 ng/ml	78	104	107	102.8
	156	182	188	103.3
	312	338	311	92.0
47 ng/ml	78	125	113	90.4
	156	203	180	88.6
	312	359	307	85.5
930 ng/ml	78	1008	999	99.1
	156	1086	1106	101.8
	312	1242	1280	103.0
967 ng/ml	78	1045	1050	100.4
	156	1123	1100	97.9
	312	1279	1271	99.3

\* (Observed value/expected value) x 100

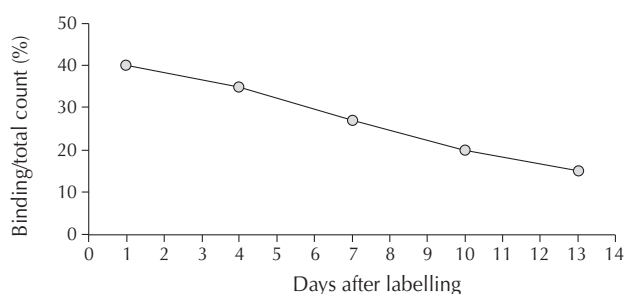
**Table II**

Coefficients of variation intra- and inter-assay of bovine pepsinogen A (bPgA) RIA

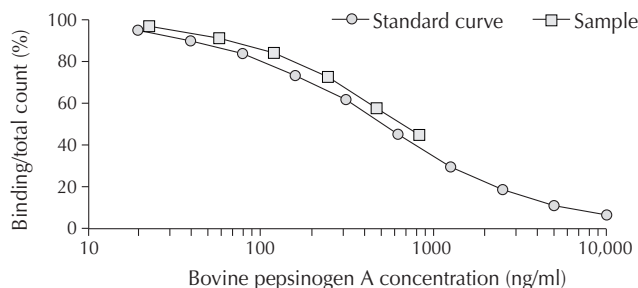
Bovine sample	Intra-assay		Inter-assay	
	bPgA concentration*	CV** (%)	bPgA concentration*	CV** (%)
Sample 1	106.63 ± 6.62 ng/ml	6.58	100.00 ± 13.39 ng/ml	13.39
Sample 2	1587.78 ± 69.31 ng/ml	4.63	1756.10 ± 105.02 ng/ml	5.98

\* Mean ± standard deviation

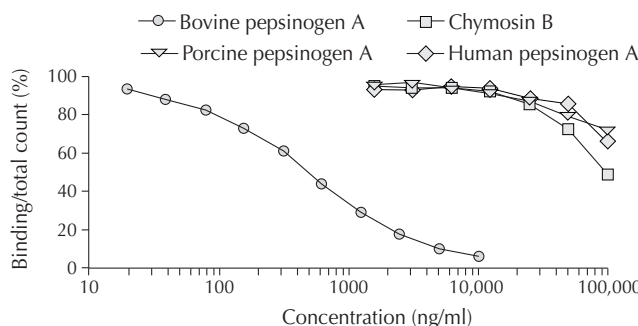
\*\* Coefficient of variation



**Figure 2:** Radio-labeled bovine pepsinogen A (<sup>125</sup>I-bPgA) stability during the two weeks of running the RIA.



**Figure 3:** Parallelism between bovine pepsinogen A (bPgA) standard curve and serial dilution of a bovine serum sample. The standard curve was calculated by the linear scale of B/B<sub>0</sub> ratio vs decadic logarithmic of the standard concentration using radiolabeled bPgA. B/B<sub>0</sub> means tracer bound / tracer bound in zero standard. The serum sample (1/1) was serially diluted at 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64.



**Figure 4:** Specificity of the bovine pepsinogen A radioimmunoassay. The standard curve was calculated by the scale of B/B<sub>0</sub> ratio vs decadic logarithmic of bPgA concentrations (expressed in ng/ml) using radiolabeled bPgA (<sup>125</sup>I-bPgA). B/B<sub>0</sub> means tracer bound / tracer bound in zero standard.

Table III

Bovine pepsinogen A (bPgA) concentrations in plasma samples taken from six calves from birth to 120 days of age

Age (days after birth)	bPgA mean concentration (ng/ml)	bPgA SD* (ng/ml)	Minim.-maxim. concent.** (ng/ml)
1	2070.6	752.1	1084.0–3058.6
7	1451.1	459.0	800.1–1902.8
14	1214.9	377.8	765.2–1689.3
21	1196.3	306.8	724.8–1538.6
28	679.7	112.4	542.8–862.5
60	815.7	139.2	707.0–1085.5
90	714.1	120.8	514.0–832.0
120	677.3	108.5	497.0–814.8

\* Standard deviation

\*\* Range of minimal–maximal concentrations

## DISCUSSION

The present study is the first report on the development of a specific RIA for bovine pepsinogen A measurements in bovine plasma samples. Three antisera with high titers were obtained. Antiserum #822 had 78% binding ratio in excess antibody (1:1000) and was used for bPgA-RIA at a final dilution of 1:250,000, yielding 40% of total counts. The advantage of the use of a specific antiserum giving high dilution titers is the possibility to perform a large number of assays with a small volume of the antiserum, along with increased sensitivity and specificity of the assay. So, it is interesting to compare the performances of the present antisera with those reported in previous publications.

In 1974, Samloff and Liebmann (29) developed an RIA for human pepsinogen A using an antiserum at a lower dilution titer (1:50,000). In 1990, the first RIA for porcine pepsinogen A was developed by use of an antiserum diluted at 1:18,000 (27). This system gave very low plasmatic concentrations which were not confirmed. Later, higher antiserum dilution titers resulting in precise plasmatic concentration measurements were described for RIA of human and porcine pepsinogens. In humans, Biemond et al. (8) described the use of antisera diluted at 1:200,000 and 1:300,000 for pepsinogen A and pepsinogen C RIA systems, respectively. A similar dilution of antiserum (1:200,000) was described by Banga-Mboko et al. (6) for porcine pepsinogen A RIA. In bovine species, the only report on pepsinogen immunoassay was described by Gomes et al. (16). They reported the development of an ELISA in which the antiserum was diluted at 1:2000, which is hundred times more concentrated than the optimal antiserum titer used in the present investigation. In the investigation carried out by Gomes et al., bovine pepsinogen ELISA had a sensitivity of 32 ng/ml and no attempt to measure pepsinogen in plasma was described. In the present report, bPgA-RIA gave a sensitivity of 2 ng/tube or 20 ng/ml of plasma. No attempt was made to improve the sensitivity, because of the high plasmatic bPgA concentrations measured in blood samples.

This is also the first report describing bovine pepsinogen A labeling with  $^{125}\text{I}$ . The obtained tracer showed to be suitable in RIA routine analysis during a two-week period. The antiserum initially bound 40% of the  $^{125}\text{I}$ -bPgA, this binding decreasing steadily till 15% after a two-week period. After two weeks, bPgA concentra-

tions measured in control samples were highly variable. In the same way, Samloff and Liebman (29) reported that the decreasing activity of the labeled human pepsinogen depends probably on time, radiation damage, and pepsinogen instability. In their study, the labeled human pepsinogen was suitable for immunoassay for 6–7 weeks only when it was re-purified on a DEAE-cellulose column before the assay.

Considering the specificity, the present assay showed 0.16% cross-reaction with porcine pepsinogen A and 0.22% with human pepsinogen A. These cross-reactions can be explained by the high percentage of amino acid homology between bPgA and the tested pepsinogen preparations. As determined by comparison of amino acid sequences, bPgA have 83.5% and 82.1% homology with porcine pepsinogen A and human pepsinogen A, respectively (30). Additionally, such cross-reactivity was only observed when high concentrations of the zymogens ( $> 10 \mu\text{g/ml}$ ) were tested. The relatively weak cross-reactivity observed in the present study confirms the necessity to develop specific RIA systems for the different existing bovine zymogens. Bovine pepsinogen A assay cross-reacted towards bovine chymosin B (bChyB) at 0.5%, when the amount of the added chymosin was higher than  $10 \mu\text{g/ml}$ . The preliminary data on bChyB concentrations in bovine circulation showed that such high levels are not found in plasma, indicating the absence of impact of bChyB concentrations on plasmatic bPgA levels measured in bovine species (data not shown).

The recovery of the assay ranged from 85.5 to 103.3%. These values are closely similar to the data reported in porcine (81.7 to 102.3%) and human assays (81.3 to 139.5%) (6, 8). The RIA showed an intra-assay coefficient of variation lower than 6.6%, and an inter-assay variation of 13.39% or lower. These values were found with a fresh tracer; they remained stable during 14 days and increased sharply thereafter.

The general validation characteristics of bPgA-RIA (sensitivity, specificity, accuracy, intra and inter-assay coefficients of variations) being satisfactory, the present assay was considered suitable for measurement of bPgA in bovine plasma. Thus, bPgA-RIA was used to determine bPgA concentrations in plasma samples collected from six newborn calves serially sampled from birth to four months of age. Relatively high levels (1084.0 to 3058.6 ng/ml, mean 2070.6) were found in calves at birth, the concentration decreasing steadily until 120 days of age (497.0 to 814.8 ng/ml, mean 677.3 ng/ml).

Because of the high variability of pepsinogen concentrations between species, comparison of the present study with those carried out in other species must be made with great caution (3, 20, 34). Nevertheless, it is interesting to situate the present values in the reported data for other species found in the literature. Thus, no great discrepancy was found between the present values and those obtained by Banga-Mboko et al. (6) who reported that, in porcine, the mean values in serum were about 290 ng/ml for 21-day-old piglets and 383.5 ng/ml (249–570 ng/ml range) for finishing pigs at 213 days of age. In normal dogs, Suchodolski et al. (33) reported a range from 18 to 129 ng/ml for pepsinogen A. In humans, in which specific RIA have been developed for pepsinogens A and C, some data are available in samples collected under both physiological and pathological conditions. The reported values in healthy subjects range between 50 to 200 ng/ml for pepsinogen A, and vary around 15 ng/ml for pepsinogen C (8, 19, 29).

The measurement of the two forms of serum pepsinogen (A and C) by RIA and the use of the ratio A/C in humans revealed a great interest of this approach in clinical investigations on ulcers or gastric cancer (18, 22, 38). This suggests that in bovine the measurement by RIA of another zymogen such as chymosin or pepsinogen C could

be of great interest for the differential diagnosis of parasite diseases. This second form of pepsinogen (PgC) has been reported in bovine only in a few studies, but it is neither available on the market, nor in the scientific domain. It was reported that the proportion of bovine pepsinogen C in the extract of gastric mucosa was 30 times lesser than pepsinogen A (13), suggesting additional difficulties in its purification. This observation could explain the reason why, in a previous study on pepsinogen purification (31), the present authors only obtained the pepsinogen A form in large amounts after extraction, ammonium sulfate fractionation, DEAE ceramic and hydroxyapatite columns. Further investigation with an adapted protocol of purification is needed to isolate bovine pepsinogen C and develop a specific radioimmunoassay. Meanwhile, bPgA-RIA is an available precious tool, which can give reliable information on the status of bovine gastric mucosa.

## ■ CONCLUSION

RIA for the measurement of bovine pepsinogen A in blood is sensitive, specific, repeatable and reproducible. The assay can therefore be used to assess accurately pepsinogen A concentrations in bovine plasma. Measured bPgA concentrations are elevated in young calves. Further studies are needed to set up reference values of serum pepsinogen A concentrations in growing calves and adults of different breeds. These reference values are necessary to compare the concentrations in different gastric disorders such as helminthosis in animals kept under various environmental and diet conditions.

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## Résumé

**Sidikou D.I., Remy B., Beckers J.F.** Développement du dosage radioimmunologique du pepsinogène A bovin

Le pepsinogène A est le zymogène le plus abondant présent dans la circulation sanguine des ruminants, et son dosage enzymatique est utilisé pour établir un diagnostic lors de lésions digestives. La présente étude a été menée pour mettre au point un dosage radioimmunologique (RIA) spécifique du pepsinogène A plasmatique chez les bovins. Les auteurs ont purifié en grandes quantités trois isoformes non dénaturées, possédant une activité élevée, du pepsinogène A bovin. Ces préparations homogènes ont été utilisées pour la production d'antisérums spécifiques chez des lapins Blancs Néozélandais, et trois antisérums avec des titres élevés ont été obtenus. Dans la présente étude l'antisérum n° 822 a été utilisé à une dilution finale de 1/250 000. La limite de détection du dosage a été de 20 ng/ml et l'exactitude a été comprise entre 85,5 et 103,3 p. 100. Les coefficients de variation intradosage (répétabilité) et inter-dosages (reproductibilité) ont été inférieurs respectivement à 6,6 et 13,4 p. 100. La capacité du RIA à détecter le pepsinogène A dans le sang a été évaluée en mesurant ses concentrations plasmatiques chez le veau (n = 6) de la naissance à 4 mois d'âge. La valeur moyenne du pepsinogène A (moyenne ± déviation standard) dans le plasma des veaux a été de  $2\,071 \pm 752$  ng/ml à un jour d'âge. Cette concentration a diminué progressivement pour atteindre environ  $1\,196 \pm 307$  ng/ml à 21 jours et  $677 \pm 109$  ng/ml à 120 jours. La présente étude rapporte pour la première fois la mesure par RIA des concentrations du pepsinogène A chez les bovins. D'autres études utilisant cette technique devraient être réalisées afin de confirmer ces valeurs et de déterminer les niveaux du pepsinogène A chez des bovins plus âgés dans différentes conditions physiologiques et pathologiques, comme les infestations par les parasites gastro-intestinaux.

**Mots-clés :** Bovin – Pepsinogène – Technique radioimmunologique – Plasma sanguin.

## Resumen

**Sidikou D.I., Remy B., Beckers J.F.** Desarrollo de un radio inmunoensayo para el pepsinógeno bovino A

El pepsinógeno A es el cimógeno más abundante de la sangre y su medida enzimática se utiliza para el diagnóstico de lesiones gástricas. El presente estudio se llevó a cabo para desarrollar un radioinmuno ensayo (RIA) específico al pepsinógeno A en el plasma bovino. Los autores purificaron grandes cantidades de tres iso-formas no desnaturalizadas del pepsinógeno bovino A, con gran actividad proteolítica. Estas preparaciones homogéneas se utilizaron para producir un anti suero específico en conejos Blancos de Nueva Zelanda y se obtuvieron tres anti sueros con altos títulos. En el presente ensayo se utilizó el anti suero No 822 a una dilución final de 1/250 000. El límite de detección del ensayo fue de 20 ng/ml y la recuperación varió de 85,5 a 103,3%. La repetibilidad (coeficiente de variación intra ensayo) fue menor de 6,6%, mientras que la reproducibilidad (coeficiente de variación inter ensayo) fue de 13,4%. La capacidad del RIA para detectar el pepsinógeno A en la sangre fue probada mediante la medida de las concentraciones en el plasma de terneros recién nacidos (n=6) en muestras en serie desde el nacimiento hasta cuatro meses de edad. El valor promedio de pepsinógeno A (promedio ± desviación estándar) en el plasma de los terneros fue de  $2071 \pm 752$  ng/ml un día después del nacimiento. La concentración disminuyó progresivamente y fue de alrededor de  $1196 \pm 307$  ng/ml al día 21 y de  $677 \pm 109$  ng/ml al día 120. El presente estudio es el primer reporte de concentraciones de pepsinógeno A en el bovino, medido mediante radioinmuno ensayo. Deben realizarse más estudios con el RIA, con el fin de confirmar estos valores y determinar los niveles de pepsinógeno en animales más viejos en condiciones tanto fisiológicas como patológicas, tal como helmintosis gastrointestinales.

**Palabras clave:** Ganado bovino – Pepsinógeno A – Técnica radioinmunológica – Plasma sanguíneo.